**UNIVERSITY OF NAPLES FEDERICO II** 



### SCHOOL POLYTECHNIC AND OF BASIC SCIENCES

Ph.D. in Chemical Sciences

### Hybrid nanostructured systems: physico-chemical properties and applications in medical and technological fields

Ph.D. Student

Noemi Gallucci

Tutor

Examiner

Prof. Filomena Sica

Prof. Luigi Paduano

Prof. Irene Russo Krauss

XXXV Cycle 2019/2022

Coordinator Prof. Angela Lombardi

### Index

Introduction	1
Section A: Coated CeO <sub>2</sub> superstructures	5
Chapter 1: Self-assembly	6
1.1 Self-assembly	7
1.1.1 Energy involved in the self-assembly	8
1.1.2 Geometrical parameters	12
1.2 Cerium Oxide Nanoparticles	14
1.2.1 Synthesis and functionalization	16
1.2.2 Properties and applications	
Chapter 2: Synthesis and Functionalization of coated CeO <sub>2</sub> NPs	
2.1 Synthesis of CeO <sub>2</sub> NPs	21
2.1.1 Materials	21
2.1.2 Synthetic protocol	21
2.2 Preparation of 3D superlattice systems	
2.2.1 Materials	25
2.2.2 3D ordered structures protocol	25
Chapter 3: Amines@CeO2 NPs: investigating the synthesis NPs	
3.1 Inorganic core	
3.1.1. Crystalline structure	
3.2.2 Chemical composition	
3.2 Organic coating	
3.3 Morphological and dimensional analyses	
3.4. Optical properties	44
Chapter 4: Ordered vs disordered coated CeO <sub>2</sub> superlattices	
4.1 OA-Amine@CeO2 NP superlattice	
4.1.1 Effect of the length of the alkyl chain of the capping agent	49
4.1.2 Effect of the inorganic core dimension	57
4.1.3 Effect of the ratio between nanoparticle suspension and oleic acid concentration	64
4.1.3.3 OA-C16@CeO2 250	64
4.1.3.4 OA-C18@CeO2 250	67
4.2 Interaction potential of coated CeO2 NPs	73
4.4. Photoluminescence properties: ordered vs disordered aggregates	82
Conclusion Section A	86
Section B: Aptasensor	90
Chapter 1: Fluorescent NPs for technological applications	
1.1 Zinc Oxide Nanoparticles	
1.1.1 Synthesis and doping	93
1.1.2 Properties and applications	94
1.2 Biosensor	96

1.2.1 Structures and types	96
1.2.2 Applications	
Chapter 2: F-doped ZnO NPs: synthesis and film deposition	100
2.1 Synthesis of ZnO NPs	101
2.1.1 Materials	
2.1.2 Synthetic protocol	
2.2 Film preparation	102
2.2.1 Materials	
2.2.2 Film preparation through spin coating method	
2.3 Aptasensor realization	105
2.3.1 Materials	
2.3.2 Preparation of the bio-recognition layer	
Chapter 3: The best F-doped ZnO NP system	107
3.1 Structural characterization of F-doped ZnO NPs	108
3.1.1 Crystalline structure	
3.1.2 Chemical composition	110
3.2 Morphological and dimensional characterization	113
3.2.1 Morphological analysis	
3.2.2 Dimensional analysis	115
3.3 Optical properties	116
3.3.1 Optical properties of F-doped ZnO NPs in suspension	
3.3.1 Optical properties of F-doped ZnO NPs at solid state	
Chapter 4: Transduecer: F-doped ZnO NP film	127
4.1 Microscopic analysis	128
4.1.1 Characterization of ZnO NP film on silicon supports through solid-state ellipsometry	
4.1.2 The choice of the best support: XRR analyses	
4.2 Macroscopic analysis	136
4.2.1 The choice of the best deposition conditions	
4.3 Design of the aptasensor	141
Conclusion Section B	143
Appendices	I
Appendix A – Scattering Techniques	II
A.1 Basic scattering theory	II
A.2 Light scattering: DLS	IV
A.2.1 Instrument	VI
A.2.2 Sample preparation and experimental set-up	VI
A.3 X-ray scattering	VI
A.3.2 Structure determination: from Wide Angle X-ray Scattering (WAXS) to Small Angle X-ray Scattering (SAX	KS) VIII
A.3.2 WAXS: sample preparation, instrument, and experiment set-up	IX
A.3.3 SAXS: sample preparation, instrument, and experimental set-up	IX
A.4 Neutron scattering: Small Angle Neutron Scattering (SANS)	IX
A.4.1 Sample preparation, instrument, and experimental set-up	XI

A.4.2 Data analysis	XII
Appendix B – Microscopy	XIII
B.1 Optical Microscopy	XIII
B.1.1 Sample and experimental set-up	XIV
B.2 Transmission Electron Microscopy (TEM)	XIV
B.2.1 Sample and experimental set-up	XIV
B.3 Cryogenic Transmission Electron Microscopy (Cryo-TEM)	XIV
B.3.1 Sample and experimental set-up	XV
B.4 Atomic Force Microscope (AFM)	XVI
B.4.1 Sample and experimental set-up	XVI
Appendix C – Spectroscopy	XVII
C.1 UV-Visible Spectroscopy	XVII
C.1.1 Band gap	XVIII
C.1.2 Sample and experimental set-up for UV-Visible measurements in suspension	XIX
C.1.3 Sample and experimental set-up for band gap determination	XIX
C.2 Fluorescence Spectroscopy	XIX
C.2.1 Relative Quantum Yield	XX
C.2.2 Sample and experimental set-up for fluorescence measurements	XXI
C.2.3 Sample and experimental set-up for relative quantum yield determination	XXI
C.3 Photoluminescence Spectroscopy	XXI
C.3.1 Synthesized NPs: experimental set-up	XXII
C.3.2 Coated CeO <sub>2</sub> superlattices: experimental set-up	XXII
C.4 X-ray Photoelectron Spectroscopy	XXII
C.4.1 Sample and experimental set-up	XXIV
C.5 Micro-Raman Spectroscopy	XXIV
C.5.1 Sample and experimental set-up	XXIV
Appendix D – Film Characterization Techniques	XXV
D.1 Reflectivity Technique	
D.1.1 Basic reflectivity theory	XXVI
D.1.2 X-Ray Reflectivity (XRR)	XXIX
D.1.3 XRR measurements: experimental set-up	XXIX
D.2 Solid State Ellipsometry	XXX
D.2.2 Instrument and experimental set-up	XXX
References	a

### Introduction

Nanomaterials, nanoscience, and nanotechnology have become common words in academia and normal life. The prefix "nano" is derived from the Greek word "nanos", meaning "very short man",<sup>1</sup> and in this context it is used to indicate particles and/or structures formed by particles with at least a dimension between 1 and 100 nm.<sup>2</sup>

The terms nanoscience and nanotechnology are closely associated and often confused. However, the difference is substantial. Nanoscience is the field that involves the study of the behavior and related questions of materials at the nanoscale and thus determines relevant laws and theoretical explanations. On the other hand, nanotechnology utilizes nanoscience knowledge to create structures, devices, and systems with novel properties and functions.<sup>3</sup>

In the last 50 years, researchers have been extensively studying how to exploit nanoparticles and nanostructured materials in different fields. Anyway, this is such a vast and varied area that comprehension of properties and applications is far to be complete. In truth, nanoscience constitutes the meeting point of different disciplines, ranging from quantum physics to supramolecular chemistry, material science to biology, from medicine to agri-food.

To understand and appreciate the diversity of nanomaterials (NMs), just think that the synthesis method, the size, or the composition are different types of classifications. Considering the synthesis method, NMs can be divided into those prepared through a bottom-up or a top-down approach (Figure 1). In the first case, synthesis occurs through chemical reactions under controlled conditions starting with ions, atoms, and molecules. In the second case, and vice versa, it starts with a bulk material crushed/broken by mechanical actions.<sup>4</sup>



Figure 1. Bottom-up and Top-down approaches.

Based on the dimension, NMs are classified as (Figure 2):<sup>5</sup>

- Zero-dimensional (0D): NMs that have nanoscale dimensions in all directions, e.g., nanoparticles;
- One-dimensional (1D): NMs that have non-nanoscale dimensions in a single direction, e.g., nanorods;
- Two-dimensional (2D): NMs that possess two dimensions having non-nanometric size range, e.g., nanosheets;
- Three-dimensional (3D): NMs that contain non-nanoscale features in any three dimensions, e.g., nanoflowers.



Figure 2. Schematic representation of 0D, 1D, 2D, 3D nanomaterials.

Finally, considering the composition, NMs are organized into four main classes (Figure 3):<sup>1</sup>

- Carbon, e.g., fullerene, carbon nanotubes, graphite, carbon dots;
- Organic, e.g., liposomes, dendrimers, polymeric nanoparticles;
- Inorganic, e.g., metal, metal oxide, ceramic;
- Composite, a combination of two or more different materials to blend the best properties of both.



**Figure 3.** Examples of Carbon (carbon nanotube), Organic (liposome), Inorganic (metal nanoparticle), and Composite-based nanomaterials.

Among the different classes of NMs, in this thesis, it was decided to turn attention to metal oxide nanoparticles, obtained through a bottom-up approach. This choice was supported by their huge

technological impact in different fields such as catalysis, photovoltaic, and electronic, but also cosmetic, medical, and agricultural.<sup>6</sup> This is because it is relatively simple and economic to modify the properties of these nanoparticles by changing their crystalline structure. It is also possible to change the electrochemical characteristics because of quantum confinement and the surface properties, which affect the conductivity and chemical activity of metal oxide nanoparticles.<sup>7</sup>

Among the various metal oxides, the choice fell on cerium oxide (CeO<sub>2</sub>) and zinc oxide (ZnO) nanoparticles.

Cerium oxide nanoparticles (CeO<sub>2</sub> NPs) have a wide number of applications, the most common being as catalysts, polishing agents, and gas sensors, but also as an anti-cancer, antioxidant, and antibacterial agents.<sup>8–14</sup> The ability of CeO<sub>2</sub> NPs to modify their oxidation state by maintaining structural integrity is the key to their large use.<sup>15</sup>

Similarly, zinc oxide nanoparticles (ZnO NPs) are widely used in many fields. For example, they are used in the rubber industry as fillers, in the pharmaceutical and cosmetic industry as components of sunscreens and toothpastes, in the energy industry as components of solar cells, and in the medical and food industry and packaging, as antibacterial agents.<sup>16–18</sup> The presence of zinc as a trace element in the muscles, bones, skin, and tissue of the teeth justifies this extensive use,<sup>18</sup> as well as the higher thermal stability, toughness, and durability of ZnO NPs with respect to other oxides, like those of titanium (TiO<sub>2</sub>), tungsten (WO<sub>3</sub>), silicon (SiO<sub>2</sub>), and iron (Fe<sub>2</sub>O<sub>3</sub>).<sup>17</sup>

In this work, we want to understand how changing the inter- and intra-particle structure is possible to amplify the intrinsic properties of NPs and thus expand their fields of application.  $CeO_2$  NPs were employed for a fundamental chemico-physical study, while ZnO NPs were examined for their possible technological application.

CeO<sub>2</sub> NPs (Section A) have been synthesized, varying synthesis temperature and capping agent, to study how the synthesis conditions could influence their physico-chemical properties. Capping agent is an amphiphilic molecule capable of modulating the shape and size of NPs in the synthesis phase, and thus varying the properties of the final NMs. In this Ph.D. work, the effect of the length of alkyl chain of primary amines was investigated, using octylamine (C8), decylamine (C10), dodecylamine (C12), hexadecylmaine (C16), and oleylamine (C18), and performing the synthesis through the thermal decomposition method at three different temperatures, 150, 200, and 250 °C. Studied the properties of coated CeO<sub>2</sub> NPs, in terms of crystallinity, composition, size, shape, and photoluminescence, self-assembly was induced by functionalizing coated NPs with oleic acid (OA) or sodium oleate (NaOA). Functionalization with NaOA has led to the formation of disordered

aggregates of coated CeO<sub>2</sub> NPs stable in water, while OA induced the formation of ordered superlattices, with structures ranging from simple face centered cubic (FCC) to the more complex pseudo hexagonal, known as C14 Frank-Kasper (C14 FK) phase. Finally, it was observed that an ordered superlattice of coated CeO<sub>2</sub> NPs amplifies by about 400% the intensity of photoluminescence compared to disordered aggregates of the same NPs.

On the other hand, in the case of ZnO NPs (Section B), the effect of several concentrations of a doping agent (from 0 to 20 % at) on the fluorescence properties was assessed, to implement this property to use the ZnO NPs as an optical transducer in the design of an aptasensor. In this work, the doping agent chosen was fluorine (F), capable of introducing itself in defects naturally present in the crystalline structure of ZnO NPs, increasing the fluorescence intensity without changing their other physico-chemical properties. The extensive physico-chemical characterization performed on the various synthesized F-doped ZnO NPs has determined that only 1% at at F concentration is required to have a relative quantum yield of the NMs of about 22%. Thus, chosen the best F-doped ZnO NPs, in terms of optical properties, a protocol for obtaining a nanostructured film capable of acting as an optical transducer has been developed. For this purpose, two types of solid support, silicon and glass, various concentrations of ZnO NPs suspension, from 0.5 to 36 mg mL<sup>-1</sup>, and different rotational speeds, from 1000 to 4000 rpm were tested. Finally, deposition protocol of a synthesized aptamer, named LA27, was defined and the release kinetics of the biomolecule from the nanostructured film was evaluated.

### **Section A**

## Coated CeO<sub>2</sub> superstructures

# **Chapter 1**

Self-assembly

### 1.1 Self-assembly

The controlled clustering of nanoparticle (NP) building blocks into defined geometric arrangements is emerging as a distinguishing feature of new soft materials with distinctive physico-chemical properties.<sup>19</sup> Clusters of NPs with a regular spatial arrangement are usually indicated with the term "superlattice",<sup>20</sup> and not only allow properties of individual NPs to be combined, but also take advantage of the interaction between neighboring NPs, which can result in new properties not present in the original constituents.<sup>21,22</sup>

The scientific interest in the organization of NPs in ordered secondary structures resides in the ability of natural and synthetic elements to spontaneously form such structures. Just think of the ice crystals, the geometry of some plants, the hexagonal structure of bee nests (Figure 1.1); or even, the lamellar structures of polymers or the different structures that surfactants can form.



Figure 1.1. Images of ice crystals (A), plant (B), and honeycomb structure (C).

Indeed, in many soft matter systems, ordered structures are observed. The structural units can be very different in nature: atoms, micelles, soft inorganic nanoparticles, and foams, but their shapes are always close to spherical.<sup>23–25</sup>

Despite progress, obtaining ordered 3D structures using NPs as building blocks is still difficult. Both because it is difficult to synthesize monodisperse spherical NPs, and because other factors, such as size, presence of organic coating, attractive and repulsive energies between NPs, between NPs and organic coating, and between NPs and environment, can affect the subsequent self-assembly.<sup>22,26,27</sup>

Normally, two general procedures can be followed to form a regular spatial arrangement of a selfassembled cluster in suspension: *i*) a one-step "*in situ*" approach, in which NPs are synthesized and assembled within the same process; *ii*) a two-step approach, in which the NPs are synthesized and then the self-assembly is induced, like the emulsion process. The latter procedure has more advantages than the former one, because NPs of the highest quality, in terms of size, shape, crystallinity, and polydispersity, can be used.<sup>22</sup> Generally, these procedures are favored by the presence of an organic coating on the NP surface. This organic shell plays a fundamental role in obtaining the superlattice of inorganic NPs.<sup>28</sup>

#### 1.1.1 Energy involved in the self-assembly

The self-assembly of coated NPs into superlattices is dominated by colloidal interactions between NPs and between NPs and the environment. However, for the formation of an ordered aggregate, the effect of kinetic barriers in the process of forming these structures must be considered. If the potential interaction is greater than 10kT, the aggregation will be rapid, and the aggregate will be disordered. Conversely, if the potential is about 2-3kT, you can have some repeatability in the bond between the NPs and then the aggregate can be ordered.<sup>22</sup> This means that understanding the energies that drive the self-assembly process will allow to control it.

The theory that describes the total interaction potential between NPs is called *Extended Derjaguin-Landau-Verwey-Overbeek theory* (XDLVO): it decomposes the total interaction potential into the sum of several contributions, van der Waals  $\Phi_{vdW}$ , electrostatic  $\Phi_{elec}$ , hydrophobic  $\Phi_{hydro}$ , and elastic  $\Phi_{elas}$  (equation 1.1):<sup>22,29</sup>

$$\Phi_{tot} = \Phi_{vdW} + \Phi_{elec} + \Phi_{hvdro} + \Phi_{elas}$$
(1.1)

In the case of magnetic NPs, a magnetic contribution must be added to the above sum.

Van der Waals interactions  $\Phi_{vdW}$  (equation 1.2) range from interatomic spacings (about 0.2 nm) up to large distances (greater than 10 nm),<sup>30</sup> due to instantaneous fluctuations in the electron density and thus somehow connected to the polarizability of the material (Figure 1.2). <sup>31–37</sup> In the case of NPs an equation accounting for the van der Waals interactions was proposed by Stolarcyk *et al.*. Such equation is a function of the distance between two NPs, and depends on the radius of the individual NP, as described in equation 1.2:<sup>22</sup>

$$\Phi_{vdW}(d) = -\frac{A}{6} \left[ \frac{2r^2}{d^2 - 4r^2} + \frac{2r^2}{d^2} + ln \left( \frac{d^2 - 4r^2}{d^2} \right) \right]$$
(1.2)

where d is the center-center distance between two NPs, A is the Hamaker constant (it depends on the interaction between the organic layer on the NP surface and the solvent), and r is the NP radius.



Figure 1.2. Schematic representation of the van der Waals interaction.

The electrostatic contribution  $\Phi_{elec}$  (equation 1.3) arises from the presence of an electric charge on the NP surface and leads, in the case of identical particles, to a repulsive interaction. The causes of the presence of the charge can be multiple, e.g., adsorption of ions onto the surface, presence of charged ligands, protonation, or deprotonation of superficial ligands, etc. The presence of these charges leads to the formation of an electric double layer, which shields the charges at large distances but leads to significant repulsive interactions at small distances (Figure 1.3).<sup>38-40</sup> The electrostatic contribution can be schematized as follows:<sup>22</sup>

$$\Phi_{elec}(d) = \frac{Q^2}{4\pi\varepsilon\varepsilon_0 \left(1+\kappa r\right)^2} \frac{exp\left(-\kappa\left(d-2r\right)\right)}{d}$$
(1.3)

where Q is the total charge on the NP,  $\varepsilon$  and  $\varepsilon_0$  are the permeability of the medium and the vacuum, respectively, and  $\kappa$  is the reciprocal of the Debye length.



Figure 1.3. Schematic representation of the electrostatic interaction.

The hydrophobic interaction  $\Phi_{hydro}$  occurs when coated NPs are dispersed in water. Alkyl chains of the organic coating, present on NPs surface, come closer to minimize interactions with water molecules. This interaction can work over a very long range, about 1 nm (equation 1.4, and Figure 1.4).<sup>26,41,42</sup>

$$\Phi_{hydro} = -4\pi r D_0 \gamma (1 - f) exp\left(-\frac{d - 2r - 2L}{D_0}\right)$$
(1.4)

where  $D_0$  is the hydrophobic decay length,  $\gamma$  is the surface tension of the ligand in water, *f* is a dimensionless parameter that reflects the hydrophobic-hydrophilic balance of the organic ligand, and *L* is the length of the alkyl chain of the ligand.



Figure 1.4. Schematic representation of the hydrophobic interaction.

The elastic interactions  $\Phi_{elas}$  are short-range interactions, due to the loss of configurational entropy of organic chains. They are repulsive when the distance between the NPs is less than the length of the alkyl chains (equation 1.5, and Figure 1.5).<sup>26,41,42</sup>

$$\Phi_{elas}(d) = \frac{2\pi r k T L^2 \rho}{MW} \phi f \left( d - 2rL \right)$$
(1.5)

where *k* is the Boltzmann constant, *T* is the temperature,  $\rho$  is the ligand density, *MW* is the molecular weight,  $\phi$  is the fraction of surface coverage by the ligand.



Figure 1.5. Schematic representation of the elastic interaction.

The right balance between the different energies, summarized in Table 1.1, is what must be optimized to obtain the desired secondary structure.

|--|

Force	Type of interaction	Elements involved in the interaction
van der Waals	attractive	coated NP surface
electrical	repulsive	electric layer near NP surface
hydrophobic	attractive	coated NP in water
elastic	repulsive	organic layers

#### **1.1.2 Geometrical parameters**

The compact structures that can be obtained by assimilating NPs to the hard-sphere are face-centered cubic (FCC), hexagonal close-packed (HPC), or body-centered cubic (BCC) structures.<sup>43</sup> Nevertheless, more complex arrangements, such as Frank-Kasper or tetrahedral close packing (TPC) phases (Figure 1.6), or even quasi-crystalline structures are detected for polymer and metallic NPs in 2D structures.<sup>24,27,44–46</sup> The Frank-Kasper phase is characterized by the arrangement of particles that form a hierarchical structure at the vertices of almost regular tetrahedrons.<sup>47</sup>



**Figure 1.6.** 3D schematic representation of face-centered cubic (FCC, green structure), hexagonal close packed (HPC, blue structure), body-centered cubic (BCC, purple structure), and tetrahedral close packing (TPC, example of C14 Frank-Kasper, gold structure) phases.

Calculating the overall energy involved in NP self-assembly in different ordered structures can be a difficult task. However, it is possible to find a rationale by considering the structural parameters of the coated NPs that go to self-assembly. In fact, not only the size of the inorganic core, which can be tuned during the synthesis but also the NP coating plays a very important role in self-assembly. Indeed, NPs are stabilized through surface functionalization with organic ligands or active surfaces. This organic coating, depending on the environment in which the NPs are dispersed and the forces

that are generated between the various constituents of the system, can, on the one hand, stabilize the NPs and avoid the self-aggregation, on the other hand, instead, they can be the driving force of the self-assembly and the formation of NP superlattices,<sup>48</sup> for example, though an emulsion process. Recently Pansu *et al.* in their study on coated gold NPs have proposed that some geometrical parameters can affect the formation of different secondary structures of coated NPs, such as the radius of the inorganic core  $r_c$ , the length of the alkyl chains of the coating molecules  $l_s$ , and the distance between two neighboring NPs  $\delta$  (Figure 1.7).<sup>43</sup>



**Figure 1.7.** Schematic representation of geometric parameters: radius of inorganic core  $r_c$ , length of alkyl chain  $l_s$ , distance between two neighboring NPs  $\delta$ .

By modulating the ratio  $\delta/l_s$  and  $l_s/r_c$ , it is possible to induce the formation of a NP cluster with a certain crystalline periodicity rather than another or to have the coexistence of several structures (Figure 1.8).<sup>43</sup>



Figure 1.8. Schematic representation of the structure diagram for soft NPs.

To study and rationalize the process of self-assembly aimed at obtaining ordered 3D structures stable in water, it was chosen to work with cerium oxide nanoparticles (CeO<sub>2</sub> NPs), one of the most promising metal oxides. The ability of CeO<sub>2</sub> NPs to modify their oxidation state by maintaining structural integrity is the key to their large use.<sup>15</sup>

### **1.2 Cerium Oxide Nanoparticles**

Cerium (Ce) is one of the most reactive and abundant rare elements of the lanthanide series and can exist both in the +3 and +4 oxidation states. When cerium combines with oxygen, two different oxides can be obtained: *i*) cerium dioxide CeO<sub>2</sub>, *ii*) cerium sesquioxide Ce<sub>2</sub>O<sub>3</sub>.<sup>49,50</sup> CeO<sub>2</sub> crystallizes in the fluorite crystal structure at room temperature and pressure, with space group *Fm3m*. Cerium cations are arranged in a face-centered cubic structure and the O<sup>2-</sup> anions are positioned in the octahedral interstices. Each cerium cation is coordinated with eight oxygen anions, while each oxygen anion is coordinated with four cerium cations (Figure 1.9 A).<sup>51,52</sup> On the other hand, the stable crystalline form of Ce<sub>2</sub>O<sub>3</sub> is hexagonal, with space group *P3ml*, in which each cerium cation is coordinated with seven oxygen anions (Figure 1.9 B).



**Figure 1.9.** Fluorite type structure of CeO<sub>2</sub> (A) and sesquioxide A-type structure of Ce<sub>2</sub>O<sub>3</sub> (B), red and white balls indicate cerium and oxygen, respectively.

Generally, cerium oxide nanoparticles (CeO<sub>2</sub> NPs) have a fluorite-like crystalline structure, as in the case of the bulk material. In this case, the crystals have defects due to the coexistence between the trivalent (Ce<sup>3+</sup>) and the tetravalent (Ce<sup>4+</sup>) states. This causes the spontaneous formation of oxygen vacancies (V<sub>o</sub>) (Figure 1.10), according to the following reaction:

$$CeO_2 \rightarrow CeO_{2-x} + \frac{x}{2}O_2$$
 (1.6)

During this reaction, two electrons from the oxygen atoms are transferred to two ions  $Ce^{4+}$ , which are reduced to  $Ce^{3+}$ , and oxygen or hydroxyl are released from the surface. The reverse process occurs when  $Ce^{3+}$  is oxidized to  $Ce^{4+}$ .<sup>10</sup>



Figure 1.10. Fluorite type structure of CeO<sub>2</sub> NPs without (A) and with (B) oxygen vacancy.

The generation of defects, which can be modulated during NP synthesis, leads to an increase of system entropy, and, in turn, to a reduction of Gibbs energy with advantages in term of stability.<sup>52</sup>

### 1.2.1 Synthesis and functionalization

Metal oxide nanoparticles can be synthesized by different methods following in three macro-areas: physical, chemical, and biological, as shown in Figure 1.11.<sup>53</sup>



Figure 1.11. Various methods of synthesizing CeO<sub>2</sub> NPs.

Although biological methods are "green", chemical, and physical methods are more widely used than biological ones, because of a better control of synthetic conditions. Moreover, among all the syntheses, chemical methods are the best for homogeneity of NP dimension, morphology, and composition, which significantly affect the properties of the final material.

During the synthetic process, as mentioned above, it is possible to modulate the  $Ce^{3+}/Ce^{4+}$  ratio and thus the concentration of  $V_0$  in the  $CeO_2$  NPs. As the size of the NPs decreases, the concentration of  $Ce^{3+}$ , and therefore of  $V_0$ , increases. To optimize this, it is possible to use various synthesis methods, such as, thermal decomposition,<sup>54</sup> hydrothermal,<sup>55</sup> co-precipitation method or the use of

microwaves.<sup>56–58</sup> For example, the co-precipitation method is relatively straightforward given the low solubility of CeO<sub>2</sub> NPs in water. However, using agents such as sodium hydroxide, urea, or ammonium may lead to the precipitation of crystals that are too small for application purposes.<sup>8,59</sup> For this reason, surfactants, such as Cetyltrimethylammonium Bromide (CTAB), or polymers, such as Polyvinylpyrrolidone (PVP), are often used, since they easily absorb on the surface of NPs and modulate their size as a function of their concentration.<sup>60,61</sup>

The choice of the capping agents forming the organic coating is crucial because it can guide the morphology of the NPs. For example, in a certain range of concentrations and reaction times, PVP drives the formation of nanospheres and oleic acid of nanocubes,<sup>62,63</sup> while the combined use of oleic acid and oleylamine in the thermal decomposition of cerium nitrate leads to formation of nanoplates.<sup>64</sup>

The result of any chemical synthesis method are NPs with a low or limited colloidal stability; to increase it and permit NP dispersion in water or in a solvent different from those used in the synthesis, surface modifications are necessary. There are two main kinds of functionalization methods. The first one is based on the addition of an amphiphilic molecule to the external surface of the NPs, without the removal of the capping agents. The hydrophobic interaction between the two amphiphilic molecules creates a bilayer on the surface of the NP exposing a hydrophilic surface, thus allowing the dispersion in water.<sup>65</sup> The second functionalization method is based on the substitution of the capping agent with a bi-functional molecule bearing a functional group able to bind to the NPs surface and a polar group that makes the compound soluble in water (Figure 1.12).<sup>66–69</sup>



Figure 1.12. Schematic representation of the two functionalization methods.

#### **1.2.2 Properties and applications**

The properties of CeO<sub>2</sub> NPs are closely related to their stable crystal structure. They can be easily manipulated by varying the concentration of defects in CeO<sub>2</sub> NPs because of the partial reduction of  $Ce^{4+}$  to  $Ce^{3+}$ .<sup>70</sup> The ability of CeO<sub>2</sub> NPs to modify the oxidation state in a suitable temperature range by maintaining structural integrity is the key to their success. Indeed, it determines the high catalytic activity of NPs for a variety of reactions, such as water-gas shift,<sup>71</sup> CO oxidation,<sup>72</sup> water splitting,<sup>73</sup> hydrogenation of unsaturated compounds, ring opening, isomerization reaction, and dehydration of alcohols.<sup>74</sup>

Moreover, CeO<sub>2</sub> NPs have attracted considerable interest in the field of photocatalysis, too. In fact, CeO<sub>2</sub> NPs are a wide bandgap semiconductor (3.0-3.4 eV) and can act as a photocatalyst if irradiated by UV light, similarly to TiO<sub>2</sub> NPs but with the advantage of longer lifetime of the charge carriers and higher oxygen mobility.<sup>72,75</sup>

CeO<sub>2</sub> NPs are extensively used as polishing agent for glasses, showing superior polishing properties than anhydrous ferric oxide particles,<sup>76,77</sup> are components of sunscreen cosmetics for UV filtration,<sup>78</sup> are added to coatings and pigments to protect steels and other metallic materials from corrosion.<sup>79</sup>

In recent years, CeO<sub>2</sub> NPs have attracted great interest in the biomedical field (i.e., as anticancer, antioxidant, and antibacterial agents), due to their redox properties, which can be opportunely

modulated to inhibit (with an antioxidant action) or promote (with a pro-oxidant action) the oxidation processes,<sup>12–14,80</sup> showing the ability to decrease or to increase the concentration of the Reactive Oxygen Species (ROS).<sup>81–83</sup>

Finally, CeO<sub>2</sub> NPs have acquired an increasing attention due to their peculiar behavior that places them into the promising category of "nanozymes", which are NPs with enzyme-like features. Indeed, they show superoxide dismutase, catalase, and phosphatase mimetic activity.<sup>81,82</sup>

Some of these applications are shown in figure 1.13.



Figure 1.13. Examples of CeO<sub>2</sub> NPs applications.

### **Chapter 2**

Synthesis and Functionalization of coated CeO<sub>2</sub> NPs

 $CeO_2$  NPs were prepared through the thermal decomposition method using amines with different lengths of alkyl chain, from C8 (octaylamine) to C18 (oleylamine), which makes them stable in non-polar media. Then, to obtain a 3D-ordered superlattice, NP self-assembly was induced by functionalizing CeO<sub>2</sub> NPs with two amphiphilic molecules.

In this chapter, the experimental procedures are described in detail.

### 2.1 Synthesis of CeO<sub>2</sub> NPs

#### 2.1.1 Materials

Cerium(III) nitrate hexahydrate Ce(NO<sub>3</sub>)<sub>3</sub>6H<sub>2</sub>O (> 99.999% trace metals basis purity), octylamine C<sub>8</sub>H<sub>19</sub>N (99% purity), decylamine C<sub>10</sub>H<sub>23</sub>N (95% purity), dodecylamine C<sub>12</sub>H<sub>27</sub>N (98% purity), hexadecylamine C<sub>16</sub>H<sub>35</sub>N (98% purity), oleylamine C<sub>18</sub>H<sub>37</sub>N (70% technical grade), 1-Octadecene (90% technical grade), ethanol (96% vol), chloroform ( $\geq$  99.5%, contains 100-200 ppm amylenes as a stabilizer) were purchased from Merck (Germany) and used without further purification.

### 2.1.2 Synthetic protocol

 $CeO_2$  NPs were synthesized through a slightly modified version of the thermal decomposition method introduced by Lee *et al.*.<sup>84</sup> Namely, 1.74 g of Ce(NO<sub>3</sub>)<sub>3</sub>6H<sub>2</sub>O were dissolved in 25 mL of 1-octadecene at room temperature. Upon the dissolution of the salt, a specific amount of the selected capping agent was added to the reaction mixture, as reported in Table 2.1, thus, to obtain a 1:3 salt: capping agent molar ratio.

Matarial	Aaronym	Quantity $\pm 0.01$	
Wiatchiat	Actoliyin	mL	
octylamine	C8	1.98	
decylamine	C10	2.40	
dedecylamine	C12	2.77	
hexadecylamine	C16	3.10	
oleylamine	C18	3.95	

**Table 2.1.** Quantity of different capping agents used in the synthesis.

The resulting solution was placed in a bath at 80 °C and stirred for 30 minutes. Then, aiming at studying the effect of temperature synthesis on NP properties, the solution was further heated as reported in Table 2.2 under an argon atmosphere and stirred for 1 hour (the experimental apparatus is represented in Figure 2.1).

Capping Agent	150 °C	200 °C	250 °C
octylamine	Х		
decylamine	Х	Х	
dodecylamine	Х	Х	
hexadecylamine	Х	Х	Х
oleylamine	Х	Х	Х

**Table 2.2.** Capping agents and synthesis temperatures employed.



Figure 2.1. Experimental apparatus used for the synthesis of CeO<sub>2</sub> NPs.

The reaction mixture was then slowly cooled down to room temperature and 30 mL of ethanol were added to induce the precipitation of NPs. The resulting suspension was transferred into 50 mL centrifuge tubes and centrifuged at 8000 rpm for 20 minutes. Then the supernatant was discarded, and the CeO<sub>2</sub> NPs were re-dispersed in ethanol. The centrifugation step was repeated twice to remove all the unreacted material. Finally, CeO<sub>2</sub> NPs were dispersed in chloroform.

The different NPs obtained according to this method are reported in Table 2.3 and Figure 2.2.

Table 2.3. CeO<sub>2</sub> NPs synthesized with different capping agents and at different temperatures.

<b>A</b>	Consiste Accest	Temperature
Acronym	Capping Agent	°C
C8@CeO <sub>2</sub> 150	octylamine	150
C10@CeO <sub>2</sub> 150	decylamine	150
C12@CeO <sub>2</sub> 150	dodecylamine	150
C16@CeO <sub>2</sub> 150	hexadecylamine	150
C18@CeO <sub>2</sub> 150	oleylamine	150
C10@CeO <sub>2</sub> 200	oleylamine	200
C12@CeO <sub>2</sub> 200	dodecylamine	200
C16@CeO <sub>2</sub> 200	hexadecylamine	200
C18@CeO <sub>2</sub> 200	oleylamine	200
C16@CeO <sub>2</sub> 250	hexadecylamine	250
C18@CeO <sub>2</sub> 250	oleylamine	250



**Figure 2.2.** CeO<sub>2</sub> NPs synthesized: C8@CeO<sub>2</sub> 150 **A**, C10@CeO<sub>2</sub> 150 **B**, C12@CeO<sub>2</sub> 150 **C**, C16@CeO<sub>2</sub> 150 **D**, C18@CeO<sub>2</sub> 150 **E**, C10@CeO<sub>2</sub> 200 **F**, C12@CeO<sub>2</sub> 200 **G**, C16@CeO<sub>2</sub> 200 **H**, C18@CeO<sub>2</sub> 200 **I**, C16@CeO<sub>2</sub> 250 **J**, C18@CeO<sub>2</sub> 250 **K**.

Ce concentration in NP synthesized was determined by ICP-MS in terms of mg/kg concentration. Considering a CeO<sub>2</sub> density of 7.6 g/cm<sup>3</sup> and the volume of NP core as experimentally determined by TEM images it was possible to estimate the weight of a single NP being 10<sup>-19</sup> g, corresponding to a NP molecular weight of about 299545.6 g/mol. Ce concentration is converted into NP molar concentration and used this value to determine the ratio between NP and functionalizing molecules.<sup>85,86</sup>

### 2.2 Preparation of 3D superlattice systems

### **2.2.1 Materials**

Oleic acid C<sub>18</sub>H<sub>34</sub>O<sub>2</sub> ( $\geq$  99% purity), sodium oleate C<sub>18</sub>H<sub>33</sub>NaO<sub>2</sub> (99% purity), ethanol (96% vol), and ethyl ether ( $\geq$  99.8% purity) were purchased from Merck (Germany) and used without further purification. All aqueous solutions were prepared using double-distilled Milli-Q-water, filtered using 0.20 µm filters except for samples for neutron and Cryo-TEM measurements where D<sub>2</sub>O (Merck) was used.

### 2.2.2 3D ordered structures protocol

The superlattice formation was induced by the micro-emulsion method. Thus, the interaction between the alkyl chains of the capping agent was induced, while the functionalizing molecules had to encapsulate the entire aggregate to make it stable in water (Figure 2.3). The functionalizing molecules chosen are oleic acid (OA) and sodium oleate (NaOA), to evaluate how the nature of these molecules could affect the structure of the NP aggregate.



Figure 2.3. Schematic representation of coated CeO<sub>2</sub> NP superlattice.

Coated  $CeO_2$  NPs are stored in chloroform, as mentioned above, which has a boiling temperature of 61.2 °C, so it hardly evaporates at room temperature and does not cause changes in suspension

concentration. Nevertheless, the process of functionalization was favored if the organic solvent is ethyl ether, which has a boiling temperature of 34.6 °C. Hence, to avoid variations in the concentration of organic suspensions of coated CeO<sub>2</sub> NPs, only before the functionalization a solvent change was carried out, with the procedure described in the paragraph 2.1.2.

In the case of oleic acid, a specific amount of OA (0.7 mM, in ethyl ether) was added to different amounts (14, 20, and 50 mM NPs concentrations) of coated CeO<sub>2</sub> NPs in ethyl ether, then, 10 mL of bi-distilled water were added to every dispersion. The resulting biphasic systems (Figure 2.4 A) were sonicated with a probe tip-sonicator for 5 minutes to get monophasic systems (Figure 2.4 B), which were left under stirring overnight, to remove the organic solvent.

Since NaOA is soluble in water, a 0.7 mM water solution of NaOA was added to different amounts (14, 20, and 50 mM NPs concentrations) of coated CeO<sub>2</sub> NPs dispersed in ethyl ether obtaining biphasic systems that were subjected to the same procedure described above.



Figure 2.4. Biphasic system A, final monophasic system B.

## **Chapter 3**

Amines@CeO<sub>2</sub> NPs: investigating the synthesis NPs

To obtain ordered 3D superlattices it is very important that the NPs used as building blocks have a regular nearly spherical shape, and low polydispersion size. To assess if CeO<sub>2</sub> NPs synthesized by means of the thermal decomposition method fulfill these requirements, they must be extensively characterized with different techniques. Namely, the inorganic core of NPs was analyzed by means of X-Ray Diffraction (XRD), and X-ray Photoelectronic Spectroscopy (XPS) with the aim to assess the crystalline state and the surface atomic composition, the presence of the organic coating on the NP surface was verified by means of Infrared (IR) spectroscopy; Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) measurements were performed to study the shape and size of coated NPs. Finally, optical properties of coated CeO<sub>2</sub> NPs were analyzed by means of UV-Visible, and photoluminescence spectroscopy.

### 3.1 Inorganic core

With the aim at assessing the crystalline state of  $CeO_2$  NPs XRD measurements were performed, while XPS spectroscopy was employed to determine the concentration of the different species on NP surface and in particular the  $Ce^{3+}/Ce^{4+}$  ratio. XPS analysis was carried out at CEA-Leti Research and Technology Institute in Grenoble (France) as part of the European project NFFA (ID070) for the development of nanoscience.

#### **3.1.1.** Crystalline structure

All NPs synthesized were crystalline as demonstrated by XRD analysis. The XRD patterns of CeO<sub>2</sub> NPs prepared with octylamine at 150 °C (C8@CeO<sub>2</sub> 150), and with oleylamine at 150 °C (C18@CeO<sub>2</sub> 150) or at 250 °C (C18@CeO<sub>2</sub> 250) are shown in Figure 3.1, for example.



Figure 3.1. XRD patterns of C8@CeO<sub>2</sub> 150, C18@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 250.

The values of the diffraction angle  $(2\theta)$  and Miller indices (hkl) of the peaks observed in the profile of Figure 3.1 are listened in Table 3.1. These patterns show the typical peaks of a face centered cubic structure (JCPDS 36-1451).<sup>87–89</sup> The broad halo at  $2\theta$  values of less than 28° could be due to the presence of the organic coating on the surface of CeO<sub>2</sub> NPs.

**Table 3.1.** Experimental values of the diffraction angles ( $2\theta$ , degs) and corresponding Miller indices (hkl) of diffraction peaks observed in the patterns reported in Figure 3.1.

Sample	(111)	(200)	(220)	(311)	(222)
C8@CeO <sub>2</sub> 150	28.31°	32.98°	47.20°	56.18°	58.92°
C18@CeO <sub>2</sub> 150	28.31°	32.83°	47.20°	56.18°	58.92°
C18@CeO <sub>2</sub> 250	28.16°	32.69°	47.20°	56.03°	58.92°

#### **3.2.2 Chemical composition**

The XPS analysis was performed to obtain information on the chemical composition of the surface of the CeO<sub>2</sub> NPs, and so to obtain the Ce<sup>3+</sup>/Ce<sup>4+</sup> ratio. The survey spectra of all synthesized CeO<sub>2</sub> NPs are plotted in Figure 3.2 A, to better highlight the different peaks, spectra separated according to the synthesis temperature are reported in panels B-D (T= 150 °C Figure 3.2 B, T=200 °C Figure 3.2 C, and T=250 °C Figure 3.2 D).



**Figure 3.2.** XPS patterns of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 (**A**), C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**B**), C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, and C18@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub>

All patterns, reported in Figure 3.2 B, show the main characteristic peaks of  $CeO_2$  (Ce 3d, Ce 4d, and O 1s) as well as carbon (C 1s) (Multipak software). All NPs synthesized at 200 °C (Figure 3.2 C) show the O 1s peak as well as the C 1s. The Ce peaks are clearly visible only for NPs synthesized with shorter chain amines as capping agents (C10 and C12) when long chain amines were used (C16

and C18) Ce peaks overlap in a single peak. Moreover, for NPs synthesized at 250 °C the signal-tonoise ratio of XPS spectra decreases as observed in Figure 3.2 D. These differences can probably be due to the depth of sampling. The size of the NPs may be too small compared to the length of the capping agent covering them, and this can make analysis difficult.

To quantify the species (Table 3.2) of interest the main peaks were considered. In Figure 3.3 are reported the O *Is* and C *Is* peaks.



**Figure 3.3.** O *1s* XPS spectra of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**A**), O *1s* XPS spectra of C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, and C18@CeO<sub>2</sub> 200 (**B**), O *1s* XPS spectra of C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 (**C**), and

C *1s* XPS spectra of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, C18@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, C18@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 (**D**).

The O *Is* peak is split in two (Figure 3.3 A, B, and C). The first one at about 527 eV is due to the O atoms bound to Ce ones, and the other, between 529 and 531 eV is due to the O atoms bound to C atoms. The relative intensity of the two peaks changes from one sample to the other. For CeO<sub>2</sub> NPs

synthesized with the shorter chain amines (C8, C10, and C12), the peak O-Ce is more intense than the O-C one, the opposite occurs for NPs synthesized with the longer chain amines (C16, and C18). The peak at about 286 eV in the C *1s* spectrum (Figure 3.3 D) is also more visible for CeO<sub>2</sub> NPs synthesized with shorter alkyl chains than for those obtained using C16 and C18 amines. Moreover, the other visible peak in the C *1s* spectrum at about 282 eV is due to the C-C and C-H bonds of the alkyl chains of the organic coating.

Sample	Ce	O (O-Ce)	O (O-C)	С
Sample	%at	%at	%at	%at
C8@CeO <sub>2</sub> 150	$13 \pm 3$	$24 \pm 5$	$15 \pm 3$	$50 \pm 10$
C10@CeO <sub>2</sub> 150	$18\pm4$	$30\pm 6$	$18\pm4$	$35\pm7$
C12@CeO <sub>2</sub> 150	$9\pm 2$	$20\pm4$	$12 \pm 2$	$60 \pm 12$
C16@CeO <sub>2</sub> 150	$7.4\pm1.5$	$14 \pm 3$	$14 \pm 3$	$65\pm13$
C18@CeO <sub>2</sub> 150	$4.6\pm0.9$	$11 \pm 2$	$43\pm9$	$42\pm 8$
C10@CeO <sub>2</sub> 200	$17 \pm 3$	$32\pm 6$	$14 \pm 3$	$37 \pm 7$
C12@CeO <sub>2</sub> 200	$11 \pm 2$	$24\pm 5$	$9\pm2$	$57 \pm 11$
C16@CeO2 200 *	/	/	$53 \pm 11$	$47\pm9$
C18@CeO2 200 *	$0.20\pm0.04$	/	$6.3\pm1.3$	$90\pm20$
C16@CeO <sub>2</sub> 250 *	$0.40\pm0.08$	/	$7.3 \pm 1.4$	$90\pm20$
C18@CeO <sub>2</sub> 250 *	/	/	$23 \pm 5$	$77 \pm 15$

**Table 3.2.** Atomic concentrations of Ce, O ("\*" indicates that the O concentration is total), and C obtained from XPS spectra.

From the data shown in Table 3.2, it is interesting to note that the concentrations of Ce and C do not follow any trend, while the results obtained from the integration of the O *Is* peaks agree with the variations in the relative intensity of the peaks themselves. For samples synthesized with C16 and C18 as capping agents at 200 or 250  $^{\circ}$ C (\* in Table 3.2), the O concentration is total. For these samples, it is not possible to quantify the Ce concentration and consequently, it is impossible to discriminate the oxygen bound to cerium and/or carbon.

To determine the  $Ce^{3+}/Ce^{4+}$  ratio it was necessary to integrate the curves of the Ce 3d shown in Figure 3.4.


**Figure 3.4.** Ce *3d* XPS patters of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**A**), C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, and C18@CeO<sub>2</sub> 200 (**B**), C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 (**C**).

The spectrum of Ce is much more complex than the previous ones. For each component of Ce  $3d_{5/2}$  – Ce  $3d_{3/2}$  doublet, five peaks were used to fit all these contributions, each of them being assigned to one of the Ce oxidation states (Ce<sup>3+</sup> and Ce<sup>4+</sup>) as indicated in Table 3.3. The five peaks are clearly visible for C8@CeO<sub>2</sub> 150 (black line in Figure 3.4 A), C10@CeO<sub>2</sub> 150 (red line in Figure 3.4 A), C12@CeO<sub>2</sub> 150 (blue line in Figure 3.4 A), C18@CeO<sub>2</sub> 150 (light blue line in Figure 3.4 A), C10@CeO<sub>2</sub> 200 (green line in Figure 3.4 B), and C12@CeO<sub>2</sub> 200 (dark cyan line in Figure 3.4 B) samples, for C16@CeO<sub>2</sub> 150 (purple line in Figure 3.4 A) the peaks are less visible, but the atomic concentration has been determined, for samples C16@CeO<sub>2</sub> 200 (orange line in Figure 3.4 B), C18@CeO<sub>2</sub> 200 (pink line in Figure 3.4 B), C16@CeO<sub>2</sub> 250 (navy line in Figure 3.4 C) and C18@CeO<sub>2</sub> 250 (violet line in Figure 3.4 C) the signals are not detectable, and the Ce concentration has not been determined. Again, this may be due to the depth of sampling.

Sample	Binding Energy / eV	Oxidation State	Area / CPS	Ce <sup>3+</sup> /Ce <sup>4+</sup> ratio
	898.1	IV	6649	-
	888.7	IV	4563	
C8@CeO <sub>2</sub> 150	884.4	III	5215	0.36
	882.2	IV	5580	
	880.3	III	807	
	898.2	IV	8134	
	888.7	IV	5749	
C10@CeO <sub>2</sub> 150	884.5	III	6667	0.35
	882.2	IV	7243	
	880.1	III	850	
	898.4	IV	4999	
	889.0	IV	3622	
C12@CeO <sub>2</sub> 150	884.4	III	6078	0.52
	882.3	IV	3573	
	879.9	III	311	
	898.8	IV	3505	
	889.3	IV	2529	
C16@CeO <sub>2</sub> 150	884.6	III	3240	0.42
	882.9	IV	2483	
	881.7	III	384	
	897.5	IV	2925	
	888.1	IV	1959	
C18@CeO <sub>2</sub> 150	883.2	III	2266	0.37
	881.6	IV	1824	
	879.7	III	194	
	897.8	IV	12723	
	888.5	IV	7654	
C10@CeO <sub>2</sub> 200	884.4	III	14239	0.57
_	882.0	IV	10570	
	880.2	III	3582	
	897.9	IV	8216	
	888.7	IV	4044	
C12@CeO <sub>2</sub> 200	884.8	III	6912	0.46
	882.2	IV	7321	
	880.6	III	2078	

**Table 3.3.** Binding energy of the peak, cerium oxidation state, integrated area, and  $Ce^{3+}/Ce^{4+}$  ratio.

The  $Ce^{3+}/Ce^{4+}$  ratio varies from 0.35 to 0.57, with a lower value for sample C10@CeO<sub>2</sub> 150 and a higher value for sample C10@CeO<sub>2</sub> 200. Therefore, focusing on the syntheses performed in the presence of decylamine (C10), by increasing the synthesis temperature the  $Ce^{3+}/Ce^{4+}$  ratio increases as well, the opposite occurs for the syntheses carried out in the presence of dodecylamine (C12), where the ratio was 0.52 and 0.46 at 150 and 200 °C, respectively. On the other hand, keeping the temperature of synthesis constant to 150 °C, the  $Ce^{3+}/Ce^{4+}$  ratio was the same for systems synthesized

in the presence of octylamine (C8,  $Ce^{3+}/Ce^{4+}=0.36$ ) and decylamine (C10,  $Ce^{3+}/Ce^{4+}=0.35$ ), has a maximum peak for the synthesized system in the presence of dodecylamine (C12,  $Ce^{3+}/Ce^{4+}=0.52$ ), and decreases again for synthesized NPs in the presence of hexadecylamine (C16,  $Ce^{3+}/Ce^{4+}=0.42$ ) and oleylamine (C18,  $Ce^{3+}/Ce^{4+}=0.37$ ). These data show no relationship between the temperature or the capping agent nature and the ratio  $Ce^{3+}/Ce^{4+}$ , at least for the synthesis conditions considered.

### **3.2 Organic coating**

To further confirm the presence of the organic coating on the NPs surface, whose evidence was clear from the halo at low  $2\theta$  values in XRD spectra, and from C-C and C-H signals present in XPS spectra, IR spectroscopy measurements were performed (Figure 3.5).



**Figure 3.5.** IR spectra of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**A**), C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, and C18@CeO<sub>2</sub> 200 (**B**), C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 (**C**).

The measurements were carried out in a 4000-400 cm<sup>-1</sup> range, where the peaks of both the organic coating and the CeO<sub>2</sub> should be visible. Indeed, the characteristic peak of CeO<sub>2</sub> falls at about 460 cm<sup>-1</sup>,<sup>90</sup> with a second peak at about 770 cm<sup>-1</sup>.<sup>91</sup>

For all systems are visible both the peaks of the organic and inorganic parts. The bands at 460 and 720 cm<sup>-1</sup> corresponding to cerium oxide are observed. The wavenumber shift could be due to the presence of the organic coating. Intense bands in the 2800-3000 cm<sup>-1</sup> region, due to CH stretching, and 1460-1500 cm<sup>-1</sup> region, due to CH<sub>2</sub> bending confirm a significant presence of the organic coating. It is noteworthy that all the amines used, except oleylamine (C18), are saturated. Therefore, the presence in all the spectra of the band corresponding to CH<sub>2</sub> bending could indicate the capacity of CeO<sub>2</sub> to induce unsaturation.

## 3.3 Morphological and dimensional analyses

The shape and size of  $CeO_2$  NPs are important features for formation of ordered 3D superlattices. As mentioned in Chapter 1, these parameters can be modulated in the synthesis phase, varying, for example, the synthesis temperature and the length of the alkyl chain of the capping agents.

From an overall observation, through TEM images, of different  $CeO_2$  NPs synthesized at three different temperature and considering amines with different lengths of the alkyl chains as capping agents, shape, size, and tendency to self-aggregation strongly depend on these synthesis conditions. In fact, as the length of the alkyl chain and the synthesis temperature increase, the size of  $CeO_2$  NPs decreases as well as the tendency to self-aggregation, as shown in Figure 3.6.



Figure 3.6. TEM image of coated CeO<sub>2</sub> NPs (scalebar: 50 nm).

37

To determine which synthetic conditions, allow monodisperse NPs with a regular and homogeneous shape to be obtained, a point characterization for all synthesized systems has been performed, combining TEM and DLS measurements.

TEM images in the first line of the Figure 3.6 show that CeO<sub>2</sub> NPs synthesized at 150 °C had a greater tendency to self-aggregation than others (second and third lines in Figure 3.6). Nevertheless, by assimilating the NPs to spheres, a statistical analysis of the TEM images has been conducted to determine their average radius  $\langle R \rangle$ , for example, the Figure 3.7 is shown the TEM image of C18@CeO<sub>2</sub> 150.



Figure 3.7. TEM image of C18@CeO<sub>2</sub> 150 (scalebar: 20 nm).

CeO<sub>2</sub> NPs synthesized at 150 °C exhibit irregular morphology. Only C10@CeO<sub>2</sub> 150 and C18@CeO<sub>2</sub> 150 samples seem to have a more geometric shape, but still irregular. Through a statistical analysis

of TEM images, the average size of the inorganic core was determined. Results are reported in Figure 3.8.



Figure 3.8. Average size of inorganic core  $\langle R \rangle$  of C8@CeO<sub>2</sub> 150 (black circle), C10@CeO<sub>2</sub> 150 (red circle), C12@CeO<sub>2</sub> 150 (blue circle), C16@CeO<sub>2</sub> 150 (purple circle), and C18@CeO<sub>2</sub> 150 (light blue circle).

The C10@CeO<sub>2</sub> 150 has a larger average size (~ 10 nm) than the others.  $\langle R \rangle$  decreases in the case of synthesis with C8 (~ 6.5 nm), and then becomes constant and equal to about 3.6 nm for system C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150. These results indicate that a moderate synthesis temperature could be sufficient to allows NPs with a mean radius equal to or less than 10 nm, although with an irregular shape, to be obtained.

To verify whether the tendency to aggregate was an artifact of the preparation of the TEM samples, which provide for the evaporation of the solvent, DLS measurements have been carried out (Figure 3.9 A).



**Figure 3.9.** Hydrodynamic radius distribution of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**A**), C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, and C18@CeO<sub>2</sub> 200 (**B**) in chloroform.

The hydrodynamic radius distributions confirm the tendency to self-aggregate also in solution for all CeO<sub>2</sub> NPs synthesized at 150 °C, however, the size of the aggregates decreases with the length of the alkyl chain of the capping agent. The self-aggregation tendency decreases further with the increase of the synthesis temperature, as shown by the hydrodynamic radius distributions in Figure 3.9 B. This is evident for all NPs synthesized with the same capping agent, but the strongest effect is observed in the case of C10@CeO<sub>2</sub>, with a  $R_h$  of about 920 and 51 nm for synthesis at 150 and 200 °C, respectively. This difference is also visible in TEM images, as shown in Figure 3.6.

To determine the average size  $\langle R \rangle$  of the inorganic core for NPs synthesized at 200 °C, a statistical analysis of TEM images (second line in Figure 3.6) was carried out. For example, a TEM image of C10@CeO<sub>2</sub> 200 is shown in Figure 3.10.



Figure 3.10. TEM image of C10@CeO<sub>2</sub> 200 (scalebar: 20 nm).

The morphology observed for these samples is still irregular, only nanoparticles  $C18@CeO_2 200$  has a spherical shape. From the statistical analysis of these images, the average size has been determined, which turns out to be for all NPs between 3 and 4 nm, as shown in Figure 3.11.



**Figure 3.11.** Average size of inorganic core *<R>* of C10@CeO<sub>2</sub> 200 (green square), C12@CeO<sub>2</sub> 200 (dark cyan square), C16@CeO<sub>2</sub> 200 (orange square), and C18@CeO<sub>2</sub> 200 (pink square).

The mean radii of the inorganic core of C16@CeO<sub>2</sub> 250 and C18@CeO<sub>2</sub> 250 are, instead, smaller than all other samples (third line in Figure 3.6). In fact, they turn out to be 2.9 and 2.5 for C16@CeO<sub>2</sub> 250 and C18@CeO<sub>2</sub> 250, respectively. Form the TEM images, shown in Figure 3.12, it is also evident that the shape of C18@CeO<sub>2</sub> 250 NPs is spherical, and the NPs are monodispersed.



Figure 3.12. TEM images of C18@CeO<sub>2</sub> 250 (scalebar: 20 nm).

Confirmation that the C18@CeO<sub>2</sub> 250 NPs were monodispersed was obtained through DLS measurements (Figure 3.13).



**Figure 3.13.** Hydrodynamic radius distribution of C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 in chloroform.

Hydrodynamic radii show that the C16@CeO<sub>2</sub> 250 system still tends to self-aggregate, while sample C18@CeO<sub>2</sub> 250 is monodispersed in solution. Furthermore, considering the  $R_h$  value and the core dimension as determined by TEM, a thickness of the coating layer of about 2.5 nm was calculated, in agreement with oleylamine (C18) length in its full-length extension (2.0 nm).<sup>92</sup>

From this careful analysis, the best candidate for obtaining ordered 3D superlattice in suspension is C18@CeO<sub>2</sub> 250.

# 3.4. Optical properties

Among the various properties of  $CeO_2$  NPs, the optical properties are a distinctive feature of the cerium oxide. Thus, an extensive study on the photoluminescence of the nanoparticles prepared was conducted. Such measures have been carried out at CEA-Leti Research and Technology Institute in Grenoble (France) as part of the European project NFFA (ID070) for the development of nanoscience.

By way of example in Figure 3.14 the absorption spectrum of C18 system is shown.



Figure 3.14. UV-Visible spectrum of C18@CeO<sub>2</sub> 250 in chloroform.

Photoluminescence measurements were performed by energizing all samples with a laser at a wavelength of 325 nm. Figure 3.15 shows the obtained photoluminescence spectra, organized into three panels with respect to the synthesis temperature.



**Figure 3.15.** Photoluminescence spectra of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**A**), C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 200, and C18@CeO<sub>2</sub> 200 (**B**), C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 (**C**).

The samples have a wide emission band in the 350-800 nm range. It is evident that the C16@CeO<sub>2</sub> 200, C18@CeO<sub>2</sub> 200, C16@CeO<sub>2</sub> 250, and C18@CeO<sub>2</sub> 250 samples exhibit the highest photoluminescence intensity (in the order of  $10^4$  counts). Moreover, in the case of the synthesis performed at 200 °C, the intensity increases as the length of the alkyl chain of the capping agent increases, while the opposite occurs in the case of the synthesis at 250 °C. However, while the NPs synthesized with C16 at two temperatures exhibit a photoluminescence maximum at 525 nm, for CeO<sub>2</sub> NPs synthesized with C18 the maximum peaks shift from 562 to 522 nm, for 200 and 250 °C, respectively. This blue shift is typical of semiconductor nanoparticles when the size decreases and the diameter is less than 10 nm.<sup>93,94</sup>

However, the other systems also have a slight photoluminescence, as visible in zoomed spectra reported in Figure 3.16.



**Figure 3.16.** Photoluminescence spectra of C8@CeO<sub>2</sub> 150, C10@CeO<sub>2</sub> 150, C12@CeO<sub>2</sub> 150, C16@CeO<sub>2</sub> 150, and C18@CeO<sub>2</sub> 150 (**A**), C10@CeO<sub>2</sub> 200, C12@CeO<sub>2</sub> 200 (**B**).

Considering all NPs synthesized at 150 °C (Figure 3.16 A), also in this case, as the size of the inorganic core decreases, there is a blue shift. This trend is not observed for samples  $C10@CeO_2 200$  and  $C12@CeO_2 200$ , probably due to the phenomenon of self-aggregation, which leads to the formation of disordered aggregates.

This characterization demonstrated how it is possible to modulate the shape, size, and tendency to self-aggregation of  $CeO_2$  NPs by varying the synthesis temperature and the length of the alkyl chain of the capping agent. Furthermore, a correlation has been shown between the maximum photoluminescence peak and the size of the inorganic core, at least for the C18@CeO<sub>2</sub> 250 monodisperse and spherical system.

# **Chapter 4**

Ordered vs disordered coated CeO<sub>2</sub> superlattices

In this chapter, the results obtained from the self-assembly of coated CeO<sub>2</sub> NPs will be presented and discussed. The self-assembly, as described in Chapter 2, is realized without removing the first organic layer from the surface of the NPs. Two functionalizing molecules, oleic acid (OA), and sodium oleate (NaOA) were used for this purpose. From the results obtained from the characterization of the synthesized NPs, which show the presence of large aggregates for the systems synthesized at 150 °C, it was chosen to functionalize the NP synthesized at 200 and 250 °C.

The resulting superlattices were characterized by Small Angle X-ray Scattering (SAXS), cryogenic Transmission Electron Microscopy (cryo-TEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and Small Angle Neutron Scattering (SANS). SAXS analysis was performed at the beamline B21 of the Diamond Light Source (Didcot, UK). SANS measurements were carried out at the KWS-2 instrument, and cryo-TEM images were acquired at Heinz Maier-Leibnitz Source (Garching, Germany).

In addition, the experimental potential, obtained by imposing the experimental distance between two neighboring NPs, was compared with the minimum potential calculated by considering the different contributions of the interactions, as described by the XDLVO theory presented in Chapter 1.

## 4.1 OA-Amine@CeO<sub>2</sub> NP superlattice

Amine-coated  $CeO_2$  NPs have all been functionalized with oleic acid (OA), which is one of the most widely used molecules for the synthesis and the functionalization of metal oxide nanoparticles.<sup>95–97</sup>

In the following paragraphs, the effect of the length of the alkyl chain of the capping agent, the size of the inorganic core, and the ratio between the concentration of the nanoparticle suspensions and the functionalizing molecule on the NP self-assembly process is presented.

#### 4.1.1 Effect of the length of the alkyl chain of the capping agent

To study the effect of the alkyl chain of the capping agent on the NP self-assembly process, all suspensions of coated  $CeO_2$  NPs at a concentration of 50 mM have been functionalized with the same amount of OA, to have a NPs:OA ratio 70:1 mol:mol.

The functionalization with OA leads in all the cases to the formation of aggregates in an aqueous suspension. To determine the morphology of these aggregates, SAXS and cryo-TEM measurements were conducted. The SAXS patterns of OA-C10@CeO<sub>2</sub> 200, OA-C12@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 200, and OA-C18@CeO<sub>2</sub> 200 are shown in Figure 4.1.



Figure 4.1. SAXS patterns of OA-C10@CeO<sub>2</sub> 200, OA-C12@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 200, and OA-C18@CeO<sub>2</sub> 200 in H<sub>2</sub>O.

The slope at low Q values of all SAXS curves indicates the presence of NP aggregates. Furthermore, the curves of OA-C10@CeO<sub>2</sub> 200 and OA-C12@CeO<sub>2</sub> 200 systems are similar, however, the presence of peaks is visible for OA-C16@CeO<sub>2</sub> 200 and OA-C18@CeO<sub>2</sub> 200 systems.

To better study the coated  $CeO_2$  organization, in all the cases the form factor of a sphere has been subtracted.



**Figure 4.2.** SAXS pattern of OA-C10@CeO<sub>2</sub> 200 in H<sub>2</sub>O (**A**), cryo-TEM image of OA-C10@CeO<sub>2</sub> 200 in D<sub>2</sub>O (scalebar:100 nm) (**B**), SAXS pattern of OA-C12@CeO<sub>2</sub> 200 H<sub>2</sub>O (**C**), and cryo-TEM of OA-C12@CeO<sub>2</sub> 200 D<sub>2</sub>O (scalebar: 80 nm) (**D**).

The SAXS curve of OA-C10@CeO<sub>2</sub> 200 present a slope of  $Q^{-3}$  (Figure 4.2 A), typical of NP aggregates.<sup>98</sup> They are also visible from the cryo-TEM image from which an average distance between two NPs of about 6 nm (shown by the red line in Figure 4.2 B) is determined. Also, for OA-C12@CeO<sub>2</sub> 200 sample disordered aggregates are visible, although subtracting from SAXS data the form factor of a sphere, in this case, a peak is shown at a value of scattering vector Q of about 0.08 (Figure 4.2 C), corresponding to an average distance between NPs of about 7.8, value in accordance with that determined by the statistical analysis of cryo-TEM image  $d=7\pm 2$  nm, shown by the red line in Figure 4.2 D.

The same analysis was performed for OA-C16@CeO<sub>2</sub> 200 sample, SAXS curve and cryo-TEM image are shown in Figure 4.3.



Figure 4.3. SAXS pattern in H<sub>2</sub>O (A), and cryo-TEM image in D<sub>2</sub>O (scalebar: 80 nm) (B) of OA-C16@CeO<sub>2</sub> 200.

As for OA-C12@CeO<sub>2</sub> 200, the SAXS pattern of OA-C16@CeO<sub>2</sub> 200 (Figure 4.3 A) also presents, after subtracting the sphere from factor, a peak at about 0.08 Å<sup>-1</sup>, corresponding to a distance between NPs of 7.8 nm. This value is in accordance with that obtained from the statistical analysis of cryo-TEM images (Figure 4.3 B),  $d=7.2\pm1.2$  nm. Interestingly, in other areas of the grid analyzed by means of cryo-TEM there are regions where clusters of NPs seem to assume an ordered arrangement (red squares in Figure 4.4 A, B, and C). They coexist with single NPs (Figure 4.4 D). Through DLS analysis the dimension of the aggregates was determined,  $R_h=470\pm40$  nm, in agreement with aggregates formed through the micro-emulsion method.<sup>48</sup>



**Figure 4.4.** Cryo-TEM image of OA-C16@CeO<sub>2</sub> 200 in different zone of grid analyzed, scalebar: 200 nm (**A**), scalebar: 80 nm (**B**), scalebar: 80 nm (**C**), and scalebar: 30 nm (**D**) in D<sub>2</sub>O.

These results show that the OA-C16@CeO<sub>2</sub> 200 sample is very heterogeneous, with coexisting single NPs, disordered and ordered aggregates in suspension.

SAXS curve and cryo-TEM image of OA-C18@CeO<sub>2</sub> 200 are shown in Figure 4.5.



Figure 4.5. SAXS pattern in H<sub>2</sub>O with  $Q/Q_1$  ratio (A), and cryo-TEM image D<sub>2</sub>O (scalebar:80 nm) (B) of OA-C18@CeO<sub>2</sub> 200.

The analysis of SAXS patter of OA-C18@CeO<sub>2</sub> 250 reveled Bragg reflections (Table 4.1) comparable to both a face centered cubic (FCC) and a Frank-Kasper with a hexagonal unit cell (C14 FK) phase, as can be seen from the comparison between the experimental data  $Q/Q_1$  and those present in the literature for the two phases, FCC and C14 FK (Table 4.1).<sup>43</sup>

**Table 4.1.** Comparison between the experimental values of OA-C18@CeO<sub>2</sub> 200 sample obtained from SAXS analysis and the theoretical values of FCC and C14 FK structures.<sup>43</sup>

OA-C18@CeO <sub>2</sub> 200		FCC		C14 FK	
Q Å <sup>-1</sup>	$Q/Q_1$	Q/Q.	ı hkl	$Q/Q_1$	hkl
0.06	1	1	111	1	100
0.07	1.17	1.15	200	1.14	101
0.12	2	2	222	2	200
0.20	3.33	3.26	440	3.32	205

However, the SAXS curve shape, and the analysis of the cryo-TEM images, from which a distance of about 7.9 nm, and an angle of about 90° from two neighboring NPs are derived, are more consistent

with an FCC phase.<sup>43</sup> Nevertheless, the undefined peaks of the SAXS data suggest the coexistence of several ordered or even disordered phase.

A comparison of the distance between two neighboring NPs obtained from SAXS and cryo-TEM data for all systems is given in Table 4.2.

**Table 4.2.** Comparison between the distance obtained from SAXS  $d_{SAXS}$  and cryo-TEM image  $d_{TEM}$  analysis of OA-C10@CeO<sub>2</sub> 200, OA-C12@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 200, OA-C18@CeO<sub>2</sub> 200.

Sample		$d_{SAXS}$	$d_{TEM}$	
	Sample	nm	nm	
	OA-C10@CeO <sub>2</sub> 200		$6\pm 2$	
	OA-C12@CeO <sub>2</sub> 200	7.8	$7\pm2$	
	OA-C16@CeO <sub>2</sub> 200	7.8	$7.2 \pm 1.2$	
	OA-C18@CeO <sub>2</sub> 200	$6\pm3$	$7.9\pm0.8$	

The average distances between two neighbouring NPs determined by means of SAXS or statistical analysis of cryo-TEM images are similar for all the systems. Nevertheless, the experimental data show a different behaviour of the various systems in the self-assembly process, leading to the formation of different hierarchical clusters.

To understand this trend, the ratios between the surface-surface distance and the length of the alkyl chain of the capping agent,  $\delta / l_s$  and between the length of the alkyl chain of the capping agent and the radius of the inorganic core,  $\frac{l_s/r_c}{r_c}$ , proposed by Pansu *et al.* to describe the aggregates structures

of coated gold nanoparticles (coated-Au NPs),<sup>43</sup> were calculated and reported in Table 4.3. Subsequently, these ratios were compared with the structure diagram obtained for coated gold nanoparticle (shown in Chapter 1, Figure 1.8) to understand if the model proposed by Pansu *et al.*<sup>43</sup> was also applicable to the superlattices of coated cerium oxide nanoparticles (Figure 4.6).

**Table 4.3.** Experimental values of surface-surface distance  $\delta$ , length of alkyl chain of capping agent  $l_s$  (calculated using Tanford's equation,<sup>99</sup> inorganic core  $r_c$ ,  $\delta / l_s$ , and  $l_s / r_c$  ratios of OA-C10@CeO<sub>2</sub> 200, OA-C12@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 200, OA-C18@CeO<sub>2</sub> 200.

Sample	δ	$l_s$	$r_c$	8/1.	$l_s/r_c$	
Sumple	nm	nm	nm	$0/t_S$		
OA-C10@CeO <sub>2</sub> 200	0	1.42	3	0	0.47	
OA-C12@CeO <sub>2</sub> 200	0.6	1.67	3.8	0.36	0.44	
OA-C16@CeO <sub>2</sub> 200	1.6	2.18	4.4	0.73	0.50	
OA-C18@CeO <sub>2</sub> 200	0.5	2.44	4.2	0.20	0.58	



Figure 4.6. Structure diagram of coated-Au structure (gold), and of OA-C10@CeO<sub>2</sub> 200 disordered aggregates (green), OA-C12@CeO<sub>2</sub> 200 disordered aggregates (green), OA-C16@CeO<sub>2</sub> 200 (orange), OA-C18@CeO<sub>2</sub> 200 FCC structure (pink).

By comparing the ratios for which coated-Au NPs form ordered superlattices and the parameters obtained for coated-CeO<sub>2</sub> NP systems, an agreement can be noted for disordered OA-C10@CeO<sub>2</sub> 200 and OA-C12@CeO<sub>2</sub> 200 aggregates. Based on the parameters obtained for OA-C16@CeO<sub>2</sub> 200 system the ordered structures observed in the cryo-TEM images should correspond to a FK structure. On the other hand, the parameters calculated for the OA-C18@CeO<sub>2</sub> 200 system should give formation of disordered aggregates, whereas a FCC superlattice is observed experimentally. In this respect, it is wort to note that not all the SAXS peaks of OA-C18@CeO<sub>2</sub> 200 could be attribute to a FCC structure, suggesting the presence of aggregates of different types. Therefore, the structure diagram proposed by Pansu well describes the formation of disordered aggregates, but not those ordered for coated-CeO<sub>2</sub> NPs. Probably the different nature of inorganic core and the obtaining of the superlattice that can be obtained with coated-CeO<sub>2</sub> NPs.

These results, show that as the length of the alkyl chain of the capping agent increases, coated-CeO<sub>2</sub> NPs tend to form ordered superlattices, although disordered aggregates and single functionalized nanoparticles also coexist.

#### 4.1.2 Effect of the inorganic core dimension

Another parameter that can affect the self-assembly process is the size of the inorganic core of NPs. As demonstrated in Chapter 3, the core size of coated-CeO<sub>2</sub> NPs varies significantly as the synthesis temperature changes: the inorganic core radius of NPs synthesized in the presence of C16 and C18 decreases from 4.4 and 4.2 nm to 2.9 and 2.5 nm, respectively, by increasing the synthesis temperature from 200 °C to 250 °C. All these NPs were functionalized, and their behavior compared to study the effect of the inorganic core size (Figure 4.7).



Figure 4.7. SAXS patterns of OA-C16@CeO<sub>2</sub> 200, OA-C18@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 250, and OA-C18@CeO<sub>2</sub> 250 in H<sub>2</sub>O.

From a first qualitative comparison of the SAXS curves, it would seem that the two systems OA-C16@CeO<sub>2</sub> 200 (orange curve) and OA-C16@CeO<sub>2</sub> 250 (navy curve) there are no variations, instead different peaks are present for the OA-C18@CeO<sub>2</sub> 200 (pink curve) and OA-C18@CeO<sub>2</sub> 250 systems (violet curve).

SAXS curve and cryo-TEM images of OA-C16@CeO<sub>2</sub> 250 sample are shown in Figure 4.8.



**Figure 4.8.** SAXS pattern in H<sub>2</sub>O (**A**), cryo-TEM image of C14 FK phase in D<sub>2</sub>O (scalebar: 80nm) (**B**, and **C**), enlarged section of region marked in C (**D**) of OA-C16@CeO<sub>2</sub> 250.

The analysis of SAXS pattern of OA-C16@CeO<sub>2</sub> 250 reveled Bragg reflections (Table 4.4) typical of a hexagonal phase. In fact, the experimental values of  $Q/Q_1$  (Table 4) are comparable to that of a Frank-Kasper phase with a hexagonal unit cell (C14 phase). The presence of the hexagonal phase is also confirmed by cryo-TEM images, in particular the enlarged section (Figure 4.8 D) showed the honeycomb structure, typical of Frank-Kasper (FK) phases.<sup>43,46,47</sup>

OA-C16@	@CeO <sub>2</sub> 250	C14	FK
Q Å <sup>-1</sup>	$Q/Q_1$	Q/Q	ı hkl
0.06	1	1	100
0.12	2	2	200

**Table 4.4.** Comparison between the experimental values of OA-C16@CeO<sub>2</sub> 250 sample obtained from SAXS analysis and the theoretical values of phase C14 Frank-Kasper<sup>43</sup>

From this data, the cell parameters were calculated,  $a=b\sim10.5$  nm, and  $c\sim17.1$  nm.

However, the presence of single functionalized CeO<sub>2</sub> NPs is detected by TEM analysis (Figure 4.9).



Figure 4.9. TEM image of OA-C16@CeO<sub>2</sub> 250 (scalebar: 20 nm).

SAXS curve and cryo-TEM images of C18@CeO<sub>2</sub> 250 sample are shown in Figure 4.10.



Figure 4.10. SAXS pattern in H<sub>2</sub>O (A), and cryo-TEM image in D<sub>2</sub>O (scalebar: 60 nm) of OA-C18@CeO<sub>2</sub> 250.

The analysis of SAXS pattern of OA-C18@CeO<sub>2</sub> 250 revealed, also in this case, Bragg reflections typical of a hexagonal phase, with the experimental values of  $Q/Q_1$  comparable to that a C14 FK phase (Table 4.5). In addition, the analysis of Cryo-TEM images show the presence of a distorted compact hexagonal structure with lattice parameters  $a=b\sim9.0$  nm,  $c\sim14.7$  nm and  $\theta\sim115^{\circ}$ .

**Table 4.5.** Comparison between the experimental values of OA-C18@CeO<sub>2</sub> 250 sample obtained from SAXS analysis and the theoretical values of phase C14 Frank-Kasper<sup>43</sup>

OA-C18@	$CeO_2 250$	C14	FK
QÅ-1	$Q/Q_1$	Q/Q i	hkl
0.062	1	1	100
0.118	1.892	1.879	9103
0.204	3.286	3.321	205

However, in TEM images an organization of OA-C18@CeO<sub>2</sub> 250 NPs with an angle of about 90° between neighboring NPs is also visible (Figure 4.11).



Figure 4.11. TEM image of OA-C18@CeO<sub>2</sub> 250 (scalebar: 20 nm).

The ratios  $\delta/l_s$ , and  $l_s/r_c$  have also been determined for these systems and are reported in Table 4.6.

**Table 4.6.** Experimental values of distance between two neighboring NPs from cryo-TEM  $d_{TEM}$ , surface-surface distance  $\delta$ , length of alkyl chain of capping agent  $l_s$  (calculated using Tanford equation), inorganic core  $r_c$ ,  $\delta/l_s$ , and  $l_s/r_c$  ratios of OA-C16@CeO<sub>2</sub> 250, OA-C18@CeO<sub>2</sub> 250.

Sample	d <sub>TEM</sub> nm	$\delta$ nm	<i>ls</i> nm	r <sub>c</sub> nm	$\delta/l_s$	ls/r <sub>c</sub>
OA-C16@CeO <sub>2</sub> 250	$7.5 \pm 0.2$	2 1.7	2.18	2.9	0.77	0.75
OA-C18@CeO <sub>2</sub> 250	$7.4 \pm 0.8$	3 2.2	2.44	2.5	0.90	0.98

Considering the ratios presented in Table 4.6, a structure diagram has been constructed, like that shown in the Figure 4.6, to compare the morphology of the structures obtained for the different superlattices of coated  $CeO_2$  NPs (Figure 4.12).



Figure 4.12. Structure diagram of OA-Amine@CeO<sub>2</sub> NP superlattices.

From the structure diagram shown in Figure 4.12, it is clearly visible that coated CeO<sub>2</sub> NPs are structured in ordered hierarchical structure for  $l_s/r_c > 0.5$ , for  $\delta/l_c < 0.7$  they have a FCC structure, for  $\delta/l_c > 0.7$  a C14 FK structure. However, in all the cases coexistence of different structures is observed: ordered and disordered aggregates as well as single NPs for OA-C16@CeO<sub>2</sub> 200 and OA-C18@CeO<sub>2</sub> 200; C14 FK structure and single NPs for OA-C16@CeO<sub>2</sub> 250; C14 FK and FCC ordered structures for OA-C18@CeO<sub>2</sub> 250.

These data show that the order of clusters formed by coated  $CeO_2$  NPs increases as the length of the alkyl chain of the capping agent increases and the size of inorganic core decreases, also leading, in the latter case, to the coexistence of different ordered phases.

#### 4.1.3 Effect of the ratio between nanoparticle suspension and oleic acid concentration

The effect of the concentration of the NP suspensions, and so the ratio between NPs:OA (mol:mol), into NP self-assembly was also investigated. For NPs synthesized with either C16 or C18 at 250 °C, 50 mM, NPs:OA 70:1 mol:mol (sample 1), 20 mM, NPs:OA 30:1 (sample 2), and 14 mM, 20:1 mol:mol (sample 3) suspensions were investigated. The previous data refer to 50 mM suspensions.

#### 4.1.3.3 OA-C16@CeO2 250

SAXS profiles of OA-C16@CeO2 250 at three different concentrations are shown in Figure 4.13 A.



**Figure 4.13.** SAXS patterns of OA-C16@CeO<sub>2</sub> 250\_1, OA-C16@CeO<sub>2</sub> 250\_2, and OA-C16@CeO<sub>2</sub> 250\_3 in H<sub>2</sub>O (**A**) and cryo-TEM image of OA-C16@CeO<sub>2</sub> 250\_2 (scalebar: 80 nm) (**B**), cryo-TEM image of OA-C16@CeO<sub>2</sub> 250\_3 (scalebar: 80 nm) (**C**) in D<sub>2</sub>O, and FTT calculated on C image (**D**).

The SAXS profiles of the three systems seem to be completely different because the Bragg peaks are at different Q values. Nevertheless, the  $Q/Q_1$  ratios for OA-C16@CeO<sub>2</sub> 250\_2, and OA-C16@CeO<sub>2</sub> 250\_3 agreed with those of OA-C16@CeO<sub>2</sub> 250\_1, and so with a C14 FK phase (Table 4.7).

**Table 4.7.** Comparison between the experimental values of OA-C16@CeO<sub>2</sub> 250\_1, OA-C16@CeO<sub>2</sub> 250\_2 and OA-C16@CeO<sub>2</sub> 250\_2samples obtained from SAXS analysis and the theoretical values of phase C14 Frank-Kasper.<sup>43</sup>

OA-C16@CeO <sub>2</sub> 250_1		OA-C16@CeO <sub>2</sub> 250_2		OA-C16@0	C14 FK		
<i>Q</i> Å <sup>-1</sup>	$Q/Q_1$	$egin{array}{c} Q \  m \AA^{-1} \end{array}$	$Q/Q_1$	$egin{array}{c} Q \  m \AA^{-1} \end{array}$	Q/Q1	$Q/Q_1$	hkl
0.069	1.00	0.080	1.00	0.079	1.00	1.00	100
0.120	2.00					2.00	200
				0.160	2.03	2.03	112
		0.201	2.50			2.56	203
				0.216	2.74	2.74	114

Cell parameters calculated from these data are  $a=b\sim9.5$ , and 9.4 nm, and  $c\sim15.5$ , and 15.3 nm for OA-C16@CeO<sub>2</sub> 250\_2 and OA-C16@CeO<sub>2</sub> 250\_3, respectively. On the other hand, from analysis of cryo-TEM images reported in Figure 4.14  $\theta = 128\pm8^{\circ}$  and  $120\pm3^{\circ}$ , and  $\delta = 7.4\pm0.7$  nm and 7.6±0.6 nm, for OA-C16@CeO<sub>2</sub> 250\_2 and OA-C16@CeO<sub>2</sub> 250\_3, respectively, were determined.



**Figure 4.14.** Cryo-TEM image of OA-C16@CeO<sub>2</sub> 250\_2 (**A**), and OA-C16@CeO<sub>2</sub> 250\_3 (**B**) (scalebar: 30 nm) in D<sub>2</sub>O.

However, in Cryo-TEM images of all three sample single NPs and disordered aggregates are also present, but probably in such a low amount to not affect SAXS data.

## 4.1.3.4 OA-C18@CeO2 250

SAXS profiles and cryo-TEM images of OA-C16@CeO<sub>2</sub> 250 at three different concentrations are shown in Figure 4.15 A.



**Figure 4.15.** SAXS patterns of OA-C18@CeO<sub>2</sub> 250\_1, OA-C18@CeO<sub>2</sub> 250\_2, and OA-C18@CeO<sub>2</sub> 250\_3 in H<sub>2</sub>O (**A**), cryo-TEM image of OA-C16@CeO<sub>2</sub> 250\_1 (scalebar: 100 nm) (**B**), cryo-TEM image of OA-C16@CeO<sub>2</sub> 250\_2 (scalebar: 100 nm) (**C**), and cryo-TEM image of OA-C16@CeO<sub>2</sub> 250\_3 (scalebar: 100 nm) (**D**) in D<sub>2</sub>O.

SAXS profiles of OA-C18@CeO<sub>2</sub> 250\_1 and OA-C18@CeO<sub>2</sub> 250\_2 are perfectly stackable, while that of OA-C16@CeO<sub>2</sub> 250\_3 seems to show a different organization, due to a different NPs:OA ratio. Nevertheless, the cryo-TEM images of the three different systems show the coexistence of several ordered phases. For this reason, the characterization of samples will be done separately. OA-C18@CeO<sub>2</sub> 250\_1 has been described above. Instead, the SAXS curve and cryo-TEM images of OA-C18@CeO<sub>2</sub> 250\_2 are shown in Figure 4.16.



**Figure 4.16.** SAXS pattern in H<sub>2</sub>O (**A**), cryo-TEM image in D<sub>2</sub>O (scalebar: 100 nm) (**B**), enlarged section of region marked in B (**C**), and FTT calculated on C image (**D**) of OA-C18@CeO<sub>2</sub> 250\_2.

SAXS curve presents features in line with that of OA-C18@CeO<sub>2</sub> 250\_1. In fact, the ratio  $Q/Q_1$  is comparable with that of a C14 FK phase (Table 4.8), and the angle between two NPs is about 115°,
typical of a pseudo-hexagonal phase. For OA-C18@CeO<sub>2</sub> 250\_2, it was possible to calculate the Fourier transform (FTT) of selected areas of cryo-TEM images (Figure 4.16 D) thanks to the presence of individual layers of NPs. They further confirm the presence of a C14 FK phase.

**Table 4.8.** Comparison between the experimental values of OA-C18@CeO<sub>2</sub> 250\_2 sample obtained from SAXS analysis and the theoretical values of phase C14 Frank-Kasper.<sup>43</sup>

OA-C18@0	$CeO_2 250_2$	C14 FK
$egin{array}{c} Q \ { m \AA}^{-1} \end{array}$	$Q/Q_1$	$Q/Q_1$ hkl
0.063	1	1 100
0.117	1.862	1.879103
0.203	3.238	3.321205

Interestingly, by decreasing the NP concentration to 14 mM, NPs:OA 20:1 mol:mol (OA-C18@CeO<sub>2</sub> 250\_3), the SAXS pattern changes with respect to systems at higher NP concentrations (Figure 4.17).



**Figure 4.17.** SAXS pattern in H<sub>2</sub>O (**A**), cryo-TEM image in D<sub>2</sub>= (scalebar: 100 nm) (**B**), enlarged section of region marked in B (**C**), and FTT calculated on C image (**D**) of OA-C18@CeO<sub>2</sub> 250\_3.

The SAXS pattern of OA-C18@CeO<sub>2</sub> 250\_3 revealed Bragg reflections consistent with an FCC (Table 4.9) symmetry and a unit cell dimension  $a\sim$ 14.3 nm. The presence of an FCC phase is detectable in the TEM images of this sample and the corresponding FTT (Figure 4.17 D). Also, the cryo-TEM image reveals features in line with SAXS data (Figure 4.17 B and C). The region highlighted in Figure 4.18 C shows orthogonal NP rows with a distance between two neighboring NPs of about 7.15 nm, consistently with the projection along the *c* axis of an FCC lattice having an axis  $a\sim$ 14.3 nm.

OA-C18@C	CeO <sub>2</sub> 250_3	FCC
Q Å <sup>-1</sup>	$Q/Q_1$	Q/Q1 hkl
0.077	1	1 111
0.088	1.14	1.15 200
0.126	1.63	1.63 220
0.149	1.93	1.91 311
0.195	2.53	2.58 420

**Table 4.9.** Comparison between the experimental values of OA-C18@CeO<sub>2</sub> 250\_3 sample obtained from SAXS analysis and the theoretical values of phase C14 Frank-Kasper.<sup>43</sup>

TEM and cryo-TEM images suggest that FCC and C14 FK phases coexisted in OA-C18@CeO<sub>2</sub> 250 systems irrespective of the concentration of NP suspensions. However, the C14 FK phase prevails at higher concentrations, while the FCC phase at lower ones. These findings can be explained by considering that, differently from the hard spheres, the conformational flexibility provided by the soft shell of decorated NPs can favor a greater adaptation to the local constraints thus inducing the coexistence of two phases (FCC and C14 FK) with comparable thermodynamic stability.<sup>100</sup>

The ordered superlattices obtained under all the conditions considered are stable with the variation of the temperature. For this purpose, the SAXS patterns of sample OA-C18@CeO<sub>2</sub> 250\_2 at different temperatures are shown in the Figure 4.18.



Figure 4.18. SAXS patterns at different temperatures of OA-C18@CeO<sub>2</sub> 250\_2.

Furthermore, the OA-C18@CeO<sub>2</sub> 250 system is stable for longer time, as can be seen from a DLS analysis (Figure 4.19).



Figure 4.19. Hydrodynamic radius distribution over time of OA-C18@CeO<sub>2</sub> 250\_1, OA-C18@CeO<sub>2</sub> 250\_2, and OA-C18@CeO<sub>2</sub> 250\_3 in H<sub>2</sub>O.

### 4.2 Interaction potential of coated CeO<sub>2</sub> NPs

The self-assembly of coated NPs into superlattices is dominated by colloidal interactions. The theory that describes the total interaction potential between NPs is the *Extended Derjaguin-Landau-Verwey-Overbeek theory* (XDLVO), as described in Chapter 1.

In this thesis work, the total interaction potential of coated-CeO<sub>2</sub> NPs as well as individual contributions to it were determined for two neighboring nanoparticles in a range distance 5-30 nm, by using MATLAB calculation software. In this way, an interaction potential curves were obtained and from the minimum of the curve it was possible to calculate the theoretical distance between two neighboring NPs. Subsequently, by imposing the experimental distance determined by SAXS and cryo-TEM measurements, the potential of the systems was calculated and compared with the theoretical values.

It is worth to underline that it has been considered the simplest monodimensional case of two interacting coated-CeO<sub>2</sub> NPs.

The parameters used in the calculations are a combination of experimental, theoretical, and extrapolated data. For example, for calculation of the Hamaker constants (*A*) for ligands that interact through the vacuum were calculated from constant for chains C10, C12, C16, and C18 extrapolated from tabulated data of Hamaker constants for alkyl chains of different lengths.<sup>31</sup> For the elastic contribution, it was chosen to approximate the parameter  $\phi$  to 1, assuming that the CeO<sub>2</sub> NPs were completely covered by the capping agent. For the osmotic contribution, the individual interaction parameters of Flory-Huggins ( $\chi$ ) were calculated, considering the molar enthalpy of vaporization, using the theory of Hildebrand.<sup>101</sup> Finally, for the hydrophobic contribution, the hydrophobic decay length was assumed equal to 1 nm, while the hydrophobic efficiency factor equal to 0, considering the maximum hydrophobic interactions. Table 4.10 shows the values used for the different systems.

**Table 4.10.** Value of inorganic core radius  $r_c$ , length of alkyl chain of capping agent  $l_s$ , Hamaker constant *A*, and Flory-Huggin's parameter  $\chi$ .

Somplo	$r_c$	$l_s$	A 10 <sup>-21</sup>	
Sample	nm	nm	J	χ
OA-C10@CeO <sub>2</sub> 200	3.0	1.42	2.2	7.00
OA-C12@CeO <sub>2</sub> 200	3.8	1.67	2.7	7.31
OA-C16@CeO <sub>2</sub> 200	4.4	2.18	3.9	7.20
OA-C18@CeO <sub>2</sub> 200	4.2	2.44	4.4	12.70
OA-C16@CeO <sub>2</sub> 250	2.9	2.18	3.9	7.20
OA-C18@CeO <sub>2</sub> 250	2.5	2.44	4.4	12.70

The trend of the various contributions to the total potential is the same for all samples, as an example, the graph of sample OA-C10@CeO<sub>2</sub> 200 is shown in Figure 4.20.



Figure 4.20. Total interaction potential of OA-C10@CeO<sub>2</sub> 200 (green line) using the XDLVO theory.

**Table 4.11.** Values of minimum total potential  $\Phi_{tot}$ , surface-surface theoretical distance  $\delta_t$ , theoretical distance between two neighboring nanoparticles  $d_t$  of OA-C10@CeO<sub>2</sub> 200, OA-C12@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 250, OA-C18@CeO<sub>2</sub> 250.

Sampla	$\varPhi_{tot}$	$\delta_t$	$d_t$
Sample	J/kT	nm	nm
OA-C10@CeO <sub>2</sub> 200	-11.33	1.17	7.17
OA-C12@CeO <sub>2</sub> 200	-13.79	0.69	8.29
OA-C16@CeO <sub>2</sub> 200	-21.86	0.69	9.49
OA-C18@CeO <sub>2</sub> 200	-51.87	0.56	8.96
OA-C16@CeO <sub>2</sub> 250	-21.94	0.81	6.61
OA-C18@CeO <sub>2</sub> 250	-51.90	0.55	5.55

For samples synthesized at 200 °C, a well-defined trend is observed, that is, as the length of the alkyl chain increases, the minimum potential moves to lower and lower values and to smaller and smaller surface-surface distances. This means that the attractive interactions predominate. To better appreciate this trend the curve of the total potentials of these systems are reported in Figure 4.22. In addition, the theoretical values of the systems OA-C16@CeO<sub>2</sub> 250 and OA-C18@CeO<sub>2</sub> 250 are very close to those of OA-C16@CeO<sub>2</sub> 200 and OA-C18@CeO<sub>2</sub> 200, for this reason, the curves of these two systems have not been reported in the Figure 4.21.



**Figure 4.21.** Total potential  $\Phi_{tot}$  calculated using XDLVO theory.

Finally, by imposing the experimental distance between two neighboring coated  $CeO_2$  NPs, the value of the minimum potential has been calculated and compared with theoretical values reported above (Table 4.12).

Sample	$\Phi_{totT}$	$\Phi_{totE}$
Sample	J / kT	J / kT
OA-C10@CeO <sub>2</sub> 200	-11.33	-9.48
OA-C12@CeO <sub>2</sub> 200	-13.79	-11.54
OA-C16@CeO <sub>2</sub> 200	-21.86	-18.36
OA-C18@CeO <sub>2</sub> 200	-51.87	-43.41
OA-C16@CeO <sub>2</sub> 250	-21.94	-18.36
OA-C18@CeO <sub>2</sub> 250	-51.90	-43.43

**Table 4.12.** Values of theoretical total potential  $\Phi_{totT}$ , and experimental total potential  $\Phi_{totE}$ 

The data shows that the experimental and theoretical values similar. Experimental distance led to a distance between two NPs close to the theoretical minimal of the total potential.

#### **4.3** Effect of a functionalizing molecule with a net charge

How the nature of the functionalizing molecule affects the process of self-assembly of  $CeO_2$  NPs have been studied, focusing on sodium oleate (NaOA), since it shares a very similar molecular structure with OA but bears a net negative charge. C18@CeO<sub>2</sub> 250 showed the most interesting self-assembly behaviour (i.e., the ordered 3D superlattice were stable in water suspension for about 20 days, and as the NPs:OA ratio changed, an ordered phase inversion occurred), they have been chosen to analyze the effect of the functionalizing molecule.

Three different samples were prepared, NaOA-C18@CeO<sub>2</sub> 250\_1, NaOA-C18@CeO<sub>2</sub> 250\_2, and NaOA-C18@CeO<sub>2</sub> 250\_3, to study the effect of different NPs:NaOA ratios. The ratios considered are the same used for OA-C18@CeO<sub>2</sub> 250. The suspensions obtained were analyzed by means of DLS, TEM, and SANS.

First, DLS measurements were performed on the three systems to assess the formation of suspended aggregates (Figure 4.22 A) and to assess their stability over time (Figure 4.22 B).



**Figure 4.22.** Hydrodynamic radius distribution of NaOA-C18@CeO<sub>2</sub> 250\_1, NaOA-C18@CeO<sub>2</sub> 250\_2, and NaOA-C18@CeO<sub>2</sub> 250\_3 at day 1 (**A**), and hydrodynamic radius distribution over time of NaOA-C18@CeO<sub>2</sub> 250\_1, NaOA-C18@CeO<sub>2</sub> 250\_2, and NaOA-C18@CeO<sub>2</sub> 250\_3 (**B**) in H<sub>2</sub>O.

DLS measurements show that aggregates with a hydrodynamic radius of about 21 nm are obtained independently of the NPs:NaOA ratio (Figure 4.23 A). When functionalized with NaOA NPs form smaller aggregates than when functionalized with OA. This suggests that the use of two different functionalizing molecules can affect the NP self-assembly.

Aggregate dimensions increase with time (Figure 4.23 B) differently from what occurs with OA-C18@CeO<sub>2</sub> 250. OA seems to stabilize the (ordered) NP aggregates better than NaOA.

The morphology of the NaOA-C18@CeO<sub>2</sub> 250 aggregates was characterized by means of TEM (Figure 4.23).



**Figure 4.23.** TEM images of NaOA-C18@CeO<sub>2</sub> 250\_2 scalebar: 100 nm (**A**), and scalebar: 20 nm (**B**).

All systems functionalized with NaOA presented disordered aggregates.

This finding indicates that in addition to the radius of the inorganic core and the length of the alkyl chain of the capping agent, also the charge of the functionalizing molecules plays a crucial role in NP self-assembly.

The structural features of the two kinds of aggregates were also investigated by means of SANS. As an example, the comparison between the SANS profiles of the OA-C18@CeO<sub>2</sub> 250\_3 and NaOA-C18@CeO<sub>2</sub> 250\_3 are shown in Figure 4.24.



Figure 4.24. SANS curves of OA-C18@CeO<sub>2</sub> 250\_3, and NaOA-C18@CeO<sub>2</sub> 250\_3 in D<sub>2</sub>O.

The two curves are completely different, indicating that the two systems are organized differently. Considering results obtained from SAXS and cryo-TEM analysis, the SANS data of OA-C18@CeO<sub>2</sub> 250\_3 have been fitted using a combination of two models: power law and FCC (Figure 4.25). The core radius  $r_c$  and the distance  $\delta$  between two neighboring NPs were obtained from the fitting (Table 4.13). The  $r_c$  is about 2.25 nm in good agreement with the  $\langle R \rangle$  of synthesized C18@CeO<sub>2</sub> NPs obtained from statistical analysis of TEM images, the distance value  $\delta$  is about 3.5 nm. Notably this value is less than the length of two alkyl chains (~5 nm), indicating that an interpenetration between alkyl chains of the organic layer of two neighboring NPs occurs.



Figure 4.25. SANS data of OA-C18@CeO<sub>2</sub> 250\_3 (purple circles) and fitting curve (blue line) in D<sub>2</sub>O (A); 3D schematic representation of FCC structure (B); cartoon of the amphiphilic molecule (C); schematic representation of interpenetration of two neighboring NPs (D).

SANS data of NaOA-C18@CeO<sub>2</sub> 250\_3 have been fitted by combining two other models: power law and core-shell (Figure 4.26). In this respect, the slope is about 3, typical of disordered aggregate. The core radius of NaOA-C18@CeO<sub>2</sub> 250\_3 well agree with results obtained for OA-C18@CeO<sub>2</sub> 250\_3, on the other hand, the thickness of the organic shell is about 4.7 nm. This indicates that the CeO<sub>2</sub> core is coated with two amphiphilic molecules. In this regard, by also fitting the scattering length density SLD of the shell it was possible to determine that it consists of 50% C18 and 50% NaOA (Table 4.13).



**Figure 4.26.** SANS data of NaOA-C18@CeO<sub>2</sub> 250\_3 (pink circles) and fitting curve (blue line) in D<sub>2</sub>O (**A**); cartoon of disordered aggregate (**B**); cartoon of core-shell NPs (**C**).

**Table 4.13.** Values of core radius  $r_c$ , distance between two neighboring NPs  $\delta$ , thickness, scattering length density *SLD* obtained from fitting of SANS data.

Sample	$r_c$	δ	Thickness	SLD <sub>core</sub>	SLD <sub>shell</sub>	$SLD_{solv}$	Xc18 Xo.	V	OA XNaOA	X <sub>D20</sub>	Хн20
	Å	Å	Å	10 <sup>-6</sup> Å <sup>-2</sup>	10 <sup>-6</sup> Å <sup>-2</sup>	10 <sup>-6</sup> Å <sup>-2</sup>		AOA			
OA- C18@CeO <sub>2</sub>	22.5±0.3	35±2		4.36±0.03	0.017±0.001	4.151±0.002	0.5	0.5		0.7	0.3
NaOA- C18@CeO <sub>2</sub>	25.92±0.12		47.0±0.2	4.37±0.02	0.016±0.002	4.150±0.002	0.5		0.5	0.7	0.3

SANS curves are perfectly overlapping for the three NaOA-C18@CeO<sub>2</sub> 250 samples. This means that, unlike OA-C18@CeO<sub>2</sub> 250 samples, the ratio NPs:NaOA does not affect the structure of aggregates.

#### 4.4. Photoluminescence properties: ordered vs disordered aggregates

The emission properties of CeO<sub>2</sub> NPs structured in ordered and disordered aggregates have been studied by means of photoluminescence (PLE). Figure 4.27 shows PLE maps of OA-C18@CeO<sub>2</sub> 250\_1, NaOA-C18@CeO<sub>2</sub> 250\_2, OA-OL@CeO<sub>2</sub> 250\_2 and NaOA-C18@CeO<sub>2</sub> 250\_2 (Panels A, B, D and E, respectively), along with a comparison of the PL spectra using comparable intensity units obtained at the same excitation wavelength ( $\lambda_{exc} = 380 \text{ nm}$ ). The signal-to-noise ratio of 14 mM CeO<sub>2</sub> NPs concentrations was too low to allow a satisfactory analysis of the data. Here, by "PLE map" it is meant the superposition of PL intensity spectra obtained at different values of excitation spectra, i.e., the quantity  $I (\lambda_{PL}, \lambda_{exc})$ , where I is the measured PL signal expressed in counts-per-second (cps) units,  $\lambda_{PL}$  is the photoluminescence (i.e., emission) wavelength, and  $\lambda_{exc}$  is the excitation wavelength. It is to be noted that the short wavelength spectral tails of photoluminescence (i.e., wavelength shorter than 420 nm) are absent from the maps and graphs of Figure 4.27, due to the need of using a longwave-pass sharp cut-off filter to block the excitation stray light and residual Raman emission generated by the liquid waveguide.



Figure 4.27. The upper row (Figures A, B and C) refers to the experimental data obtained for the structured (OA-C18@CeO<sub>2</sub> 250\_1) and disordered aggregates (NaOA-C18@CeO<sub>2</sub> 250\_1) suspensions with 50 mM NPs concentration. (A): PLE intensity maps of OA-C18@CeO<sub>2</sub> 250\_1, (B): PLE intensity map of NaOA-C18@CeO<sub>2</sub> 250\_1, (C) PL spectra of the two samples obtained for 380 nm excitation. The lower row (Figures D, E and F) represents the same quantities obtained for the suspensions of 20 mM concentrations, i.e. (D): PLE intensity maps of OA-C18@CeO<sub>2</sub> 250\_2, (E): PLE intensity map of NaOA-C18@CeO<sub>2</sub> 250\_2, (F) PL spectra of the two samples obtained for 380 nm excitation.

The top row of Figure 4.27 reports data all referring to the samples of suspension whose concentration of CeO<sub>2</sub> was 50 mM, while the bottom row refers to 20 mM suspensions. The first two maps (Panels A and B) allow to compare the PLE intensity maps obtained for the Frank-Kasper structured superlattice (Figure 4.27 A) and for disordered aggregates (Figure 4.27 B). Given the close similarity of the maps, it can be inferred that, the qualitative features of the excitation-emission phenomena are the same for the two systems, on the other hand a much higher PL emission is detected for the ordered superlattices with respect to disordered aggregates, as clearly shown from comparison of spectra obtained by exciting at 380 nm (Figure 4.27 C). The same behavior is envisaged for samples at 20 mM NP concentration.

The data indicate a specific relationship between the enhancement of PL intensity and the aggregation of the  $CeO_2$  nanoparticles in ordered aggregates. Some hypotheses about the nature of such correlation can be proposed.

The occurrence of an amplification of luminescence intensity caused by the aggregation of individual luminophores has been observed in some classes of organic molecules and schematized in the so-called phenomenon of AIEE (namely: "aggregation-induced emission enhancement"), firstly reported in the case of helix-shaped organic molecules.<sup>102</sup> AIEE has been described as the consequence of the suppression of non-radiative decays of excited states of the molecules, caused by aggregation-induced inhibition of molecular rotation.<sup>103,104</sup> Obviously, this mechanism cannot be invoked in cases such as ours where the luminophores are semiconductor nanoparticles, whose PL emission intensity is typically regulated by the density of non-radiative traps. However, the experimental evidence and the analogy with AIEE suggests that some aggregation-related mechanism that quenches the non-radiative recombination and/or enhances the radiative recombination charge carriers in CeO<sub>2</sub> shall be at work.

Interestingly, a similar phenomenon was described by Jassby and Wiesner in their work about the aggregation-induced PL enhancement in ZnS nanoparticles.<sup>54,105</sup> The authors found out that aggregation favors the band-edge PL of ZnS nanoparticles, a phenomenon that they proposed to be caused by the reduction of surface tension of the semiconductor nanocrystals determined by the increase in the van der Waals forces caused by particle aggregation. They in fact argued that the decrease of surface tension in the crystal, has been often associated to an increase in the PL emission due to reduction of strain caused by point defects and consequent decrease in the number of electron traps acting as non-radiative centers.<sup>54,105</sup> The role of electron traps is played by sulfur vacancies in ZnS, and more in general by oxygen vacancies in a metal oxide. Specifically, lattice strain favors the formation of O vacancies on CeO<sub>2</sub> surfaces,<sup>106</sup> so that the mechanism observed for ZnS can be suggested to occur also in our case. Moreover, as also pointed out by Jassby and coworkers,<sup>107</sup> NPs aggregation can also determine the passivation of surface traps and dangling bonds caused by the presence of neighboring particles in the close-packed Frank-Kasper aggregate, again determining a decrease in the probability of non-radiative electron-hole recombination.

As another possible interpretation for the phenomenon, it can be due by an improvement in the excitation efficiency caused by Foster resonant energy transfer. The emission of the isolated  $CeO_2$  particle is of course proportional to its absorption cross-section. Hence, isolated NPs floating in suspension will be able to convert the excitation energy into PL emission only proportionally to the photons absorbed by them. However, once the NPs are closely packed forming the Frank-Kasper superlattice, with an interparticle distance of the order 1-2 nm intercalated by the amphiphilic molecules, the absorption cross section of these latter can also contribute indirectly to PL via Förster energy transfer. This kind of indirect PL excitation is well known, for example, in case where the

emission of small luminophores, e.g., lanthanide ions, is amplified by the "antenna effect" operated by neighboring donor species.<sup>108,109</sup> In this case, the amphiphiles can act as donor species. It is worth remembering that Förster energy transfer occurs at an efficiency proportional to the inverse sixth power of the distance between donor and acceptors, so that only at very close distances the amplification effect shall be relevant.

# **Conclusion Section A**

The aim of this section of the Ph.D. project was to rationalize the geometric and energy parameters capable of driving on ordered self-assembly of coated CeO<sub>2</sub> NPs, and study how an ordered superlattice of semiconductor NPs could amplify its properties.

To achieve these objectives, CeO<sub>2</sub> NPs have been synthesized through the thermal decomposition of Ce(NO<sub>3</sub>)<sub>3</sub>6H<sub>2</sub>O, varying the synthesis temperature (150, 200, and 250 °C) and the length of the alkyl chain of the capping agent (C8, C10, C12, C16, and C18 amines). All synthesis conditions lead to the formation of crystalline NPs, with a typical fluorite structure of CeO<sub>2</sub>. The Ce<sup>3+</sup>/Ce<sup>4+</sup> ratio, which influences the properties of CeO<sub>2</sub> NPs, varies between 0.35 and 0.57. The minimum value is observed for C10@ CeO<sub>2</sub> 150 and the maximum value for the system C10@ CeO<sub>2</sub> 200.

The presence of an organic coating on the surface of CeO<sub>2</sub> NPs was evident from the good dispersion in organic solvents, from the halo at low  $2\theta$  values in XRD spectra, and from the C-C and C-H signals present in XPS spectra. However, IR analysis confirmed the presence of an organic coating on the surface of CeO<sub>2</sub> by detecting simultaneously the characteristic peaks of the metal oxide at about 460 and 770 cm<sup>-1</sup> and those of CH (2800-3000 cm<sup>-1</sup>) and CH<sub>2</sub> (1460-1500 cm<sup>-1</sup>) of the alkyl chains.

After the determination of the crystalline nature of the inorganic core, the presence of defects and the co-presence of the inorganic core and the organic coating; the shape, the size, and the tendency to self-aggregation were determined. Modular these last three parameters mean allow obtaining ordered or disordered 3D superstructures. All CeO<sub>2</sub> NPs synthesized at 150 °C, regardless of the capping agent used, exhibit irregular morphology, with an average dimension between 8 and 20 nm, and a strong tendency to self-aggregation, as confirmed by hydrodynamic radii between 70 and 920 nm. By increasing the synthesis temperature from 150 to 200 °C, the size of the aggregates in chloroform decreases significantly, as well as the average size of the individual inorganic cores, which was between 6 and 8 nm. Moreover, to have a single monodisperse coated CeO<sub>2</sub> NPs in organic suspension ( $R_h \sim 5$  nm, and  $<R > \sim 2.5$  nm), the synthesis was realized at 250 °C and using oleylamine (C18,  $l_s=2.4$  nm) as capping agent. This was also the only case where coated CeO<sub>2</sub> NPs were perfectly spherical.

To induce the self-assembly, CeO<sub>2</sub> NPs coated with a first organic layer of amines with different length of alkyl chains (C10, C12, C16, and C18) and synthesized at 200 and 250 °C, have been functionalized with oleic acid (OA), which is one of the most widely used amphiphilic molecules for the functionalization of metal oxide NPs. Functionalization of CeO<sub>2</sub> NPs coated with shorter chain amines, namely the OA-C10@CeO<sub>2</sub> 200 and OA-C12@CeO<sub>2</sub> 200 systems, leads to the formation of disordered aggregates in aqueous suspension. NPs in these systems are also in a situation of energy instability, in fact, the distance between two neighboring NPs calculated from the minimum of

potentials is different from that found experimentally. Only the longer chain amine coated systems, namely OA-C16@CeO<sub>2</sub> 200, OA-C18@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 250, and OA-C16@CeO<sub>2</sub> 200 and OA-C16@CeO<sub>2</sub> 250 present together with ordered aggregates, also disordered aggregates, and single functionalized NPs. On the other hand, the OA-C18@CeO<sub>2</sub> 200 system has a coexistence of two phases, face centered cubic phase (FCC) and a pseudo hexagonal phase Frank-Kasper (C14 FK). For these samples, OA-C16@CeO<sub>2</sub> 200, OA-C16@CeO<sub>2</sub> 250, and OA-C18@CeO<sub>2</sub> 200, also by varying the NPs:OA ratio the system structuring does not vary. OA-C18@CeO<sub>2</sub> 250, on the other hand, presents a more particular situation. When the concentration of C18@CeO<sub>2</sub> NPs is 50 or 20 mM, a C14 FK phase is most noticeable, characterized by the cell parameters  $a=b\sim9$  nm,  $c\sim14.7$  and  $\theta\sim115^\circ$ , instead for concentration of 14 mM, and FCC phase is most present, with cell parameters  $a\sim14.3$ , and  $\theta\sim90^\circ$ . The simultaneous presence of the two phases is also confirmed by the  $r_cA_s$  ratio of about 0.96 and  $\delta A_s$  of about 1.6. Furthermore, the OA-C18@CeO<sub>2</sub> 250 has stable 3D ordered superlattice in water suspension for about 20 days, with dimensions ranging between 160 and 350 nm.

The C18@CeO<sub>2</sub> 250 sample functionalized with OA was found to be the most interesting system. For these reasons, the C18@CeO<sub>2</sub> 250 system was also functionalized with sodium oleate (NaOA), to study how the functionalizing molecule with a net charge affected the process of self-assembly of CeO<sub>2</sub> NPs. The functionalization with NaOA forms disordered aggregates with a hydrodynamic radius of about 20 nm. This means that, although the same C18@CeO<sub>2</sub> 250 ( $R_h$ ~5 nm) are used in both cases, the use of two different molecules, an acid (OA), and a salt (NaOA), can affect the NP self-assembly. It is also very interesting to note how over time the two systems differ. In fact, evaluating the evolution of  $R_h$  over time, it can be observed how the functionalization with OA stabilizes the ordered 3D superlattice, with a dimension which remained constant, for 20 days. Instead, considering the same time frame, the functionalized system with NaOA tends to aggregate. The *R<sub>h</sub>* of NaOA-C18@CeO<sub>2</sub> 250 increase from about 21 to about 37 nm, for all C18@CeO<sub>2</sub>:NaOA ratio. In addition, the presence of disordered aggregates in the case of NaOA-C18@CeO<sub>2</sub> 250 means that in addition to the radius of the inorganic core and the length of alkyl chain of capping agent, another important contribution is the electrostatic one in driving the ordered self-assembly. This contribution depends on the surface charge, stronger is this charge greater is the repulsion between NPs, and therefore less the possibility of inter-digitalization between the alkyl chain of the organic coating that stabilize the formation of ordered structures.

The interpenetration of the alkyl chains in the case of the formation of ordered 3D superlattice is confirmed by the thickness of the organic shell ( $\delta$ ~3.5 nm) obtained from the fitting of the SANS data

of the OA- C18@CeO<sub>2</sub> 250 systems. This value is less than the length of two alkyl chains (~5 nm), this demonstrated that there is an interpenetration between alkyl chains of the organic layer of two neighboring NPs. A core-shell structure, instead, is observed for NaOA- C18@CeO<sub>2</sub> 250 systems.

Finally, it has been shown that an ordered organization can amplify the photoluminescence intensity of coated  $CeO_2$  NPs by about 400% compared to a disordered organization. This can be interpreted considering a decrease in the probability of nonradiative recombination between electrons and holes or an improvement in excitation efficiency due to Förster Resonant Energy Transfer.

These results will lead to the development of superlattices with novel properties that will enable the use of nanoparticles in increasingly innovative technologies.

# **Section B**

Aptasensor

# **Chapter 1**

Fluorescent NPs for technological applications

#### **1.1 Zinc Oxide Nanoparticles**

Zinc oxide (ZnO) is one of the most important metal oxide materials. It is a II-VI semiconductor with a bonding energy of 60 meV and a direct wide bandgap of about 3.37 eV.<sup>17,110</sup> Reducing its size at the nanometer level its optical properties further increase.

Zinc oxide nanoparticles can exist in three forms: rocksalt, zincblende, and wurtzite (Figure 1.1). The rocksalt is a crystalline structure characterized by a face-centered cubic packaging of  $O^{2-}$  anions, with octahedral sites occupied by  $Zn^{2+}$  cations, while tetrahedral sites are empty. On the other hand, the crystalline form of zincblende is a face-centered cubic structure of  $O^{2-}$  anions, while  $Zn^{2+}$  cations occupy the tetrahedral interstices. Finally, the wurtzite crystalline form presents a hexagonal lattice with two interconnected sub-lattices consisting of  $Zn^{2+}$  and  $O^{2-}$ , where the  $Zn^{2+}$  cations are surrounded by  $O^{2-}$  anions with a tetragonal configuration. This spatial organization generates a polar symmetry along the hexagonal axis, which is responsible for many physico-chemical properties of ZnO NPs and the generation of defects that are directly related to the optical properties of the material.<sup>17,111</sup>



Figure 1.1. Rocksalt, Zincblende, and Wurzite structures of ZnO, blue and white balls represent zinc and oxygen, respectively.

The wurtzite form is the thermodynamically stable form of ZnO. This crystalline form belongs to the space group  $P6_{3mc}$  whose elemental cell is characterized by the parameters a=0.325 nm and c=0.521 nm with a ratio c/a=1.6 very close to the value of c/a=1.633 of the perfect hexagonal elemental cell.<sup>112</sup> Nonetheless, the presence of empty octahedral sites in the crystalline structure determines the presence of intrinsic defects, such as oxygen vacancies V<sub>0</sub> or interstitial zinc Zn<sub>i</sub>, or extrinsic, as dopants.<sup>113</sup> The formation of these defects in the NP crystalline structure is strongly influenced by the NP synthesis temperature and the partial pressure of zinc and oxygen in the reaction environment. In

particular,  $Zn_i$  defects are predominant when the reaction environment is rich in zinc vapors, while  $V_o$  predominate when partial pressures of zinc and oxygen are low.<sup>114,115</sup> This latter point is crucial for the design of ZnO nanostructures because vacancies play a major role in the electrical and thermal transport as well as the mechanical behavior of materials.

#### 1.1.1 Synthesis and doping

ZnO NPs, like CeO<sub>2</sub> NPs, can be synthesized by physical, chemical, and biological methods, as shown in Figure 1.2.



Figure 1.2. Various methods of synthesizing ZnO NPs.

Different synthesis methods or variations of the same method, such as changes of precursor, temperature, pH, reaction time, or oxygen partial pressure, lead to NPs with different structural features. The sol-gel method, for example, is a wet chemical method that provides NPs with a large surface area and surface stability. In 2019, Ismail *et al*<sup>116</sup> prepared rod-like ZnO NPs by sol-gel treatment of zinc acetate dehydrate with NaOH and polyethylene glycol. Starting from the same precursor, in 2020, Chandeker *et al*.<sup>117</sup> prepared oval La-doped ZnO NPs through the combustion

method, a simple, efficient, and quick method that can take place in liquid, solid, or gas phases. Using the zinc acetate dehydrate as precursor, spherical and rod-like NPs were prepared through the hydrothermal method as well as more complex shapes, such as nanoflowers.<sup>118</sup>

To improve the performance of ZnO NPs, doping agents may be added during the synthesis. The ion radius of the doping agent must be similar to that of the ion it replaces, so the crystalline lattice does not distort.<sup>119</sup> Generally, two types of doping of a semiconductor exist: n or p. In the first case, the doping agent is an electron donor, in the second one it is an electron acceptor. In the case of ZnO NPs, n-type doping can be performed by replacing Zn with elements belonging to the III group, such as aluminum (Al), gallium (Ga), and indium (In), or by replacing oxygen atoms with elements of the VII group, such as fluorine (F) or chlorine (Cl).<sup>120,121</sup> P-type doping, on the other hand, is difficult for ZnO NPs. One of the most widely used doping agents in the case of ZnO NPs is fluorine, both because it has an ionic radius of about 0.132 nm, identical to that of oxygen (0.132 nm), both because it act as a donor, if it occupies V<sub>o</sub>, as an acceptor, if it occupies an interstitial site (Figure 1.3).<sup>122</sup> The doping of ZnO NPs with fluorine increases the electrical conductivity of the nanomaterial and its fluorescence emission.<sup>123,124</sup>



**Figure 1.3.** Schematic representation of oxygen vacancies (V<sub>o</sub>) formation, and fluorine (F) doping in a wurtzite structure. Blue, white, and purple balls indicate zinc, oxygen, and fluorine, respectively.

#### **1.1.2 Properties and applications**

The higher thermal stability, toughness, and durability of ZnO NPs with respect to NP of other oxides justifies their extensive use.<sup>17</sup> Moreover, by changing the size, morphology, and defects it is possible to change their properties and so their applications.

For example, as the size of ZnO NPs decreases their antibacterial activity increases. In fact, varying the size of NPs, varying concentrations of defects can modulate the concentration of Reactive Oxygen Species, which causing the death of bacteria, and the apoptosis of cancer cells.<sup>125</sup>

In addition to the biomedical field, ZnO NPs are also used in cosmetics, for example in sunscreens because they are UV-blocker,<sup>126</sup> and in toothpaste as anti-sensitivity agents.<sup>18</sup>

ZnO NPs are used in the construction of solar cells and LEDs because they are charge conservators and have a large bandgap.<sup>127</sup> They are also used in the rubber industry, as fillers, because they catalyze the vulcanization reaction.<sup>128</sup>

Furthermore, ZnO NPs have a high isoelectric point (about 9.5), therefore, they can establish electrostatic interactions with materials that have an inferior isoelectric point, biological molecules, such as proteins.<sup>129</sup> The excellent optical properties of ZnO NPs and the ability to easily interact with biological molecules, make this nanomaterial an excellent candidate for the realization of transducers for optical biosensors.<sup>130</sup>

Some of these applications are shown in figure 1.4.



Figure 1.4. Examples of ZnO NPs applications.

#### **1.2 Biosensor**

A biosensor is an analytical device capable of converting a biological response into a measurable signal proportional to the analyte concentration.<sup>131–136</sup>

The biological recognition system, usually consisting of a receptor protein, enzyme, or antibody, is based either on the catalytic transformation of a substance or on the detection of inhibitory enzymes or mediators. This system has the function of transferring biological information in a detectable physical or chemical signal.<sup>137</sup>

Despite the first biosensor was developed already in 1956 by Leland C. Clark Jr., the so-called Clark's electrode, consisting of two electrodes (Pt and Ag/AgCl) capable of measuring the concentration of oxygen in water and biological fluids,<sup>131</sup> an impressive growth of biosensor developments started only in the 90s, with the industrialization of production processes.<sup>132</sup> Then, biosensors spread rapidly since they overcome the limitations of chemical sensors, based on conventional analytical methodologies. In fact, chemical sensors require expensive analysis methods, long analysis times, and the preliminary use of separation techniques due to inherent difficulties in analyzing complex matrices.<sup>133,134</sup>

#### **1.2.1 Structures and types**

A typical biosensor consists of four main parts: a bioreceptor, a transducer, electronics, and a display (Figure 1.5). The bioreceptor is a biomolecule capable of selectively recognizing the analyte, this interaction is known as biorecognition. The most widely used biomolecules are enzymes, oligonucleotides, and aptamers, which are short single-chain oligonucleotide sequences consisting of between 20 and 100 nucleotides.<sup>138,139</sup> The transducer is the element able to convert the biological signal into a measurable signal, it can be of various types, for example optical, piezoelectric, electrochemical. Electronics is the part of the sensor that processes the transduced signal. The display shows the results obtained.<sup>131</sup>



Figure 1.5 Schematic representation of a biosensor.

In order to be usable, a biosensor has to satisfy some requirements. It must be sensitive to changes in analyte concentration and be selective towards a specific analyte, must be characterized by a low detection limit and high resolution, must be stable for a long time, and have a long-life cycle. All these parameters define the efficiency of the biosensor.<sup>140</sup>

Biosensors can be classified according to different criteria, the most widely used criterion is based on the physico-chemical transduction method, such as electrochemical, mass, thermal, optical.<sup>141,142</sup> The most used biosensors are electrochemical ones, because they have a low cost, a very low detection limit, high selectivity, ease of construction and use, and can be used even for small sample volumes. Mass biosensors exploit mass variations to detect the analyte of interest; thermal ones are based on detection and quantification of thermal variations that occur during the biorecognition processes, finally, optical biosensors are based on the measurement of the light absorbed or emitted after the occurrence of the biorecognition phenomenon. In recent years, thanks to the synthesis of increasingly performing NPs, which can be used as transducers, optical biosensors have widely developed, because they feature high sensitivity and ease of use.<sup>141,143</sup>

An important issue to consider in the design of a biosensor is the instability of the bioreceptor, which is subject to denaturation. Increasing the stability of biomolecules is one of the main objectives in the development of a biosensor. This can be achieved by a proper choice of the optical protocol to immobilize the biomolecule (i.e., enzyme, antibody, oligonucleotide, aptamer) on the active transducer sites, such as ZnO NPs.

Immobilization strategies can be physical or chemical and can also improve the efficiency of the biomolecule, for example in the case of enzymes, immobilization on nanostructures improves resistance to changes in pH and temperature.<sup>144</sup>

Chemical immobilization involves a first step of activation of the transducer, and a second step in which the activated transducer is linked to the biomolecule, directly through a covalent bond or by means of a cross-linker, a molecule, generally glutaraldehyde or (3-aminopropyl)triethoxysilane, that stably bonds the transducer and the biomolecule.<sup>145</sup>

Physical immobilization exploits the trapping of the biomolecule in polymeric matrices or its reversible adsorption on the transducer, through van der Waals, hydrogen bonds, electrostatic or hydrophobic interactions. In the first case, the deactivation of the biomolecule can occur; on the other hand, absorption avoids the use of additional reagents and pre-functional steps and has a low cost. However, the lack of strong interactions between the transducer and the biomolecule can make the system unstable to changes in pH, temperature, and ionic force.<sup>144,146</sup>

In the case of optical biosensors using ZnO NPs as transducers and biomolecules, such as aptamers, physical immobilization seems to be the most promising study.

## **1.2.2 Applications**

Biosensors are important devices to measure an extensive spectrum of analytes like gases, organic compounds, bacteria, and ions therefore are widely used in various fields, including medical, agricultural, environmental, and food (Figure 1.6).<sup>135</sup>



Figure 1.6 Examples of biosensors applications.

Biosensors are used in the medical field because they quickly and accurately detect markers of highly dangerous diseases for humans, such as tumors, allowing an early diagnosis to be formulated, but are also used for the detection of high blood glucose values in diabetics.<sup>139</sup>

In the food field, they are used because they can detect in real-time the gases released from spoiled food.<sup>147</sup>

Finally, biosensors are used in the environmental field because they can detect pollutants, such as pesticides, potentially toxic elements, pathogens, toxins, in different types of environments, such as water, soil, air.<sup>148</sup> But are also used for the detection of chemical or bacteriological weapons.<sup>135</sup>

# Chapter 2

F-doped ZnO NPs: synthesis and film deposition

ZnO NPs were prepared by means of a wet phase synthesis using different amounts of fluorine (F), from 0 to 20 % at, as doping agent to evaluate the optimal F amount able to produce an increase of the fluorescence emission of NPs. The NPs that showed the best optical properties were than used to set up an aptasensor, through deposition on silicon or glass supports.

In this chapter, the adopted procedures are described in detail.

### 2.1 Synthesis of ZnO NPs

#### 2.1.1 Materials

Zinc acetate dihydrate (ZAD) Zn(CH<sub>3</sub>COO<sub>2</sub>)·2H<sub>2</sub>O ( $\geq$  99% purity), ammonium hydrogen difluoride NH<sub>4</sub>HF<sub>2</sub> ( $\geq$  99.999% purity), sodium hydroxide KOH ( $\geq$  85% purity), quinine sulfate monohydrate C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>·H<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O (90% purity), methanol ( $\geq$  99.8% purity) were purchased from Merk (Germany) and used without further purification.

#### 2.1.2 Synthetic protocol

Doped ZnO NPs were synthesized through a modified version of the wet method introduced by Beek *et al.*<sup>149</sup> The precursor salt ZAD has been solubilized in methanol to obtain a 0.11 M solution. The solution was heated at 60 °C, a temperature close to the boiling temperature of methanol, 64.7 °C. The appropriate amount of doping salt was added to the system as detailed in Table 2.1.<sup>122</sup> When the system temperature stabilized, a 0.4 M KOH solution was added drop by drop as a basifying agent.

C	$ZAD \pm 0.0001$	MeOH for ZAD $\pm 0.0001$	$\text{KOH} \pm 0.0001$	MeOH for KOH $\pm 0.0001$	$NH_4HF_2\pm0.0001$
Sample	g	g	g	g	g
F0	1.2342	39.8270	0.6038	27.7106	
F1	2.6402	83.9602	1.3202	47.3301	0.0034
F2.5	2.6402	83.9602	1.3202	47.3301	0.0085
F5	2.6402	83.9602	1.3202	47.3301	0.0171
F7.5	2.6402	83.9602	1.3202	47.3301	0.0256
F10	2.6398	83.9605	1.3202	47.3298	0.0342
F15	2.6401	83.9598	1.3201	47.3302	0.0513
F20	2.6402	83.9960	1.3198	47.3301	0.0684

Table 2.1. Quantity of reagents used in the synthesis.

The resulting solution was stirred for 2 h at 60 °C. The experimental apparatus is shown in Figure 2.1.



Figure 2.1. Experimental apparatus used for ZnO NPs synthesis.

The reaction mixture was then slowly cooled down to room temperature, placed in 50 mL centrifuge tubes and centrifuged at 5000 rpm for 5 minutes, the supernatant was separated from ZnO NPs. The NPs were re-dispersed in methanol, and the centrifugation step was repeated twice to wash out all the unreacted materials. Finally, undoped and doped ZnO NPs were dispersed in methanol.

## 2.2 Film preparation

#### 2.2.1 Materials

F0 and F5 ZnO NPs synthesized as described in paragraph 2.1.2 have been used for film preparation. Glass and silicon supports were used.

#### 2.2.2 Film preparation through spin coating method

To obtain an aptasensor that meets the requirements necessary for its correct use, it is necessary that the transducer layer is suitable for the interaction with the biomolecule. This requires that the ZnO NP film be as homogenous as possible. This phase has therefore required several tests, both for the choice of the good preparation method, for the support to be used, and for the optimal conditions of deposition.

After the first preliminary tests of NP film production by drop casting and spin coating, it was decided to use the second technique for the realization of NP films. Indeed, spin coating is the most widely used technique for production of thin films.<sup>150–152</sup> This procedure can be divided into four steps: deposition, spin-up, spin-off, and evaporation (Figure 2.2). The centrifugal force facilitates the distribution of the solution on the support and the evaporation of the solvent. However, to achieve a complete evaporation of the solvent, the support can also be heated at a temperature slightly above the boiling temperature of the solvent in which the NPs are dispersed.



Figure 2.2. Spin coating procedure to create a NP film.

To optimize the deposition process several parameters were changed, namely the type of support (glass or silicon), the NP concentration (five different concentration) and the rotation speed (four different speed), while the time was kept constant and equal to 60 s (Table 2.2).

Aaronym	NP suspensio concentration	Speed rotation
Actonym	mg mL <sup>-1</sup>	rpm
C1_P1	0.5	1000
C1_P2	0.5	2000
C1_P3	0.5	3000
C1_P4	0.5	4000
C2_P1	1.0	1000
C2_P2	1.0	2000
C2_P3	1.0	3000
C2_P4	1.0	4000
C3_P1	2.0	1000
C3_P2	2.0	2000
C3_P3	2.0	3000
C3_P4	2.0	4000
C4_P1	3.0	1000
C4_P2	3.0	2000
C4_P3	3.0	3000
C4_P4	3.0	4000
C5_P1	6.0	1000
C5_P2	6.0	2000
C5_P3	6.0	3000
C5_P4	6.0	4000
C6_P1	9.0	1000
C6_P2	9.0	2000
C6_P3	9.0	3000
C6_P4	9.0	4000
C7_P1	36.0	1000

 Table 2.2. NP films, NP suspension concentrations, and speed rotation.
# 2.3 Aptasensor realization

# 2.3.1 Materials

For the realization of the aptasensor, the NP films that showed the best surface properties, in terms of degree of coverage, roughness, and fluorescence emission, was used. The chosen biomolecule is the aptamer LA27, with a sequence 3'-CGATAGATTGTTGTAAGACAATCTTCC-5 able to selectively recognize Lipopolysaccharides and provided by CEINGE (Finanziamento per la Ricerca di Ateneo, Progetto LipAptaNanoSens, 000008 ALTRI\_Cda\_75\_2021\_FRA\_LINEA\_B-VITIELLO).

# 2.3.2 Preparation of the bio-recognition layer

The bio-recognition layer is prepared through the physical interaction between the transducer, in this case, ZnO NPs, and the biomolecule, LA27, as described in section 1.2.1 of Section B.

Aptamer deposition on NPs film occurred through the drop-casting technique.<sup>153</sup> This technique can be divided into three steps: biomolecule deposition, solvent evaporation, film densification (Figure 2.3).



Figure 2.3. Drop-casting procedure to create the aptasensor.

For the preparation of the bio-reconnaissance layer (Figure 2.4), 300  $\mu$ L of a 20  $\mu$ M solution of aptamer in water were deposited on the NP film. Evaporation of the solvent took place in a controlled environment at 25 °C.



Figure 2.4. Schematic representation of the aptasensor.

To assess the stability of the system in an aqueous environment, the supports were immersed in a water bath for different times, 1, 3, 6, and 24 hours. Subsequently, to quantify the eventual aptamer and /or ZnO NPs detached from the support, the water was recovered and freeze-dried. The freeze-dry was then dissolved in 3 mL of water and analyzed by UV-Visible spectroscopy.

# **Chapter 3**

The best F-doped ZnO NP system

The aim of this part of the project was to obtain the F-doped ZnO NPs that had the best fluorescence properties, to be used as an optical transducer in the realization of an aptasensor. For this reason, the wet chemical method was chosen for the synthesis of F-doped ZnO NPs. All the syntheses, with varying concentrations of the doping agent, were successful. To identify the degree of doping able to improve the NP fluorescence properties without altering other properties, an extensive physico-chemical characterization of all systems was performed. Namely, the crystalline structure, as well as the presence of reaction by-products, has been evaluated through X-Ray Diffraction (XRD) measurements; the concentrations of the various atomic species and of the doping agent were determined through X-ray Photoelectron Spectroscopy (XPS) measurements; the dimensional and morphological characterization was performed by means of Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM); finally, the NP optical properties were analyzed both in solution and in the solid state by UV-Visible, fluorescence, and photoluminescence spectroscopies.

# 3.1 Structural characterization of F-doped ZnO NPs

The XRD and XPS characterization aimed at determining the crystalline structure and the concentration of the atomic species on ZnO NP surface was carried out at the CEA-Leti Research and Technology Institute in Grenoble (France) as part of the European project NFFA (ID071) for the development of nanoscience.

### **3.1.1 Crystalline structure**

All F-doped ZnO NPs were characterized by means of XRD to determine whether they were crystalline or amorphous, and in the former case which crystalline structure they present. The diffraction spectra are reported in Figure 3.1.



**Figure 3.1.** XRD patterns of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs. Dot lines indicate ZnO peaks, stars indicate ZnF<sub>2</sub> peaks.

The values of the diffraction angles (2 $\theta$ ), the cell dimensions (*a*, *b*, *c*, *a*, *β*, and *γ*), and Miller indices (*hkl*) of the peaks observed in the diffraction spectra of Figure 3.1 are listed in Table 3.1. These patterns show the typical peaks of a wurtzite structure (JCPDS 36-1451). The crystalline sizes were found to range from 2 to 4 nm, which were calculated for the most intense peak (101) using the Scherrer equation as given below:<sup>154</sup>

$$D = \frac{0.9\lambda}{\beta \cos\theta} \tag{3.1}$$

where *D* is the crystalline size (nm),  $\beta$  is the full width of the direct line at half of the maximum (101) intensity,  $\lambda$  is the X-ray wavelength of Cu K<sub> $\alpha$ </sub> = 0.154 nm, and  $\theta$  is the Bragg angle.

Sampl	(100)	(000)	(101)	(100)	(110)	(100)	(200)	(110)	(201)		b			β	
e	(100)	(002)	(101)	(102)	(110)	(103)	(200)	(112)	(201)	Å	Å	Å			
F0	31.56	34.16	36.21	47.31	56.43	62.80	66.43	67.85	68.86	3.2	3.2	5.2	89.9	89.9	119.9
	°	°	°	°	°	°	°	°	°	5	5	1	3	1	9
F1	31.56	34.07	36.21	47.22	56.43	62.80	66.27	67.85	69.03	3.2	3.2	5.2	90.7	89.4	120.6
	°	°	°	°	°	°	°	°	°	7	8	1	0	9	6
F2.5	31.64	34.32	36.21	47.30	56.50	62.80	66.35	67.77	69.03	3.2	3.2	5.2	90.7	89.6	120.7
	°	°	°	°	°	°	°	°	°	6	8	1	7	4	7
F5	31.54	34.32	36.05	47.22	56.59	62.80	66.43	67.85	69.11	3.2	3.2	5.2	90.6	89.3	120.3
	°	°	°	°	°	°	°	°	°	5	7	1	7	0	3
F7.5	31.54	34.24	36.13	47.28	56.51	62.80	66.43	67.85	69.11	3.2	3.2	5.2	90.4	89.5	120.0
	°	°	°	°	°	°	°	°	°	4	7	0	8	8	5
F10	31.64	34.32	36.30	47.28	56.59	62.80	66.35	67.92	69.11	3.2	3.2	5.2	89.8	90.1	119.9
	°	°	°	°	°	°	°	°	°	5	4	0	1	2	3
F15	31.54	34.32	36.21	47.30	56.51	62.80	66.51	67.92	69.11	3.2	3.2	5.2	90.0	89.9	120.1
	°	°	°	°	°	°	°	°	°	5	5	0	7	0	6
F20	31.72	34.32	36.29	47.28	56.59	62.88	66.43	67.92	69.11	3.2	3.2	5.2	90.0	90.0	120.0
	°	°	°	°	°	°	°	°	°	5	5	0	1	4	5

**Table 3.1.** Experimental values of the diffraction angles ( $2\theta$ , deg), corresponding Miller indices (*hkl*), and the cell dimensions of diffraction peaks observed in the profiles reported in Figure 3.1.

Experimental data of doped versus undoped samples show no significant shift of diffraction peaks, suggesting no significant distortion of the crystalline lattice of ZnO because of the presence of the doping agent. However, in the diffraction spectra of samples obtained using a high amount of doping salt (from F7.5 to F20 ZnO samples), the presence of diffraction peaks typical of  $ZnF_2$  (reported as stars in Figure 3.1) in the tetragonal crystalline structure with spatial group  $P4_2/mnm$  is observed.

The formation of  $ZnF_2$  may be a consequence of the dissociation of the fluorine-based doping salt, NH<sub>4</sub>FHF, in H<sup>+</sup>, HF<sub>2</sub><sup>-</sup>, and HF species. HF in the presence of  $Zn(CH_3COO)_2$ , the inorganic precursor of ZnO NPs, can lead to the formation of ZnF<sub>2</sub>, according to the following reaction (3.2):<sup>155</sup>

$$Zn(CH_3COO)_2 + 2HF \rightarrow ZnF_2 \tag{3.2}$$

#### **3.1.2 Chemical composition**

The XPS analysis was performed to obtain information on the chemical composition of undoped and doped ZnO NPs. The spectra reported in Figure 3.2 show the characteristic peaks of ZnO (Zn 2p, Zn 3s, Zn 3p, Zn 3d, and O 1s), in all the samples, while in the samples with the highest nominal concentration (F15 and F20) of fluorine the F 1s peak is clearly visible. Carbon contamination (C 1s) was detected in all samples, probably due to the presence of unconverted reagents and/or to the presence of reaction by-products not removed during the purification.



Figure 3.2. XPS patterns of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs.

To quantify the species of interest, the main peaks were considered, namely the peaks of Zn *3p*, O *1s*, F *1s*, and C *1s*.



**Figure 3.3.** Zn *3p* (**A**), O *1s* (**B**), F *1s* (**C**), and C *1s* (**D**) XPS spectra of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs.

The spectrum of Zn 3p (Figure 3.3 A) has two peaks: one at about 87 eV due to the zinc bound to oxygen and one at about 91 eV due to the zinc bound to fluorine. The XPS spectrum of O 1s (Figure 3.3 B) also presents two peaks: one between 529 and 530 eV due to the oxygen bound to zinc, the other one at about 532 eV due to the oxygen bound to carbon. In all the cases, the intensity of the O-Zn peak is higher than that of the O-C peak, except for samples F15 and F20. The peak of F is visible and, therefore, its concentration is quantifiable only for the highest nominal concentration samples (Figure 3.3 C). Finally, the presence of C 1s (Figure 3.3 D) may be due to the presence of an unconverted reagent. Spectra in Figure 3.3 D also highlight the presence of another contaminant, K 2p, in samples with the highest F concentration.

By integration of these peaks, the atomic composition of the different F-doped ZnO NPs is determined, as reported in Table 3.2.

Sampla	Zn	O (O-Zn)	F	С	O (O-C)
Sample	%at	%at	%at	%at	%at
F0	31 ± 6	$31 \pm 6$		$14 \pm 3$	$24 \pm 5$
F1	$33 \pm 6$	$27 \pm 5$		$18 \pm 3$	$22 \pm 4$
F2.5	$33\pm7$	$25 \pm 5$		$18 \pm 4$	$23\pm5$
F5	$36\pm7$	$27 \pm 5$	$1.5\pm0.3$	$17 \pm 3$	$19 \pm 4$
F7.5	$39\pm8$	$27 \pm 5$	$1.7\pm0.3$	$15 \pm 3$	$17 \pm 3$
F10	$40\pm 8$	$29\pm 6$	$7 \pm 1$	$15 \pm 3$	$16 \pm 3$
F15	$31\pm 6$	$13 \pm 3$	$8\pm 2$	$14 \pm 3$	$24\pm5$
F20	$35\pm7$	$19 \pm 4$	$7\pm1$	$17 \pm 3$	$23\pm5$

Table 3.2. Atomic concentrations of Zn, O, C, and F were obtained from the XPS spectra.

All F-doped ZnO NPs have the same atomic surface concentration of Zn, as well as the concentration of C and O bound to C. Other than the concentration of O bound to Zn for samples F15 and F20, which have a lower concentration of O-Zn than the other samples. This means that for these samples the surface  $V_0$  are greater. The fluorine concentration was, however, only determined for samples F5 to F20, as mentioned above. This concentration is approximately 1.5 % at for samples F5 and F7.5, and equal to about 7/8 % at for samples F10, F15, and F20. This probably indicates that a concentration of 7/8 % at is the maximum concentration of dopant that these ZnO NPs may have.

# 3.2 Morphological and dimensional characterization

The size and shape of individual NPs was determined by means of TEM. However, as the sample preparation involves a solvent evaporation step that can cause NP aggregation, the size of samples in solution was analyzed also by means of DLS.

#### **3.2.1 Morphological analysis**

Morphological analysis was performed for all F-doped ZnO NPs, for example in Figure 3.4, micrograph images of F0, F5, and F15 ZnO NPs are shown.



Figure 3.4. TEM images of F0 (A), F5 (B), and F15 (C) ZnO NPs (scalebar: 50 nm).

TEM images clearly show that ZnO NPs tend to self-aggregate indipendently of their composition, even if aggregate size increases with the percentage of fluorine. Individual F-doped ZnO NPs have an irregular morphology, but by approximating them to spheres, we determined their size through a statistical analysis of the images. The average radius of the individual nanoparticles is 3 nm and does not vary with the atomic percentage of the doping agent (Figure 3.5), indicating that the synthesis conditions are such to make the size of the individual F-doped ZnO NPs reproducible. The dimensions

of individual F-doped ZnO NPs were in agreement with the crystalline sizes obtained from XRD analysis.



**Figure 3.5.** The radius of F0 (black circle), F1 (red square), F2.5 (blue up-triangle), F5 (green down-triangle), F7.5 (purple rhombus), F10 (orange left-triangle), F15 (pink right-triangle), and F20 (wine hexagon) ZnO NPs obtained from statistical analysis of TEM images of Figure 3.4.

### **3.2.2 Dimensional analysis**

To determine whether the tendency to aggregation depends on the evaporation of the solvent for the TEM analysis or whether F-doped ZnO bare NPs naturally aggregate in suspension, DLS measurements were performed (Figure 3.6).



**Figure 3.6.** Hydrodynamic radius distribution of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs in methanol.

Hydrodynamic radius distributions reveal the presence of a single population in all the samples.  $R_h$  increases moving from sample F0 to sample F2.5, then remains constant for samples F5 and F7.5.  $R_h$  of F10 and F15 is smaller than those of samples with less fluorine, being about 28 nm, while for sample F20 a population with a hydrodynamic radius value of about 62 nm is observed. The process of self-aggregation of bare NPs in solution is not a controllable process. For this reason, the values of  $R_h$  obtained do not follow any trend.

# **3.3 Optical properties**

The optical properties of F-doped ZnO NPs have been analyzed in suspension and in the dried state.

UV-Visible and fluorescence measurements were carried out on methanol dispersions of F-doped ZnO NPs. To calculate the band gap, the Tauc plot method was used. In addition, photoluminescence measurements were performed to evaluate the optical properties of NP films, since they could be exploited as optical transducers for the development of aptasensors. The last measurements were carried out at the CEA-Leti Research and Technology Institute in Grenoble (France) as part of the European project NFFA (ID071) for the development of nanoscience.

# 3.3.1 Optical properties of F-doped ZnO NPs in suspension

The UV-Vis spectra of F-doped ZnO NPs suspended in methanol, the solvent in which the NPs were synthesized and stored, are reported in Figure 3.7.



Figure 3.7. UV-Visible spectra of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs in methanol.

All F-doped ZnO NPs absorb in the UV region with an absorption peak between 332 and 347 nm, due to the passage of the carriers of excess charge from the valence band to the conduction band. The maximum wavelength of F0, F1, and F2.5 ZnO NPs, for which XPS analysis has not detected an F concentration, increases as the  $R_h$  of suspended aggregates increases, as observed by Xu *et al.* for

undoped ZnO NPs with a dimension between 15 and 40 nm.<sup>156</sup> F5 and F7.5 ZnO NPs (effective F concentration of 1.5, and 1.7 % at, respectively) have a maximum at about 343 nm, as well as the same F concentration shift the maximum peak in F10, F15, and F20 ZnO NPs samples at about 333 nm. It is interesting to note that the increased concentration of doping agent causes a decrease in the absorption maximum, as observed by Singh *et al.* doping ZnO NPs with Mn and observing a blue shift from 366 to 352 nm, increasing the Mn concentration from 1 to 4%.<sup>157</sup> The same trend has also been observed by Hammad *et al.* for Co-doped ZnO NPs.<sup>158</sup>

Fluorescence spectra were recorded in the 400-800 nm range by exciting each sample at the maximum absorption wavelength as determined by UV-Vis measurements, which are 339, 343, 347, 343, 343, 333, 332, and 333 nm for F0, F1, F2.5, F5, F7.5, F10, F15, F20 ZnO NPs, respectively (Figure 3.8).



Figure 3.8. Fluorescence spectra of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs in methanol.

All emission spectra show a single band centered at about 530 nm that can be attributed to the presence of point defects, such as oxygen vacancies or interstitial oxygen. Indeed, point defects strongly affect the fluorescence properties of nanocrystals. The crystalline structures of ZnO NPs are characterized by various defects, which are directly related to emission peaks. An emission band between 500 and 520 nm is attributed to oxygen vacancies  $V_0$ ; a band between 550 and 560 nm to interstitial oxygen  $O_i$ ; a band between 400 and 440 nm to zinc vacancies  $V_{Zn}$  and interstitial zinc  $Zn_i$ .<sup>159–162</sup>

This band is often called the yellow-green band,<sup>163</sup> in fact, the suspensions of F-doped ZnO NPs illuminated with Wood light emit a strong yellow color (Figure 3.9).



Figure 3.9. F5 ZnO NPs suspension in methanol (A), F5 ZnO NPs suspension in methanol under the Wood light (B).

To quantify the effect of the doping agent on the emission properties of ZnO NPs, the relative quantum yield  $\Phi$  of each system has been calculated with respect to quinine sulfate monohydrate. The quinine sulfate monohydrate has been chosen as the standard because the absorption and emission peaks fall in the same range of those of F-doped ZnO NPs, as can be seen in Figure 3.10.



Figure 3.10. Comparison of the absorption (blue) and emission (red) spectra of F-doped ZnO NPs (solid lines) and of quinine sulfate monohydrate (dotted lines).

The quantum yield  $\Phi$  was calculated as:

$$\boldsymbol{\Phi} = \left(\frac{A_s}{A}\right) \left(\frac{F}{F_s}\right) \left(\frac{n}{n_s}\right)^2 \phi_s \tag{3.3}$$

where  $A_s$  is the absorption of the standard, A is the absorption of the sample, F is the integral of the area below the fluorescence peak of the sample,  $F_s$  is the integral of the area below the emission peak of the standard, n, and  $n_s$  are the reflective indices of the solvent in which the sample and the standard are solubilized, respectively, and  $\phi_s$  is the quantum yield of the standard ( $\phi_s = 0.59$ ).<sup>164</sup> The results obtained are reported in Table 3.3.

Comula	${\it \Phi}$
Sample	%
F0	$7 \pm 1$
F1	$19 \pm 1$
F2.5	$19 \pm 2$
F5	$22 \pm 2$
F7.5	$13 \pm 2$
F10	$7\pm2$
F15	$6\pm1$
F20	$5 \pm 1$

**Table 3.3.** Relative quantum yield for all F-doped ZnO NPs samples.

Relative quantum yield increases with the fluorine content up to the sample F5, which by XPS analysis has an atomic percentage of F on the NP surface of 1.5 % at, then decreases by further increasing the fluorine content, with quantum yields of F15 and F20 lower than that of the undoped sample. So, a minimum amount of dopant is not only sufficient but also optimal to improve the optical properties of ZnO NPs.

### 3.3.1 Optical properties of F-doped ZnO NPs at solid state

The energy of the band gap is an important parameter for the definition of the properties of a semiconductor. Tauc plots for the different F-doped ZnO NPs are shown in Figure 3.11, while the corresponding band gap values are reported in Table 3.4.



Figure 3.11. Tauc plot of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs.

Sample	$E_g$ eV		
F0	$3.31\pm0.01$		
F1	$3.31\pm0.01$		
F2.5	$3.32\pm0.03$		
F5	$3.27\pm0.01$		
F7.5	$3.27\pm0.01$		
F10	$3.17\pm0.02$		
F15	$3.20\pm0.05$		
F20	$3.18\pm0.01$		

Table 3.4. Band gap values obtained from the Tauc Plot for all F-doped ZnO NPs samples.

The band gap of sample F0, about 3.31 eV, is slightly lower than that of ZnO bulk (3.37 eV),<sup>17,110</sup> this can be attributed to the presence of defects in the crystalline structure of NPs. The values of samples F1 and F2.5 ZnO NPs are equal to that of sample F0 ZnO NPs, which confirms the results of XPS that do not detect the presence of F in these samples. For samples, F5 and F7.5 *Eg* is 3.27 eV, this decrease compared to previous sample values is due to the presence of the doping agent, 1.5 and 1.7 % at from XPS analysis, respectively. The presence of a doping agent creates a new valance band that causes a decrease in the band gap.<sup>165</sup> The band gap for samples F10, F15, and F20 is even lower, probably due to a greater concentration of F. These values agree with the shift of the maximum absorption peaks observed for F-doped ZnO NPs in suspension.

Finally, to assess whether F-doped ZnO NPs preserve their fluorescence properties even when dried, photoluminescence measurements were performed. All samples were excited using a 325 nm wavelength laser, and the emission spectra were collected in the 330-1000 nm range (Figure 3.12).



Figure 3.12. Photoluminescence spectra of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs,  $\lambda_{exc}$ =325 nm.

All samples have a well-defined peak at about 380 nm and a broad band between 560-590 nm. In addition, from the F7.5 to the F20 samples the presence of an additional peak at about 780 nm is detected. The first peak close to 400 nm could be due to the presence of defects such as  $V_{Zn}$  or  $Zn_i$ . The broad band, on the other hand, is also present in the emission spectra of suspended F-doped ZnO NPs, and it is the yellow-green band. The peak at about 780 nm may be due to the presence of the reaction by-product  $ZnF_2$  in the samples, according to XRD measurements.

Considering the yellow-green emission band, it can be observed that fluorescence intensity varies for different samples, even if no clear trend can be identified (Figure 3.13). The highest fluorescence intensity is observed for samples F2.5 and F5, it decreases for samples F7.5, F15, F0, and F10, is almost nothing for samples F1 and F20 ZnO NPs.



Figure 3.13. Photoluminescence spectra in the range of 450-700 nm of F0, F1, F2.5, F5, F7.5, F10, F15, and F20 ZnO NPs,  $\lambda_{exc}$ =325 nm.

This data cannot be compared with the relative quantum yield, but nevertheless highlights that sample F5 ZnO NPs has very good fluorescence properties. The comparison cannot be made because for the determination of relative quantum yield the area below the emission curve is considered and not the intensity, as has been done in this case, both because it would have been necessary to also perform measurements with the same instrument for the standard. For these reasons, ZnO NPs with a nominal content of fluorine of 5% (and an actual content of 1.5%) were exploited for the optimization of an F-doped ZnO NP film to be used as a transducer.

# **Chapter 4**

# Transducer: F-doped ZnO NP film

The F5 ZnO NPs, with a nominal content of fluorine of 5% (and an actual content of 1.5%), were proved to have the best optical properties among the F-doped ZnO NPs characterized, thus they were exploited for the optimization of a F-doped ZnO NP film to be used as a transducer. An undoped ZnO NP film was studied as well for sake of comparison.

The films have been studied both microscopically, by means of solid-state ellipsometry and X-Ray Reflectometry (XRR), and macroscopically, by means of optical, Atomic Force, and Raman microscopies. The microscopic and macroscopic analysis allowed the best support and the best conditions to obtain the nanostructured film to be identified, respectively.

Then, a protocol was established for the physical immobilization of the aptamer LA27, the biomolecule chosen as bioreceptor, on the nanostructured film and the interaction between the aptamer and the film in an aqueous environment was investigated.

### 4.1 Microscopic analysis

Films prepared with the spin-coating method employing different concentrations of NPs and speed rotation programs, as described in paragraph 2.2.2, were characterized by means of solid-state ellipsometry, and XRR. These measurements were carried out at the Institut Max von Laue-Paul Langevin (ILL) in Grenoble (France).

#### 4.1.1 Characterization of ZnO NP film on silicon supports through solid-state ellipsometry

F-doped ZnO NPs were recovered from the synthesis at 3 mg mL<sup>-1</sup> concentration (C4), and they were first used at this concentration when forming F5 and F0 ZnO films on silicon. Two rotation speeds were tested, 1000 rpm (P1) and 3000 rpm (P3). The supports thus obtained are shown in Figure 4.1.



**Figure 4.1.** Photo of C4\_P1\_F0 (**A**), C4\_P3\_F0 (**B**), C4\_P1\_F5 (**C**), C4\_P3\_F5 (**D**) ZnO NPs film on silicon supports.

A first qualitative analysis shows that the films obtained using the P1 program (A and C photos in Figure 4.1) are more heterogeneous than those obtained using the P3 program (B and D photos in Figure 4.1). To achieve a significant characterization of the film thickness, the measurements were performed on at least twenty different points of the support. Thickness average values are reported in Table 4.1.

Donosition condition	F0 Thickness	F5 Thickness	
Deposition condition	nm	nm	
C4_P1	$30 \pm 9$	$33 \pm 7$	
C4_P3	$13 \pm 1$	$19 \pm 1$	

**Table 4.1.** Thickness values for C4\_P1\_F0, C4\_P3\_F0, C4\_P1\_F5, and C4\_P3\_F5 ZnO NPs film on silicon supports.

Quantitative characterization confirms that both films obtained with 3000 rpm speed rotation are more homogeneous and thinner than those obtained with the lower speed rotation.

Keeping the speed rotation constant at 3000 rpm (P3), the effect of an NP concentration is tested on film properties, by analyzing samples obtained using suspensions at 3.0 (C4), 2.0 (C3), 1.0 (C2), and 0.5 (C1) mg mL<sup>-1</sup> NP concentration. The average thickness values are reported in Table 4.2.

Deposition condition	F0 Thickness	F5 Thickness
	nm	nm
C4_P3	$13 \pm 1$	$19 \pm 1$
C3_P3	$9 \pm 1$	$12 \pm 1$
C2_P3	$7 \pm 1$	$8\pm1$
C1_P3	$7 \pm 1$	$8 \pm 1$

**Table 4.2.** Thickness values for C4\_P3\_F0, C3\_P3\_F0, C2\_P3\_F0, C1\_P3\_F0, C4\_P3\_F5, C3\_P3\_F5, C2\_P3\_F5, and C1\_P3\_F5 ZnO NPs film on silicon supports.

Plots of thickness values as a function of the NP concentration used for film formation (Figure 4.2) clearly indicate that at small NP concentrations (0.5 and 1.0 mg mL<sup>-1</sup>) film thickness does not change with NP concentration, then it increases with increasing NP concentration.



**Figure 4.2.** Thickness values as a function of NP concentration, 0.5, 1.0, 2.0, 3.0 mg mL<sup>-1</sup> of F0 (blue triangles) and F5 (green rhombus) ZnO films at 3000 rpm (P3).

With the aim at obtaining as thinner as possible ZnO NP films, the lowest NP concentrations are selected, and the speed rotation was increased further to 4000 rpm (P4). Thickness average values

reported in Table 4.3 indicate that an increase in speed rotation does not reduce the thickness of the nanostructured film.

**Table 4.3.** Thickness values for C2\_P4\_F0, C1\_P4\_F0, C2\_P4\_F5, and C1\_P4\_F5 ZnO NPs film on silicon supports.

Deposition condition	F0 Thickness	F5 Thickness	
Deposition condition	nm	nm	
C2_P4	$8 \pm 1$	9 ± 1	
C1_P4	$7 \pm 1$	$7 \pm 1$	

These initial analyses show that a good NP film would require low F-doped ZnO NPs suspension (0.5 or 1.0 mg mL<sup>-1</sup>) and high-speed rotation (3000 or 4000 rpm). Nevertheless, the analysis of the film through solid-state ellipsometry can lead to a significant error in the determination of the characteristics of the film itself; both because it is a point analysis, and because it allows obtaining only the thickness of the deposited layer. Moreover, both the silicon and glass supports have a certain thickness. This thickness causes, in the case of glass supports, the reflection of the radiation used during ellipsometry measurements on the lower face of the support. This problem makes it impossible to perform solid-state ellipsometry measurements on the nanostructured films on glass supports. For this reason, a better analysis of the choice of the best support to use for the deposition of F-doped ZnO NPs was performed through XRR measurements.

### 4.1.2 The choice of the best support: XRR analyses

Since low concentrations (C1=0.5, or C2=1 mg mL<sup>-1</sup>) of F-doped ZnO NP suspensions and a speed rotation of P3 = 3000 rpm seemed to be the optimal conditions to obtain a thin and homogenous nanostructured film on silicon support, XRR measurements were initially performed on the sample C1\_P3\_F0 on a silicon support.

In Figure 4.3 A Reflectivity *R* is plotted as a function of the Scattering Vector  $q_z$ , and the resulting Scattering Length Density *SLD* profile is reported in Figure 4.3 B.



**Figure 4.3.** *R* as a function of  $q_z$  (red circles) and the best fit to the data (black line) (**A**); Scattering length density (SLD) plotted as a function of distance Z (red line) (**B**) of C1\_P3\_F0 ZnO NP film on silicon support.

Considering the silicon support consists of  $Si/SiO_2$  and fitting the experimental data the values of thickness *T*, roughness *R*, and scattering length density *SLD* reported in Table 4.4 were obtained.

Table 4.4. Thickness *T*, roughness *R*, and scattering length density *SLD* obtained by fitting XRR data.

Lovon	Т	R	<i>SLD</i> 10 <sup>-4</sup> nm <sup>-2</sup>	
Layer	nm	nm		
Si		$0.50\pm0.07$	$2.00\pm0.02$	
$SiO_2$	$2.11\pm0.01$	$0.50\pm0.09$	$1.90\pm0.02$	
ZnO	$1.54\pm0.06$	$0.36\pm0.01$	$1.37\pm0.02$	

The SLD profile and the higher value of the thickness of  $SiO_2$  compared to that of ZnO NPs show that the surface of the support is not completely covered by the film of NPs. So, although the measurements of solid-state ellipsometry showed that low concentrations of NP solutions were sufficient to obtain a film covering the entire support, XRR analysis indicates that nonetheless, support coverage was incomplete. For this reason, for the choice of the best support to be used, silicon or glass, nanostructured films obtained from 3 mg mL<sup>-1</sup> NP suspensions (C4) were characterized.

Figure 4.4 shows XRR data obtained for F0 and F5 ZnO NP films on both silicon and glass supports.



**Figure 4.4.** XRR profiles of C4\_P3\_F0 on silicon support (blue line), and on glass support (pink line) (**A**), and XRR profiles of C4\_P3\_F5 on silicon support (green line), and on glass support (purple line) (**B**).

A qualitative analysis of the comparisons of the XRR data of the films obtained with the same preparation conditions on the two different supports shows that the films obtained are quite different. The different positions where the curve reaches an almost constant value of reflectivity of the films deposited on silicon and glass are indices of the presence of a different number of layers. Moreover, an increase in the reflectivity of the films deposited on silicon compared to those deposited on glass at intermediate values of  $q_z$  indicates a greater presence of material on the support. This being considered, the XRR data of the nanostructured films on glass support were fitted considering a single layer of F-doped ZnO NPs, while for those on silicon supports, a two-layer model was used (Figure 4.5, and Table 4.5).



Figure 4.5. XRR experimental data (circles) and the best fit (black lines) of C4\_P3\_F0\_on silicon support (A), C4\_P3\_F0\_on glass support (B), C4\_P3\_F5\_on silicon support (C), and C4\_P3\_F5\_on glass support (D). Corresponding SLD curves are shown in the inserts.

C 1 -	τ	Т	R	SLD 10 <sup>-4</sup>	
Sample	Layer	nm	nm	nm <sup>-2</sup>	
	Si		$0.2 \pm 0.1$	$1.89\pm0.09$	
C4 E0 on silicon support	SiO <sub>2</sub>	$1.45\pm0.02$	$0.28\pm0.01$	$1.98\pm0.08$	
C4_F0_on sincon support	ZnO_1	$7.26\pm0.05$	$1.68\pm0.06$	$3.16\pm0.07$	
	ZnO_2	$15 \pm 2$	$5.9\pm0.8$	$2.0 \pm 0.1$	
C4 E0 on glass support	Glass		$0.36\pm0.01$	$4.29\pm0.04$	
C4_10_011 glass support	ZnO	$10.5\pm0.1$	$2.42\pm0.05$	$3.22\pm0.05$	
	Si		$0.1 \pm 0.1$	$1.89\pm0.09$	
C4 E5 on silicon support	SiO <sub>2</sub>	$0.4 \pm 0.2$	$0.25\pm0.03$	$2.15\pm0.05$	
C4_F5_on sincon support	ZnO_1	$11.2\pm0.2$	$2.5\pm0.2$	$3.22\pm0.06$	
	ZnO_2	$14 \pm 3$	8.6 ± 1.4	$2.4\pm0.2$	
C4 E5 on glass support	Glass		$0.10\pm0.06$	$2.09\pm0.01$	
C+_1'J_011 glass support	ZnO	$13.7\pm0.1$	$3.56\pm0.05$	$2.52\pm0.01$	

**Table 4.5.** Results of the fit to the XRR data concerning thickness *T*, roughness *R*, and scattering length density *SLD* of C4\_P3\_F0 and C4\_P3\_F5 on silicon and glass supports.

The data show that for both undoped and doped ZnO NPs two layers of different thickness and roughness are obtained on silicon as support, while a single layer is on the glass support. For both undoped and doped ZnO NPs the first layer, closer to the surface of the support, is thinner and more homogeneous than the second one. In addition, the thickness of the second layer values obtained through the XRR fitting agrees with the thickness values obtained through the solid-state ellipsometry. In the case of glass supports, instead, a single layer with a thickness of about 10.5 and 13.7 nm, and a roughness of about 2.42 and 3.56 nm for undoped and doped ZnO NP films are obtained. Therefore, the formation of two layers of ZnO NPs of heterogeneous thickness and roughness cannot be controlled in the case of deposition on silicon support, instead, a more homogenous nanostructured film is obtained in the case of deposition of F-doped ZnO NPs to be used as an optical transducer for the realization of an aptasensor.

# 4.2 Macroscopic analysis

Optical Microscopy, Atomic Force Microscopy (AFM), and Raman microscopy measurements were performed in collaboration with Dr. Stefano Luigi Oscurato and Dr. Marcella Salvatore at the Physic Department "Ettore Pacini" of University of Naples Federico II with the aim at determining the coverage of the glass support and therefore the best protocol, in terms of ZnO NP suspensions concentrations and speed rotation, for film formation.

## 4.2.1 The choice of the best deposition conditions

The above-described characterization has shown that microscopic properties of the films do not depend on the kind of ZnO NPs, F0 or F5, therefore, a comprehensive analysis of F0 films was performed, by changing NP concentration (C1 = 0.5, C4 = 3.0, C5 = 6.0, and C6 = 9.0 mg mL<sup>-1</sup>) and for each concentration the speed rotation (P1 = 1000, P2 = 2000, P3 = 3000, and P4 = 4000 rpm); then the best conditions were used for the preparation of a film composed of F5 ZnO-NPs.

All the films have been analyzed under an optical microscope, from which it is possible to determine the degree of coating. For example, in Figure 4.6 two images of the C4\_P1 and C6\_P1 systems are shown.



Figure 4.6. Optical image of C4\_P1 (A), and C6\_P1 (B) on glass supports, scalebar: 100 µm.

The degree of coverage is determined by comparing the area occupied by the aggregates visible in the image (highlighted by the red circle in Figure 4.6 B) with the total area of the image analyzed. It has been calculated by analyzing at least four images from different areas of the film, using the ImageJ

software. In Figure 4.7 the degree of coating of the films was reported as a function of the concentration of the ZnO NP suspension used for each speed rotation used.



Figure 4.7. Degree of coating as a function of the concentration of ZnO NP suspension.

Using a NP suspension at a very low concentration ( $C1 = 0.5 \text{ mg mL}^{-1}$ ) a low degree of coating is obtained in agreement with XRR results. While at low NP concentrations, the effect of rotation speed is neglectable, as the concentration of NPs increases, it affects the degree of coating, with fast rotations (3000 and 4000 rpm) determining a low coverage and slow rotations (1000 and 2000) determining an increasing coating of the glass support. Based on these observations, a film using a very concentrated NP suspension, C7= 36 mg mL<sup>-1</sup>, and a low-speed rotation, P1 = 1000 rpm was prepared and characterized.

Comparison of optical microscopy images of this film with those recorded on films obtained from lower NP concentrations (Figure 4.8) suggests that coverage increases, but the glass support appears to be covered by increasingly larger aggregates, as the NP concentration of employed suspensions increases.



**Figure 4.8.** Optical image of C4\_P1 (**A**), C6\_P1 (**B**), and C7\_P1 (**C**) on glass supports, scalebar: 100 μm.

The formation of these aggregates is likely due to the drying of NPs and subsequent solubilization of NP carried out to obtain suspensions with concentrations higher than 3 mg mL<sup>-1</sup>. With the aim at avoiding the deposition of ZnO NP aggregates, the suspensions of ZnO NPs were sonicated with a tip sonicator at 60% power for 5 minutes before deposition on the glass support. Optical microscopy (Figure 4.9) indicates a significant improvement in the film: an almost complete support coverage is achieved.



**Figure 4.9.** Optical image of C7\_P1 before (**A**), and after (**B**) sonication on glass supports, scalebar: 100 μm.

This improvement is also highlighted by the observation of the films under the Wood light, as shown in Figure 4.10.



Figure 4.10. Photo of C7\_P1 before (A), and after (B) sonication on glass supports under Wood light.

To confirm these results, the C7\_P1 film obtained by sonicating the starting suspension was further characterized by means of AFM and Raman microscopy.

The morphology of the film was determined by AFM analysis (Figure 4.11).



Figure 4.11. 2D AFM image, 20x20 µm (A), and 3D AFM image, 20x20 µm (B) of C7\_P1 film.

AFM images indicate a complete coverage of the support. Moreover, the presence of areas with different heights, lower than about 70 nm and higher than about 225 nm are present, proves that the resulting film is quite wrinkled.

By means of Raman microscopy (Figure 4.12 A), the Raman spectrum of the film was obtained (Figure 4.12 B) and compared with that of dried ZnO NPs (Figure 4.12 C). The overlap of the peaks of the two spectra, verified in different points of the support, confirms the exclusive presence of ZnO NPs on the support that covers it entirely.



**Figure 4.12.** Raman image of C7\_P1 film (**A**), Raman spectrum of C7\_P1 film (**B**), and Raman spectrum of ZnO NPs powder.
### 4.3 Design of the aptasensor

The realization of an aptasensor, or more generally of a biosensor, involves the interaction of the biomolecule with the transducer, which in this case is the film of F-doped ZnO NPs.

To assess the effect of the film features on its interaction with the biomolecule, the aptamer LA27 was bound not only on the optimized film C7\_P1\_sonicate but also on film C4\_P1. Moreover, aptamer interaction with undoped and doped NP has been investigated as well using the C7\_P1\_sonicate\_F5 film.

LA27 was deposited on the films through drop-casting, as described in paragraph 2.3.2. Then, to assess whether the aptamer is stably bound to the ZnO NP films, they were placed in a water bath for different times, 1, 3, or 6 hours, the water was recovered, and the aptamer content, eventually dissociated from the nanostructured film, determined by means of UV-Visible spectroscopy.<sup>166–168</sup> Through UV-Visible spectroscopy, it is also possible to verify the possible dissociation of ZnO NPs caused by interaction with the LA27 aptamer or dispersion in water. The UV-Visible spectrum of the water in contact with the C4\_P1 film reported in Figure 4.13 shows a main peak at 260 nm typical of DNA oligonucleotides, while the absence of absorption peaks between 332 and 347 nm rules out any significant detachment of ZnO NPs.



Figure 4.13. UV-Visible spectrum of contact water of the C4\_P1 system after 1 hour of contact.

Considering a deposition of 300  $\mu$ L of a 20  $\mu$ M LA27 aptamer solution, and 3 mL of water for the redispersion of the lyophilized sample, for each film, a percentage of aptamer detachment was determined and reported in Table 4.6.

Sampla	Contact time	Detachment of LA27
Sample	h	%mol
C4_P1	1	90
	3	90
	6	99
C7_P1_sonicate	1	45
	3	60
	6	60
C7_P1_sonicate_F5	1	35
	3	65
	6	65

Table 4.6. Percentage of the detachment of the LA27 from nanostructured films.

From the analysis of Table 4.6 an almost complete detachment emerges for the system in which the ZnO NPs only partially cover the support (C4\_P1), while higher aptamer retention is achieved when the support is completely covered (C7\_P1\_sonicate). Moreover, by comparing the systems obtained with undoped and doped NPs minimal detachment changes are observed. These data suggest lower kinetic detachment of the LA27 aptamer from F-doped ZnO NP films with respect to other biomolecules and film.<sup>169</sup>

Preliminarily, it is possible to propose the physical immobilization as a potential deposition strategy of LA27 aptamer on ZnO nanostructured films, since the interaction between LA27 and the surface with a high coating is quite stable and is not affected by the nature of both doped and undoped NPs.

# **Conclusion Section B**

The aim of this part of the Ph.D. project was to optimize the optical properties of ZnO NPs by atomic doping with fluorine and to develop a protocol to produce nanostructured films acting as an optical transducer matrix able to interact with the aptamer LA27. The ZnO NP optimization was necessary for the proper functioning of a biosensor because the transducer layer has the task of converting the interaction between the biomolecule and the analyte into a measurable signal.

ZnO is a semiconductor whose thermodynamically stable crystalline structure is wurtzite, which is characterized by the presence of defects such as oxygen vacancies  $V_o$ , zinc vacancies  $V_{Zn}$ , zinc interstitial Zn<sub>i</sub>, and oxygen interstitial O<sub>i</sub>. These defects are directly related to the optical properties of ZnO NPs, and their presence can be appropriately modulated by varying the NP synthesis conditions and/or through appropriate doping at the atomic level, such as insertion of fluorine F atoms in the crystalline structure.

ZnO NPs were synthesized by a wet-phase method in reflux at 60 °C, using ammonium hydrogen difluoride as doping agent at different concentrations, from 0 to 20 % at. In all the cases, crystalline ZnO NPs, in the stable form of wurtzite, were synthesized, indicating that the presence of the doping agent does not alter the crystalline structure of the ZnO. However, at high F concentrations, from 7.5 to 20 % at, ZnF<sub>2</sub> is formed as a reaction by-product probably determining the presence of a low-intensity peak (at 780 nm) in the photoluminescence spectra of the corresponding NP systems.

To determine the actual concentration of the species within the doped ZnO NPs, XPS measurements have been performed. From the analysis of the XPS spectra, it was possible to quantify in all the cases the atomic percentage of Zn and O, differentiating between the O linked to Zn and that linked to C. The presence of fluorine was detected only for the highest F concentration samples, with atomic concentrations of 1.5 % at. for sample F5, 1.7 % at. for F7.5, 7% at. for F10 and F20, and 8% at. for sample F15, respectively.

Combined TEM and DLS analysis indicated that all ZnO NPs have an irregular shape and a significant tendency to aggregate. By considering the single NP as a sphere, the mean radius was determined through a statistical analysis of TEM images, obtaining a value of about 3 nm for all the synthesized NPs.

The concentration of the doping agent, the morphology, and the size can significantly affect the optical properties of ZnO NPs. For this reason, and for sake of comparison with the properties of the corresponding ZnO NP films, the optical properties of all ZnO NPs were studied both in solution and at the solid state and were quantified both as relative quantum yield and band gap values.

When suspended in methanol, ZnO NPs have an absorption peak with a maximum between 332 and 347 nm, and an emission band centered at about 530 nm, the so-called yellow-green band due to the presence of  $V_o$  or  $O_i$ . The same emission band is also present in the photoluminescence spectra, together with a peak at about 780 nm, already mentioned above, and a peak at about 380 nm, probably due to  $V_{Zn}$  or Zn<sub>i</sub>. The band gap values obtained are in accordance with the fluorine concentrations determined by XPS measurements. In fact, the band gap for samples F0, F1, and F2.5, for which the concentration of fluorine was found to be zero, is 3.31 eV close to the value of bulk ZnO (3.37 eV). The minimum difference between the two values may be due to the presence of defects in the crystalline structure of ZnO NPs do not present in the bulk material. The band gap for samples F5 and F7.5 is 3.27 eV, and for F10, F15, and F20 samples is 3.17 eV. The band gap decreases as the fluorine concentration increases. On the other hand, the system with the best optical properties in terms of relative quantum yield, and no alteration of the other physico-chemical properties with respect to the undoped NPs, is the F5 sample, with a relative quantum yield value of about 22%.

Therefore, this system was used for the deposition of nanostructured films on either glass or silicon, using the undoped system, F0, as a comparison.

By means of solid-state ellipsometry and XRR analyses, it was determined that the best support for the deposition of ZnO NPs is glass, while a thorough analysis by optical microscopy indicated that high concentrations of NP dispersions and low rotational speeds lead to the formation of a film that completely covers the support, is satisfactorily homogenous and scarcely wrinkled. Moreover, AFM and Raman microscopy confirmed the film was composed of ZnO NPs.

Finally, the aptamer LA27 was physically immobilized on the ZnO NP film, and the success of the immobilization strategy was evaluated by means of UV-Visible spectroscopy, with the higher degree of support coverage determining a stronger interaction between the aptamer and the film. It was also demonstrated that this interaction is not affected by the presence of the doping agent, since a detachment percentage of about 60% occurs after 6 hours in an aqueous environment for both the films made up of undoped and doped NPs.

This work proposed a oerspective platform for the realization of an optical aptasensor. From the development of the best F-doped ZnO NP system to the optimization of the interaction between the nanostructured transducer and the recognition biomolecule.

# Appendices

# **Appendix A – Scattering Techniques**

The term "scattering" in physics means a variation in the direction of motion of a particle due to collision with another particle.<sup>170</sup> Scattering techniques, therefore, exploit this property of particles to investigate structure and dynamics of materials;<sup>171</sup> with the great advantage being non-invasive and possibly non-destructive.

Scattering techniques use a variety of probing radiation, such as visible light, X-rays, and neutrons to analyze matter in a large range of length scales, from Angstrom to microns.<sup>171,172</sup> The theory governing visible light, X-rays, or neutron scattering is the same, even though visible light and X-rays interact with electron present within the sample, while neutrons interact with nuclei. The difference is in the wavelength, in fact, the wavelength of visible light is in the order of hundreds of nanometers, X-rays in the 0.1-0.2 nm range, and neutrons in the 0.5 nm range.<sup>171,173</sup>

### A.1 Basic scattering theory

Although scattering techniques have developed separately, the fundamental equations are the same formalism regardless of the source used.

A scattering experiment can be described as a plane wave with a defined intensity, wavelength, and direction, incoming on the sample. Because of the interaction between the sample and the incident plane wave, a scattered wave is emitted, which can be described as a plane wave, in the condition of detection of the diffuse wave at a large distance from the sample. The interaction can be of two types: *(i)*. inelastic when the scattered wavelength has a different energy that the incident, *(ii)*. elastic, the scattered wavelength has the same energy as the incident.<sup>174</sup>

The interaction between the incident  $k_i$  and scattered  $k_s$  waves generates a vector, known as scattering vector q, which can be expressed as follows:<sup>175</sup>

$$q = \frac{4\pi n}{\lambda} \sin\theta \tag{1}$$

where *n* is the reflective index of the medium,  $\lambda$  is the wavelength, and  $\theta$  is the half of the angle forms between the projection of the incident wave and scattered wave (Figure A.1).



Figure A.1. Schematic representation of a scattering experiment.

The measurable parameter in a scattering experiment is the intensity (I), that is related to the product of two factors:<sup>171</sup>

$$I(q) \sim S(q) \cdot P(q) \tag{2}$$

where S(q) is the structure factor, and P(q) is the form factor.

The structure factor S(q) represent the spatial arrangement of the scattering elements, and it is expressed as follow:<sup>173</sup>

$$S(q) := \frac{1}{N} \left\langle \sum_{j=1}^{N} \sum_{k=1}^{N} e^{iq \cdot r} jk \right\rangle$$
(3)

where N is the scatters number and  $r_{jk} := r_j - r_k$  is the distance between two scatters.

The form factor P(q), instead, is related to the size and shape of scattering particles. For example, for solid sphere with *R* radius, the P(q) expression is:<sup>171</sup>

$$P(q,R) = \int_{0}^{\infty} \frac{3}{(qR)^{3}} \left[ \sin(qR) \cdot (qR) \right]$$
(4)

However, the intensity measured in a scattering experiment depends not only on the structure of the individual scattering elements and their interaction, but also from the interaction between the probe radiation and the matter expressed by the scattering length b:<sup>173</sup>

$$I(q) \approx \frac{d\sigma}{d\Omega} \approx b^2 \tag{5}$$

III

where  $\frac{d\sigma}{d\Omega}$  is the differential scattering cross section, and  $\Omega$  is the scattering solid angle. The differential scattering cross section is the probability that the incident wave is scattered by the sample within a certain solid angle,  $\Omega$ , and it is proportional to  $b^2$ . The scattering length *b* can have either positive or negative values, where a negative value signifies a change of the scattering phase.<sup>173</sup> Depending on the interaction with the radiation, *b* varies with the change of the probe used, in the following paragraphs will be explained for the three different probes.

# A.2 Light scattering: DLS

Why do clouds appear white and the sky blue? Because of the interaction of light with matter. The visible light spectrum is in the range of 400-800 nm, that is, from violet to red. When the scattering particles are smaller than the wavelength of the incident light, the radiation spread is equal in all directions, Rayleigh scattering, which is why the sky is blue, instead the clouds appear white because the particles that constitute them have bigger dimensions than the wavelength of the incident light, therefore the diffusion of the radiation is different in the three directions, scattering of Mie. If this is true, why does the sky appear red at sunset? Because in addition to the size of the scattering particles, the angle of incidence is also important. These observations of daily life make it clear how important and exploitable is visible light for the characterization of colloidal systems.

In a scattering experiment, a polarized light in one direction, for example z, is used as a source. The electric field of the incident wave  $E(t,x) = E_0 cos(\omega t - kx)$  causes the scattering center to oscillate. This induced dipole oscillates with the same frequency, which results in the emission of a scattered spherical wave.<sup>173</sup> Since the intensity is measured and not the electric field it is necessary to use the following relationship:<sup>173</sup>

$$I = \varepsilon_0 c \left\langle E^2 \right\rangle \tag{6}$$

Whereas, moreover, the ratio of the scattered intensity to the incident intensity  $\frac{I_s}{I_0}$ , and  $c = \lambda v$ , the differential scattering cross-section is obtained:<sup>173</sup>

$$\frac{d\sigma}{d\Omega} = \frac{I_s}{I_0} R^2 = \frac{16\pi^4}{\lambda^4} \alpha^2 \sin^2\phi \tag{7}$$

IV

where R' is the direction which is tilted against the direction of the polarization of the incident wave by an angle  $\phi$ , and  $\alpha$  is the polarizability. The polarizability, however, is not a measurable parameter, therefore a relationship with the refractive index *n* is used to determine it:<sup>173</sup>

$$\alpha = \frac{1}{4\pi} \frac{V}{N} (n^2 - 1) \tag{8}$$

where *N* is the number of scattering particles in a volume *V*. For a solute-solvent system under diluted conditions, the term in brackets becomes  $(n^2 - n_0^2) = (\Delta \alpha)^2$ , with  $(n^2 - n_0^2) \approx 2n_0 \left(\frac{dn}{dc}\right)c$ . Therefore, the differential cross-section of a point scatter is:<sup>173</sup>

$$\frac{d\sigma}{d\Omega} = b^2 = \frac{16\pi^4}{\lambda^4} (\Delta\alpha)^2 \sin^2\phi \tag{9}$$

With the technique of Dynamic Light Scattering (DLS) it is also possible to determine the dynamic of the systems, as the instantaneous intensity of the sample is measured in the order of nanoseconds is considered, extremely shorter than the characteristic motion times of the particles in solution. The instantaneous intensity values are used to construct the intensity autocorrelation function  $g^2(t) = \langle I_s(0) \cdot I_s(t) \rangle$ , from which the diffusion coefficient *D* can be determined. In particular, *D* is obtained by converting  $g^2(t)$  in the field autocorrelation function  $g^1(t) = \langle E_s(0) \cdot E_s(t) \rangle$  through Siergert equation:<sup>175–177</sup>

$$g^{2}(t) = 1 + \beta \left| g^{1}(t) \right|^{2}$$
(10)

where  $\beta$  is the coherence factor of scattered radiation, and depends on geometric factor, characteristic of the instrumental apparatus used for the measurements. For short time intervals (ms)  $E_s(t) \sim E_s(0)$ so  $g^{I}(t) = 1$  while, considering  $\beta = 1$ ,  $g^{2}(t) = 2$ . Instead, for long time intervals (s)  $g^{I}(t) = 0$  and  $g^{2}(t) = 1$ .<sup>175–177</sup>

The expression of  $g^{1}(t)$  is closely dependent on the characteristics of the systems. For example, in the ideal case of particles dispersion having all the same shape and dimensions:<sup>175–177</sup>

$$g^{I}(t) = e^{-\Gamma t} \tag{11}$$

If, however, the particles have different size:

V

$$g^{I}(t) = \int_{0}^{\infty} G(\Gamma) e^{-\Gamma t} d\Gamma$$
(12)

with  $\Gamma = D \cdot q^2$ .

Moreover, if the continuous medium hypothesis is satisfied, the interaction between the particles is negligible and the particles are spherical, through the Stokes-Einstein equation is possible to determine the hydrodynamic radius  $R_h$ :<sup>175–177</sup>

$$D = \frac{kT}{6\pi\eta R_h} \tag{13}$$

where k is the Boltzmann constant, T the temperature, and  $\eta$  is the viscosity of the medium.

#### A.2.1 Instrument

The DLS measurements were performed using a home-made instrument, composed of a Photocor compact goniometer, an SMD 6000 Laser Quantum 50 mW light source operating at 532.5 nm, a photomultiplier (PMT-120-OP/B), and a correlator (Flex02-01D) from Correlator. Com.

#### A.2.2 Sample preparation and experimental set-up

The experiments were carried out at a constant temperature (25.0 $\pm$ 0.1) °C using a thermostatic bath, and at a scattering angle  $\theta$  of 90°, with the only exception of amine coated CeO<sub>2</sub> NPs analyzed at 120°. In any case, reflective index and viscosity of the solvent used were considered (Table A.1).

Solvent	Deflective index	Viscosity
	Kenecuve muex	Р
Chloroform	1.448	0.0053
Methanol	1.329	0.0054
Water	1.336	0.0089

Table A.1. Reflective index and viscosity at 25 °C for chloroform, methanol, and water.

# A.3 X-ray scattering

X-rays, as in the case of visible light, interact with the electrons in the sample, and as the scattering of electromagnetic radiation on the matter depends on the frequency. The difference with light

scattering is that it goes from low to high frequencies, so from Rayleigh to Thomson scattering, so a frequency-dependent atomic structure factor or scattering length is obtained.<sup>173,174,176,177</sup> The scattering cross-section for Thomson scattering for a single electron, considering an unpolarized incident electron field, can be written as:<sup>173</sup>

$$\frac{d\sigma}{d\Omega} = r_{el}^2 \frac{1 + \cos^2\theta}{2} \tag{14}$$

where  $r_{el}$  is the electron radius. When considering Small Angle X-ray Scattering (SAXS), with correspondingly small values of  $\theta$ , the equation 14 can be written as:<sup>173</sup>

$$\frac{d\sigma}{d\Omega} = r_{el}^2 Z^2 \tag{15}$$

with Z number of electrons in the atom. Therefore, considering the equation 9, the scattering length is: $^{173}$ 

$$b = r_{\rho l} Z \tag{16}$$

In the X-ray scattering, each electron in the scattering sample scatters like an induced dipole scatterer and emits X-rays. X-rays scattered by different electrons arrive at the detector with different phases. These phase differences must be considered when amplitudes of the scattered waves are added together to calculate the resultant scattered amplitude. In the case of the Born approximation, the field incident on a scatter is not perturbated by the presence of the other scatterers, the intensity can be calculated as in the case of a single electron. Nevertheless, scattering studies are most useful when the scattered waves have a definite relationship with each other. In this case, the scattering amplitude A(q) is:<sup>177</sup>

$$A(q) = (I_0)^{1/2} F(q)$$
(17)

where  $I_0$  is the incident intensity, and F(q) is a structure factor. For a continuous charge distribution  $\rho(r)$ , F(q) can be written:<sup>177</sup>

$$F(q) = \int_{V} \rho(r) e^{i(q \bullet r)} dV$$
(18)

Therefore, the scattering intensity I(q) is the product of the scattering amplitude A(q) and its complex conjugate  $A^*(q)$ :<sup>177</sup>

$$I(q) = A(q) \cdot A^{*}(q) = |A(q)|^{2} = F(q)F^{*}(q)I_{0} = I_{0}|F(q)|^{2}$$
(19)

In this discussion, only the elastic scattering was considered, which, in the case of X-rays, represent the strongest component. However, X-rays can indeed transfer a small amount of energy to the electron. This phenomenon is known as Compton-modified scattering.<sup>178</sup>

# A.3.2 Structure determination: from Wide Angle X-ray Scattering (WAXS) to Small Angle X-ray Scattering (SAXS)

By combining the definition of q (equation 1) with the Bragg law (equation 20, Figure A.2), it can be demonstrated that the scattering vector is inversely proportional to the characteristic length of the sample d.

$$\lambda = 2dsin\theta \tag{20}$$



Figure A.2. Schematic representation of Bragg law.

This relation demonstrates how it is possible to investigate from the crystalline structure of a single particle to the structure of an aggregate of several particles to the variation of the angle.

The WAXS technique specifically relies on the analysis of Bragg peaks scattered to wide angles. The diffraction pattern generated allows for the determination of the phase structure, and the crystallite size. The crystallite size is determined through the Debye-Scherrer equation:<sup>179</sup>

$$D = \frac{0.9\lambda}{\beta \cos\theta} \tag{21}$$

VIII

where *D* is the crystalline size (nm),  $\beta$  is the full width of the direct line at half of the maximum intensity,  $\lambda$  is the X-ray wavelength of Cu K<sub> $\alpha$ </sub> = 0.154 nm, and  $\theta$  is the Bragg angle.

Passing from wide to small angle, information about the structure of particles in solution can be obtained. In fact, the ordered structures, such as body-centered cubic (BCC), face-centered cubic (FCC), hexagonal, etc., can be determined through SAXS measurements. In the presence of ordered structures, the angular dependence of the scattered intensity shows specific Bragg patterns. The relevant positions of Bragg peaks are unique for different structures:<sup>171</sup>

$$d = \frac{2\pi}{q} \tag{22}$$

#### A.3.2 WAXS: sample preparation, instrument, and experiment set-up

WAXS measurements were conducted in CEA-Leti Research and Technology Institute (Grenoble, France) laboratories, using the Empyrean instrument from Malvern. The X-ray source is Cu,  $\lambda$ =1.54 Å, 45 kV, and 40 mV. Powders of all synthetic NPs, both CeO<sub>2</sub> and ZnO, were analyzed in the 20 range between 10 and 80 degrees. The patterns of coated CeO<sub>2</sub> NPs are reported in the range of 10-60°, while those of F-doped ZnO NPs in the range of 20-80° to better highlight the characteristic peaks of the two crystals.

#### A.3.3 SAXS: sample preparation, instrument, and experimental set-up

SAXS analysis was performed at the beamline B21 of the Diamond Light Source (Didcot, UK). The beamline configuration was with a beam energy of 12.4 keV, a sample-to-detector distance of 3.7 m. These configurations allowed to collect the data for the scattering vector in the range between 0.0026-0.34 Å<sup>-1</sup>. All samples of coated CeO<sub>2</sub> NP superlattices were analyzed in water at 25 °C. The form factor of a sphere was subtracted from samples where it was considered necessary, to better highlight the Bragg peaks.

# A.4 Neutron scattering: Small Angle Neutron Scattering (SANS)

Neutrons are non-charged particles having mass of 1.675·10<sup>-27</sup> kg, spin of ½ and a magnetic moment of -1.9132 nuclear magnetons. Due to their spin and magnetic moment, neutrons can interact in two different ways with the nuclei, giving rise to the nuclear and magnetic scattering. The nuclear scattering involves complex nuclear interactions between the spin states and the magnetic moments

of the interacting nucleus and the neutrons. Furthermore, neutrons are differently scattered from isotopes of a same element since they present different nuclear properties.<sup>174,177</sup>

Neutrons can be produced in nuclear reactors, resulting from chain reactions involving <sup>235</sup>U fission, spallation sources, where accelerated protons or electrons hit a heavy nucleus, can be as well exploited for neutrons production. In both type of sources, neutrons are obtained with a very high energy (hot neutrons) and need to be thermalized to be used for the scattering experiments. Thus, the produced neutrons are scattered by a moderator, which is composed by light nuclei. The inelastic collisions between the neutrons and moderator atoms reduce the neutron energy. If the moderator is enough large, the equilibrium condition at the moderator temperature for the produced neutrons energy and wavelengths can be controlled. According to their temperature, neutrons can be classified in hot, thermal, or cold neutrons. Among them, thermal neutrons are characterized by energy corresponding to about 20 meV, which corresponds to a wavelength close to that of X-rays, but with much less energy. The energies associated to atomic motions arising from vibrations, rotations, and translations in solids and liquids are like thermal neutron energies. Thus, thermal neutrons represent an effective probe to have both structural and dynamic information about the sample under investigation. Furthermore, neutron scattering is particularly suitable for the characterization of biological systems, since the high energy associated to X-rays might causes sample damage, unless short time exposure is used.174,177

The interaction between neutrons and nuclei is quantified by the scattering length b, but, in contrast to light and X-ray scattering, the calculation of neutron b is difficult. As mentioned above, in fact, b depends in an unsystematic way on the nucleus into which the neutrons are dispersed. Moreover, dramatic variations in b are found for an isotopic replacement. This, and the spin dependence are visible from the differential cross-section expression:<sup>173</sup>

$$\frac{d\sigma}{d\Omega}(q) = \sum_{j,k=1}^{N} \left\langle b_j b_k e^{iq \cdot r} j_k \right\rangle$$
(23)

the values of  $b_j b_k$  depend on the given isotope and spin state. Since the individual scattering length are independent:

$$j \neq k : \left\langle b_{j} b_{k} \right\rangle = \left\langle b_{j} \right\rangle \left\langle b_{k} \right\rangle = \left\langle b \right\rangle^{2}$$
(24)

$$j = k : \left\langle b_j b_k \right\rangle = \left\langle b_j^2 \right\rangle = \left\langle b^2 \right\rangle$$
(25)

Х

which leads to:

$$\left\langle b_{j}b_{k}\right\rangle = \left\langle b\right\rangle^{2} + \delta_{jk}\left(\left\langle b^{2}\right\rangle - \left\langle b\right\rangle^{2}\right)$$
 (26)

Assuming that the spin is independent to the position of the scatter element:

$$\frac{d\sigma}{d\Omega}(q) = \left\langle b \right\rangle^2 \sum_{j,k=1}^N \left\langle e^{iq \cdot r} jk \right\rangle + N\left(\left\langle b^2 \right\rangle \cdot \left\langle b \right\rangle^2\right) \tag{27}$$

The first term of equation 27 represent the coherent part of the scattering intensity. This describes the scattering from nuclei which have the same  $\langle b \rangle$ , includes all the interference terms, and thus it contains the entire structural information. With the coherent scattering, collective properties can be study, such as the spatial arrangement of elements. The second term, instead, is the incoherent part caused by the disorder of isotopes, it is important to study the behavior of individual scatterers, such as their self-diffusion.<sup>173</sup>

The enormous variation in b varying the considered isotope allows modifying the contrast and therefore to obtain much more information in a SANS experiment. For example: (*i*). increase the contrast using hydrogenated samples and deuterated solvent; (*ii*). create or vary a contrast using a mixture of deuterated and hydrogenated particles. However, replacing isotopes can cause problems, such as changing the properties of the solvent, varying the melting point, inducing phase separation, and shifting the critical micellar concentration; therefore, it must be done with reasoning and when necessary.<sup>173</sup>

#### A.4.1 Sample preparation, instrument, and experimental set-up

SANS measurements were performed at the KWS-2 instrument located at Heinz Maier-Leibnitz Source, Garching Förschungzentrum (Germany). Neutron with a wavelength spread  $\Delta\lambda/\lambda$  of 0.1 were used. A two-dimensional array detector at three different wavelength (W)/ collimation (C)/ sample-to-detector (D) distance combinations were used to measure the neutron scattered from the samples. This configuration allowed to collect the data for the scattering vector in the range between 0.002-0.4 Å<sup>-1</sup>. The investigated systems were collected for a closed quartz cell. The raw data were corrected for detector sensitivity, blocked beam, background, and empty cell scattering, followed by the radial averaging and transformation to absolute scattered intensity I(q) using a secondary plexiglass standard. All samples of coated CeO<sub>2</sub> NP superlattices were analyzed in deuterated water at 25 °C.

#### A.4.2 Data analysis

Two types of samples were analyzed using SANS measurements: OA-C18@CeO<sub>2</sub> 250 and NaOA-C18@CeO<sub>2</sub> 250. The data have been fitting using the SasView program (version 5.0). For OA-C18@CeO<sub>2</sub> 250 sample, the data have been fitting combined two models: power law (equation 28), and FCC (equation 29), in the case of and NaOA-C18@CeO<sub>2</sub> 250, instead, power law and core-shell-sphere (equation 32) models are used.

$$I(q) = scale \cdot q^{-power} + bkg$$
<sup>(28)</sup>

with power=power law exponent.

$$I(q) = \frac{scale}{V_p} V_{lattice} P(q) Z(q)$$
<sup>(29)</sup>

where  $V_p$  is the particle volume, P(q) is the form factor of a sphere, Z(q) is the paracrystalline structure factor for a face-centered cubic (FCC) structure, and  $V_{lattice}$  is the volume of crystalline structure, and is equal to:

$$V_{lattice} = \frac{16\pi}{3} \frac{r^3}{\left(D\sqrt{2}\right)^3}$$
(30)

with *r* particle radius, and *D* distance between neighbor particles:

$$\frac{qD}{2\pi} = \sqrt{h^2 + k^2 + l^2}$$
(31)

The intensity for the core-shell-sphere is:

$$P(q) = \frac{scale}{V} F^2(q) + bkg$$
(32)

with

$$F(q) = \frac{3}{V_s} \left[ V_c \left( \rho_c - \rho_s \right) \frac{\sin(qr_c) - qr_c \cos(qr_c)}{\left(qr_c\right)^3} + V_s \left( \rho_s - \rho_{solv} \right) \frac{\sin(qr_s) - qr_s \cos(qr_s)}{\left(qr_s\right)^3} \right]$$
(33)

where  $V_s$  is the particle volume,  $V_c$  is the core volume,  $r_c$  is the radius of the core,  $r_s$  is the radius of the core and the shell,  $\rho_c$  is the scattering length density of the core,  $\rho_s$  is the scattering length density of the solvent.

# Appendix B – Microscopy

Microscopy is a technique based on the magnification of a sample not visible to the naked eye.<sup>180</sup> Microscopy is divided into three branches: optical, electron, and scanning probe. Optical and electron microscopies involve the diffraction, reflection, or refraction of an electromagnetic radiation or electron beams interacting with the sample, and the collection of the scattered radiation or another signal to create an image. This process may be carried out by wide-field irradiation of the sample, light and transmission electron microscopies, or by scanning a fine beam over the sample, confocal laser scanning microscopy and scanning electron microscopy. Scanning probe microscopy involve the interaction of a scanning probe with the surface of the interest sample.<sup>181</sup>

Each microscopy technique is characterized by a determined resolving power, which is closely related to the type of source used. The resolving power of a microscope can be defined as the minimum distance between two distinct points. In general, the resolution of an image is inversely proportional to the wavelength of the radiation that is used. At the first approximation, not considering the various optical aberrations, the following relationship, known as the Abbe principle, can be considered:<sup>182</sup>

$$d = \frac{\lambda}{2NA} \tag{1}$$

where d is the spatial resolution,  $\lambda$  is the wavelength, NA is the numerical aperture, that is a dimensionless number indicating the maximum angle useful for the system to receive or emit a radiation.

The microscopes used in this thesis work will be described below.

## **B.1 Optical Microscopy**

Optical microscopy involves passing visible light transmitted through ore reflected from the sample through a single or multiple lenses to allow a magnification of the sample.<sup>183</sup> The wavelength of visible light is included from about 380 to 740 nm. In terms of frequency, this compares to a band in the vicinity of 430-770 THz. In practice, considering the diffraction phenomenon, light can not be focused on an infinitely small spot, consequently, objects that are together at a close distance can not be distriminated and *minutiae* of the way they are distributed in a certain space can be seen in an image only to a certain degree of visibility.<sup>182</sup> This means that in the visible region of the electromagnetic spectrum, one can appreciate, for example, the adhesion of a biological cell to a surface without reaching the ability to see the cellular protein distribution at the molecular scale, or

the degree of coverage of a support but not the single particles that cover it. The magnified image can be observed directly or acquired through a camera.

#### **B.1.1 Sample and experimental set-up**

Optical images were acquired using Zeiss Axio Observer A1 microscopy. Each sample was analyzed at various points of the support. Using the ImageJ program, the degree of coverage was determined.

## **B.2** Transmission Electron Microscopy (TEM)

In Transmission Electron Microscopy (TEM) an electron beam of uniform current density is used. The electron energy is in the range from 60 to 150 keV or from 200 keV to 3 MeV in the case of the high-voltage electron microscope.<sup>184</sup>

In a TEM experiment, electrons pass through a hollow tube, where vacuum is applied, and, when they reached the sample, are partially absorbed, partially diverted, and partially transmitted. Then, they continue their path through the lenses that magnify the image. The image as obtained is further magnified by the projective lens on the screen. The rays used during the experiment have little penetration power, therefore it is necessary that the sample is fixed, dehydrated, and thin on the grid. The resolving power of TEM is 0.2 nm and provides black and white images of the sample.<sup>184</sup>

#### **B.2.1 Sample and experimental set-up**

TEM images were acquired using the FEI TECNAI G2 200 kV microscope. For all analyzed samples, approximately 10  $\mu$ L of each sample were placed on a carbon-coated copper grid and allowed to air dry before imaging. Images were taken at various magnifications for each sample, form the lowest magnification to have an overall view of the sample at the highest magnification to allow a statistical analysis of the size of the synthesized nanoparticles (coated CeO<sub>2</sub> and F-doped ZnO), and the distance between two neighboring nanoparticles in coated CeO<sub>2</sub> superlattice. The images are processed with a free digital imaging processing system, ImageJ.

# **B.3** Cryogenic Transmission Electron Microscopy (Cryo-TEM)

A variant of TEM is represented by Cryogenic Transmission Electron Microscopy (Cryo-TEM). The advantage of this type of technology consists of the possibility to observe the sample directly in their

native environment. Generally, the term Cryo-TEM refers to electron transmission microscopy measurements in thin water-based film, vitrified. This technique provides, for example, the possibility of obtaining immediate images of structures formed by amphiphilic molecules in solution. These delicate and dynamic structures require an aqueous environment to form and maintain and poorly tolerate the drying procedure typical of conventional TEM.<sup>185</sup>

In the Cryo-TEM technique, the solution to be analyzed is placed on a porous layer, the excess material is removed, the layer is placed on a normal grid for TEM, and then the sample is quickly frozen. The structures remain trapped in the vitrified film and can be observed without dehydration. Nevertheless, the contrast is a limiting factor. In fact, it is produced by the difference in electron density between the molecules and the solvent or between the various molecules that make up the structure under consideration. The resolution of this technique is 4/5 nm, then, for example, micelles are only seen as dots and only their size can be obtained. However, this technique, with this resolution, is very useful to determine the self-assembly of inorganic nanoparticles coated with organic layers. There are also limitations on maximum observable dimensions, determined by the thickness of the aqueous film. The maximum allowed size is about 500 nm, otherwise the diffusion of the electrons becomes too large and the vitrification process too slow. The liposomes and similar structures are the samples best analyzed with this kind of technique.<sup>185</sup>

#### **B.3.1 Sample and experimental set-up**

Cryo-TEM measurements were performed to characterize coated CeO<sub>2</sub> NP superlattice structures and to determine the parameters that drive the hierarchical organization. Cryo-TEM images were carried out at Heinz Maier-Leibnitz Source, Garching Forschungzentrum (Germany), on a JEOL 200 kV JEM-FS2200 with a field emission gun (FEG). Samples for Cryo-TEM were prepared by placing a 5 µL drop of superlattice suspension on a Quantifoil Multi A carbon-coated copper grid. After, the sample was cryo-fixed by rapidly immersing into liquid ethane at -180 °C in a cryo-plunge (EMGP Leica GmbH). The grid was inserted into a cryo-transfer holder (HTTC 910, Gatan, Munich, Germany), and transferred to a JEM 2200 FS EFTEM instrument (JEOL, Tokyo, Japan). Examinations were carried out at temperature around -180 °C. The transmission electron microscope was operated at an acceleration voltage of 200 kV. All images were recorded digitally by a bottommounted 16-bit CMOS camera system (TemCam-F2016, TVIPS, Munich, Germany) and processed with a free digital imaging processing system, ImageJ.

# **B.4 Atomic Force Microscope (AFM)**

Atomic Force Microscope (AFM) falls into the class of scanning probe microscopy. In fact, the AFM microscope consists of a cantilever at the end of which is mounted a tip, typically composed of silicon or silicon nitride, which has a curvature radius of the order of nanometers.<sup>186,187</sup>

During an AFM experiment, the tip is placed near the surface of the sample to be scanned. The van der Waals force acting between the tip and the sample causes a deflection of the cantilever, whose elastic constant is known, in accordance with the Hooke law. Generally, the sample is placed on a piezoelectric tube, which can move it in a perpendicular direction, z-direction to maintain a constant force between the tip and the surface of the sample, and in the plane x, y to analyze the surface. The resulting map represents the topography of the surface sample nanometers.<sup>186,187</sup>

## **B.4.1 Sample and experimental set-up**

The combined atomic force microscopy (AFM) and micro-Raman analysis were conducted with the integrated apparatus Alpha300-RA (WiTec). The system can be switched at will between AFM and confocal micro-Raman configurations, allowing a combined topographical and spectral characterization of a specified microregion of the sample. The samples topographies were studied by AFM in intermittent contact (AC) mode using a cantilever with 75 kHz resonant frequency. For the micro-Raman analysis, a laser beam at  $\lambda$ = 488 nm was used as excitation light source. The beam was focused onto the sample surface by means of a microscope objective with magnification 50x and NA= 0.75 working in epi-illumination mode. The diffraction-limited focused spot in the objective focal plane had a full width at half-maximum (FWHM) of approximately 320 nm. The light backscattered from the sample was collected by the same objective and sent to the spectrograph through a confocal optical collection path.

# Appendix C – Spectroscopy

The interaction between light and matter has allowed the development of different spectroscopic techniques able to probe different molecular characteristics.<sup>188</sup>

The interaction of electromagnetic radiation with the matter is a quantum phenomenon and depends on both the properties of the radiation and the structure of the sample. Electromagnetic radiation is characterized by a wavelength, which is the spatial distance between two consecutive peaks, the frequency, which is the number of oscillations made by the wave in 1s, and the amplitude, the maximum length of the vector that describes the wave. The element responsible for this electromagnetic phenomenon is the photon, which has properties of both a wave and a particle of zero mass. As a particle, it interacts with matter by transferring energy E:<sup>188</sup>

$$E = \frac{hc}{\lambda} = hv \tag{1}$$

with *h* Plank constant,  $\lambda$  wavelength, and *v* frequency. The energy variation  $\Delta E$  that occurs due to the interaction of radiation with matter will be:<sup>188</sup>

$$\Delta E = E_{final} - E_{start} = hv \tag{2}$$

When radiation provides energy to matter, it causes the electrons to transfer from the electronic ground state  $S_0$  to the electronic excited state  $S_1$ , energy is then absorbed by matter. Subsequently, the relaxation returns the electrons to their initial state. This relaxation can occur in various ways, causing, for example, fluorescence or heat emission.<sup>188,189</sup>

In the following paragraphs, the spectroscopic techniques used in this thesis work will be presented.

# C.1 UV-Visible Spectroscopy

UV-Visible spectroscopy is an absorption spectroscopic technique, thus determining the energy absorption following the interaction between an electromagnetic wave and the matter. Depending on the energy used for the transition, the molecule will absorb in the ultraviolet region (180-400 nm) or in the visible region (400-800 nm), in accordance with equation 1. As mentioned above, this interaction causes an electronic transition from a ground state to an excited one. The electronic transitions in molecules can be classified according to the participating molecular orbital. Only two transitions are possible using light in the UV-Visible region, namely  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$ , where *n* is the non-binding orbital,  $\pi^*$  anti-binding, and  $\pi$  binding. The possibility of the photon being XVII

absorbed by matter depends on the extinction coefficient  $\alpha$ , which in turn depends on the photon wavelength. This means that if the light with intensity  $I_0$  passes through a sample of thickness d, the resulting intensity will be  $I = I_0 e^{-\alpha d}$ . The ratio between  $I_0$  and I can be determined by the Lambert-Beer law (equation 3) from the absorbance A of the sample, which is the experimentally measurable parameter:<sup>188</sup>

$$A = \varepsilon cd = \log \frac{I_0}{I} \tag{3}$$

where  $\varepsilon$  is the molar absorption coefficient, and *c* the molar concentration of the sample. The Lambert-Beer law is only valid for low concentration.<sup>188</sup>

During a UV-Visible absorption experiment, changes in the sample and or in the immediate environment of the sample may also be evaluated due to a shift in the maximum absorption at higher value of wavelength (red shift or bathochromic effect), or at lower (blue shift or hypsochromic effect), an increase in maximum absorption (hyperchromic) or decrease (hypochromic).<sup>188</sup>

#### C.1.1 Band gap

The band gap is the energy difference between the maximum of the valence band and the minimum of the conduction band.<sup>190</sup>

The energy of the band gap is an important parameter for the definition of the properties of a semiconductor, like metal oxide nanoparticles, and it can be calculated in various ways, one of the most common ones is that proposed by Tauc in 1966.<sup>191,192</sup>

The Tauc method assumes that the absorption coefficient depends on energy according to the following relationship:

$$(\alpha \cdot hv)^{1/\gamma} = B(hv - E_g)$$
(4)

where  $\alpha$  is the absorption coefficient, *h* is the Planck constant, *v* is the photon frequency, *Eg* is the band gap energy, and *B* is a constant: the  $\gamma$  factor depends on the nature of the electron transition and it is equal to 0.5 for the direct transition band gap and to 2 for indirect transition band gap.<sup>193</sup>

The band gap energy is usually determined from diffuse reflectance spectra, which can be transformed into the corresponding absorption spectra by applying the Kubelka-Munk function (equation 5):

$$F(R_{\infty}) = \frac{K}{S} = \frac{\left(1 - R_{\infty}\right)^2}{2R_{\infty}}$$
(5)

where  $R_{\infty}$  is the ratio of the reflectance of the sample to that of the standard, *K* is the absorption coefficient, and *S* is the scattering coefficient.<sup>194</sup> Substituting  $\alpha$  with  $F(R_{\infty})$  gives the following:

$$\left(F(R_{\infty}) \cdot hv\right)^{1/\gamma} = B(hv - E_g)$$
(6)

Plotting the first term of equation 6 as a function of hv gives a graph from which the band gap can be obtained.

#### C.1.2 Sample and experimental set-up for UV-Visible measurements in suspension

UV-Visible spectroscopy measurements were performed using a Jasco V-560 UV-Vis instrument, dual-beam instrument, equipped with a deuterium lamp (190-350 nm) and a halogen lamp (330-900 nm). 1.5 mL of suspension containing the coated CeO<sub>2</sub> or F-doped ZnO NPs was placed in a quartz cuvette in order to carry out the measurements (optical path: 1 cm, band width: 2.0 nm, and scanning speed: 40 nm/min). For coated CeO<sub>2</sub> NP suspensions, chloroform was used as white, instead, for F-doped ZnO NP suspensions, methanol, respectively, storage solvents of synthesized NPs.

### C.1.3 Sample and experimental set-up for band gap determination

UV-Vis DRS measurements on powdered F-doped ZnO NPs were carried out using a UV-2600i UV-Vis spectrophotometer 230 V, equipped with an integrating sphere ISR-2600Plus operating in a wavelength range of 220-1400 nm. Barium sulfate was exploited as a reflectance standard.

#### C.2 Fluorescence Spectroscopy

Once the electron has passed into the excited state due to the absorption of the energy to return to its initial state it must disperse this energy. One of the possible ways to disperse this energy is emit light. The process that occurs between the absorption and emission of light are usually illustrated by the Jablonski diagram (Figure C.1).<sup>189</sup>



Figure C.1. Jablonski diagram.

The absorption, as already seen, brings the electrons from the state  $S_0$  to the excited states  $S_1$  or  $S_2$ . In some cases, a first relaxation from  $S_2$  to  $S_1$  is possible, known as internal conversion. Relaxation from the excited state  $S_1$  to initial state  $S_0$  causes energy emission, known as fluorescence (lifetime<10<sup>-8</sup>s). This emission is practically the mirror image of absorption. This similarity occurs because electronic excitation does not greatly alter the nuclear geometry. However, molecules in the state  $S_1$  can also undergo a spin conversion to the first triplet state  $T_1$ , intersystem crossing. Emission from  $T_1$  is called phosphorescence (lifetime>10<sup>-8</sup>s), and is generally shifted to longer wavelength, then lower energy, relative to the fluorescence.<sup>189</sup>

A general property of fluorescence is that the same fluorescence emission spectrum is generally observed irrespective of the excitation wavelength, Kasha rule. Upon excitation into higher levels, the excess energy is quickly dissipated, leaving the fluorophore in the  $S_1$  state. Because of this rapid relaxation, emission spectra are usually independent of the excitation wavelength. Exceptions exist, such as fluorophores that exist in two ionization states, or molecules that can emit directly from the  $S_2$  state.<sup>189</sup>

#### **C.2.1 Relative Quantum Yield**

Quantum yield  $\Phi$  is the number of emitted photons relative to the number of absorbed photons.<sup>189</sup> The easiest way to estimate the quantum yield of a sample is by comparison with a standard of known quantum yield, such as quinine sulfate, fluorescein, or rhodamine:<sup>164</sup>

$$\boldsymbol{\Phi} = \left(\frac{A_s}{A}\right) \left(\frac{F}{F_s}\right) \left(\frac{n}{n_s}\right)^2 \phi_s \tag{7}$$

XX

where  $A_s$  is the absorption of standard, A is the absorption of the sample, F is the integral of the area below the fluorescence peak of the sample,  $F_s$  is the integral of the area below the emission peak of standard, n, and  $n_s$  are the reflective indices of the solvent in which the sample and the standard are solubilized, respectively, and  $\phi_s$  is the quantum yield of standard.

#### C.2.2 Sample and experimental set-up for fluorescence measurements

Fluorescence spectra were recorded at 25 °C using a Horiba Scientific Fluoromax-4 spectrofluorometer equipped with a Peltier control system and 1 cm path length cells. Each F-doped ZnO NP suspension was excited to the specific absorption wavelength, determined by means of UV-Visible spectroscopy measurements (integration time: 0.1 s, excitation and emission slit width:5 nm).

#### C.2.3 Sample and experimental set-up for relative quantum yield determination

To perform a correct analysis for the determination of the relative quantum yield each measurement was performed on three independent samples of F-doped ZnO NPs and three independent samples of quinine monohydrate, used as standard. All samples were excited at the maximum absorption (maximum value of 0.3) wavelength determined by UV-Visible spectroscopy measurements, while the standard was excited at 317 nm. The instrument used to perform the fluorescence measurements to calculate the relative quantum yield is the same as described in the previous paragraph, but using the excitation and emission slit width of 2 nm.

# C.3 Photoluminescence Spectroscopy

The term luminescence indicates all the phenomena that lead to the emission of electromagnetic radiation by the matter following an excitation. If the exciting energy is of electromagnetic origin, the phenomenon is called photoluminescence.<sup>189</sup>

Photoluminescence includes the fluorescence and the phosphorescence phenomena described in detail above.

In some cases, the emission can take place after a few hours or even days.<sup>189</sup>

#### C.3.1 Synthesized NPs: experimental set-up

Photoluminescence measurements of amine coated CeO<sub>2</sub> NPs and F-doped ZnO NPs were carried out at CEA-Leti Research and Technology Institute (Grenoble, France) using an Hariba LabRAM HR instrument, equipped with a microscope objective (x40, Thorlabs NA 0.5 WD 1 mm), visible grating (100 grooves/mm), CCD detector. The measurements, on powders or deposited nanoparticles on silicon supports, were performed using a laser with a wavelength of 325 nm, power of 1 mW, at room temperature, and with an entrance slit of 300  $\mu$ m.

#### C.3.2 Coated CeO<sub>2</sub> superlattices: experimental set-up

An in-house setup for excitation-resolved photoluminescence (PLE) was used to analyze the optical properties of coated CeO<sub>2</sub> superlattice suspensions, in collaboration with Dr. Stefano Lettieri of Physical Department "Ettore Pancini" of University of Naples Federico II. The PLE setup provided wavelength-tunable incoherent light to excite and collect the photoluminescence (PL) of a sample placed in controlled environment and using a double-lens confocal optical system to collect the PL spectral intensity. Excitation light at variable central wavelength spanning in the interval from 340 nm to 420 nm (FWHM of the excitation line approximately 2 nm) was provided by a monochromatized Xe lamp, collected by a liquid waveguide and focused on the samples, which consisted of 200  $\mu$ L droplets coated CeO<sub>2</sub> aqueous suspensions dropped onto an aluminum substrate placed into the optical chamber. The PL light was collected by a multi-mode 1000 µm core optical fiber and sent to a motorized monochromator of 320 mm equivalent focal length, equipped with a cooled CCD camera for spectral acquisition. Before detection, the light emerging from the samples was filtered of the spectral components of wavelengths shorter than 420 nm to avoid the detection of spurious signals originating from the excitation light and to reduce a residual Raman signal originating from the liquid waveguide. Residual signal measured from the Al substrate and from the pure solvent (i.e., water droplets) was largely negligible with respect to the ones detected for the dispersions of functionalized CeO<sub>2</sub> NPs.

# C.4 X-ray Photoelectron Spectroscopy

X-ray Photoelectron Spectroscopy (XPS) is a spectroscopic technique that allows analyzing the surface of materials, allowing both a qualitative and quantitative analysis of the different chemical species present on the surface.<sup>195</sup>

The XPS technique is based on the photoelectronic effect. When a sample is exposed to electromagnetic radiation of adequate energy, the electrons of the atoms can be emitted. Photoemission is a complex process that can be divided into three successive stages: (*i*). the incoming photon is absorbed by an electron, which passes to an excited final state; (*ii*). the electron moves on the sample surface; (*iii*). the electron is ejected into the vacuum. During the path of the electron to the surface, the most electrons generated undergo inelastic collisions, which result in a reduction in kinetic energy. Thus, the photoemission spectra consist of two main components, namely primary and secondary electrons. The primary electrons, that do not undergo inelastic collisions, determine distinct peaks, that, in first approximation, reflet the density states of the sample. The secondary electrons in energy up to zero kinetic energy. These two types of electrons provide different types of information. The primary peaks allow the determination of the binding energy of the electron states of the sample. The resulting spectrum consists of primary electrons with a kinetic energy  $E_{kin}$  given by a following equation:<sup>195</sup>

$$E_{kin} = hv - E_b - \varphi_s \tag{8}$$

where hv is the photon energy,  $E_b$  is the binding energy of the electron, and  $\varphi_s$  is the work function of the sample, that is the minimum energy needed to extract an electron from the material in the vacuum.

The most important information is related to  $E_b$ , whose value not only depends on the chemical element from which the photoelectron is extracted but also contains information about the chemical configuration in which the photoelectron atom originates.

During a XPS experiment a high-energy (<2keV) X-ray beam produced in a linear accelerator is collimated on the sample. The electrons torn from their level of belonging cross the surface layer of the material and are re-emitted in the working chamber. The experiment requires that the atmosphere be kept in ultra-high vacuum to avoid energy losses of electrons due to collisions with other particles. The emitted electrons are detected by passing from an energy selector through a photomultiplier, which amplifies the number of electrons received to a certain energy and converts this signal into a voltage signal. By changing the electromagnetic parameters of the selector, it is possible to scan energy ranges from a few tens of eV to a few thousand of eV.<sup>195</sup>

## C.4.1 Sample and experimental set-up

Surface properties of coated CeO<sub>2</sub> and F-doped ZnO NPs were investigated by XPS analyses which were carried out using a spectrometer VersaProbe II with a monochromatic Al Kv X-ray source (hv = 1486,6 eV), a spot of 200  $\mu$ m, and a dual-beam system (Ar+ et <sup>-</sup>e) for charge compensation. The pass energy was set at 23.5 eV, leading to an overall energy resolution of 0.6 eV. Photoelectrons were collected at a take-off angle of 45°, which means a sampling depth of approximately 5 nm. Decomposition of the spectra was done using Voigt functions after Shirley's background subtraction with the Multipak software. XPS measurements were carried out at CEA-Leti Research and Technology Institute (Grenoble, France).

# C.5 Micro-Raman Spectroscopy

Raman spectroscopy is the study of the interaction between light and matter where light is inelastically scattered.

During an experiment using Raman spectroscopy, light of a single wavelength is focused onto a sample. Most commonly a laser is used as it is a powerful monochromatic source. The photons from the laser interact with the molecules of the sample and are scattered inelastically. The scattered photons are collected, and a spectrum is generated from the scattered photons.<sup>196,197</sup>

Raman micro-spectroscopy is where a Raman micro-spectrometer is used in place of a standard Raman spectrometer. A Raman micro-spectrometer consists of a specially designed Raman spectrometer integrated with an optical microscope. This allows the experimenter to acquire Raman spectra of microscopic samples or microscopic areas of larger samples. The advantages are that much less samples is required and certain effects may also be enhanced over very localized regions.<sup>196,197</sup>

# C.5.1 Sample and experimental set-up

Experimental set-up is described in paragraph B.4.1.

# **Appendix D – Film Characterization Techniques**

In this appendix will be described two techniques used for the characterization of ZnO NP films, reflectivity and ellipsometry.

# **D.1 Reflectivity Technique**

Reflectivity techniques analyze the reflective radiation from surfaces and are complementary methods to microscopy techniques, with the advantage of being able to analyze solid-liquid or liquid-liquid interfaces. In a reflectivity experiment, represented in Figure D.1, the property of a flat surfaces to reflect the incoming wave, thus scatter it at the same incident angle, is exploited. By measuring reflected wave intensity over the incident one, as function of the scattering vector q, information about the composition profile normal to the sample surface can be obtained.



Figure D.1. Schematic representation of a reflectivity experiment.

It is possible to distinguish two different kind of reflectivity experiments. Specular reflectivity is devoted to the characterization of the interface in the direction z normal to the surface. Thus, only the intensity reflected at the same angle of the incident wave is analyzed. This means that the interface is considered homogenous composition in x, y plane, and the reflective properties of the surface can

change only in the z-direction. On the other hand, off-specular reflectivity is used to measure the reflected wave at angles different from the incident one. Form this kind of experiments information about the inhomogeneities in the direction parallel to the surface are accessible.<sup>198–200</sup>

#### **D.1.1 Basic reflectivity theory**

According to the scheme in Figure D.1, an incoming wave hitting a surface through a medium  $\theta$ , it is partially reflected by the surface with the same angle  $\theta$ , and partially transmitted through the medium *I*. To describe this phenomenon, it is important to recall the concept of refractive index *n* (equation 1), which is the properties of each material to change the propagation of the wave when it passes through a material.<sup>201</sup>

$$n = 1 - \delta + i\beta \tag{1}$$

where  $\delta = \frac{\lambda^2 \rho}{2\pi}$  with  $\rho$  scattering length density, and  $i\beta$  is responsible to the absorption of the incoming wave.

The fundamental laws describing the reflection of an incident plane wave from a flat surface are reported in equations 2 and 3:<sup>201</sup>

$$\cos\theta_0 = \cos\theta \tag{2}$$

$$n_0 \cos\theta_0 = n_1 \cos\theta_1 \tag{3}$$

where  $\theta_0$ ,  $\theta$ , and  $\theta_1$  are the incident angle, reflection angle, and refraction angle, respectively, while  $n_0$  and  $n_1$  are the reflective indices of the two media in contact.

The amplitude of the incoming wave in the z-direction is defined:<sup>201</sup>

$$A_0(z) = e^{ik_0 sin\theta_0 z}$$
(4)

The amplitude in the medium 0 is the sum of the incoming wave and the reflected one:<sup>201</sup>

$$A(z) = e^{ik} 0^{sin\theta} 0^{z} + r_{0I} e^{ik} 0^{sin\theta} 0^{z}$$
(5)

The amplitude in the medium I is, instead, the transmitted wave:<sup>201</sup>

XXVI

$$A_{I}(z) = t_{0I}e^{ik_{0}sin\theta}I^{z}$$
(6)

Imposing that the  $A_1$  is the same on both side of medium *I*, and that also its derivative with respect to z is the same, the wave amplitude must vary smoothly along the interface. Hence, the reflectivity *R* can be expressed as:<sup>201</sup>

$$R = \left| \frac{k_0 \sin\theta_0 - k_1 \sin\theta_1}{k_0 \sin\theta_0 + k_1 \sin\theta_1} \right|^2 \tag{7}$$

Since reflectivity is a function of the incident angle, it is possible to avoid the existence of a critical angle  $\theta_c$ . For  $\theta_0 \le \theta_c$  the maximum interaction between the incoming wave and the surface occurs. If

the medium  $\theta$  is the vacuum ( $n_0=1$ ),  $\cos\theta_c$  is equal to  $n_I$ . Instead, if  $\cos\theta = 1 - \frac{\theta^2}{2}$  and  $n_I = 1 - \delta_I$  it is possible to obtain an approximated expression of the critical angle:<sup>201</sup>

$$\theta_c = \lambda \sqrt{\frac{\rho}{\pi}} \tag{8}$$

So, for  $\theta_0 \gg \theta_c$  reflectivity can be determined with a simple relationship:<sup>201</sup>

$$R \approx \frac{16\pi^2}{q_{z0}^4} \Delta \rho^2 \tag{9}$$

where  $\Delta \rho$  is the difference in terms of scattering length density between the two media.

During a reflectivity experiment the sample is deposited on a suitable support, and it is in contact with a medium, such as air or a solvent. Thus, the presence of one or more layers must be considered between the medium 0 and 1. A general approach is based on considering  $\rho$  as a function of z. Thus, the equation 9 becomes:<sup>201,202</sup>

$$R(q_z) = \frac{16\pi^2}{q_z^2} \left| \int_{-\infty}^{+\infty} e^{iq_z z} \frac{d\rho(z)}{dz} dz \right|^2$$
(10)

where  $\frac{d\rho(z)}{dz}$  is the Fourier transform of the scattering length density profile. If the interface is a sharp step between two materials with different scattering length density,  $\rho_1$  and  $\rho_2$ , the scattering length

density profile is given by the difference between  $\rho_1$  and  $\rho_2$  times a delta function  $\delta(z)$ . Since the Fourier transform of a delta function is 1, the expression of  $R(q_z)$  is given again by the equation 9.

However, a more realistic representation of the system can be achieved by substituting the  $\delta$  function by a function describing the interface shape. In particular, it is possible to consider that the interface is a diffuse interface. This situation can be represented with an error function of  $\rho(z)$ :<sup>201</sup>

$$\frac{d\rho(z)}{dz} = \frac{\Delta\rho}{\sigma\sqrt{2\pi}} e^{\frac{z^2}{2\sigma^2}dz}$$
(11)

with  $p(z) = \frac{\Delta \rho}{\sigma \sqrt{2\pi}} \int_{-\infty}^{z} e^{\frac{z^2}{2\sigma^2} dz}$ 

and so:

$$R(q_z) = \frac{16\pi^2}{q_z^2} \Delta \rho^2 \left(-q_z^2 \sigma^2\right)$$
(12)

The  $\sigma$  parameter is related to the characteristic of the interface between the two materials, and at its roughness. In fact, the more a surface is rough, the more interface with a second material will be diffuse, and faster will be the reflectivity decay with *q*.

The great advantage of the obtained reflectivity expression (equation 12) is that it underlines the relation between R and the composition variation along the z-direction, which allows determining the thickness and the scattering length density profile. However, it is worth to note that even the equation 12 does not represent a complete description of the system. Indeed, the R expression that was obtained does hold only for large incident angle with respect to the critical angle. To obtain an R expression for the entire q range, the optical matrix method proposed by Born and Wolf can be used.

The optical matrix method allows calculating the *R* from any number of parallel homogenous layers. This means that the sample can be represented by a stack of parallel layers, each of them are characterized by a certain thickness, and scattering length density. This method leads to an exact calculation of the *R* profile. Each layer is characterized by a matrix  $M_i$ :<sup>201</sup>

$$M_{i} = \begin{pmatrix} \cos\beta_{i} & -\left(\frac{i}{v_{i}}\right)\sin\beta_{i} \\ -iv_{i}\beta_{i} & \cos\beta_{i} \end{pmatrix}$$
(13)

XXVIII

where  $\beta_i = kn_i d_i \sin\theta$  and  $d_i$  is the thickness of the layer *i*. *R* is obtained by combining the matrices of the different layers constituting the sample. For example, for a sample with two layers it is, and a solid(s)-air(a) interface:<sup>201</sup>

$$R = \left| \frac{\left( M_{11} + M_{12} v_s \right) v_a \cdot \left( M_{21} + M_{22} \right) v_s}{\left( M_{11} + M_{12} v_s \right) v_a + \left( M_{21} + M_{22} \right) v_s} \right|^2$$
(14)

Eventually the matrix can be slightly modified to also include the roughness. Although it does not lead directly to the scattering length density profile, the optical matric method offers the advantage of exactly calculating the reflectivity at any angle.

#### **D.1.2 X-Ray Reflectivity (XRR)**

The theoretical considerations given in the previous paragraph are valid regardless of the source used in the reflectivity experiment. In this thesis, the samples, F-doped ZnO NP films, were analyzed by X-ray Reflectivity (XRR). The choice fell on XRR because the samples to be analyzed were composed of inorganic NPs.

As already seen in the case of X-ray Scattering (Appendix A), X-ray radiation is part of the broad spectrum of electromagnetic waves. X-rays can be produced by the acceleration or deceleration of electrons either in vacuum or in metallic targets. The most widely X-ray radiation used is the Cu K<sub> $\alpha$ </sub>. It has a typical wavelength of about 0.154 nm, associated to a very high frequency of the order of 1019 Hz, which is at least four orders of magnitude greater that the eigen frequency of an electron bound to a nucleus.<sup>201</sup> In addition, this technique is extremely sensitive to any defects of flatness at the scale of a tenth of nm. So, it is the ideal technique for characterizing an inorganic NP film.

#### **D.1.3 XRR measurements: experimental set-up**

XRR measurements were carried out at ILL/ESRF laboratories in Grenoble (France), using a Bruker instrument. After several tests, the final configuration, best for F-doped ZnO NP films characterization, was: 10 mm Cu mask, 1/32° slit for incident beam, 1/4° anti-scattering outgoing beam, 1/8° slit for detector, no soller, and automatic attenuator.

# **D.2 Solid State Ellipsometry**

Ellipsometry is an optical technique that allows the detection of the variation of the polarization state of a polarized light beam produced by interaction with a linear optical element. Only the change in the polarization state between incident and reflected wave is measured making the measurement independent of the intensity I of the beam, and then by any fluctuations of I.

The theory that connects the variations of the polarization state of the electromagnetic waves to other optical parameters was originally developed by Fresnel and is the same at the base of the reflectivity.

In ellipsometry the quantities that are measured are the angle  $\Psi$  and  $\Delta$  whose definition is linked to Fresnel coefficients by the fundamental equation of the ellipsometry:

$$\rho = tan\Psi e^{i\Delta} \tag{15}$$

 $tan\Psi$  is linked to the ratio of the amplitude of the coefficients of reflection, and  $\Delta$  is the difference between the two shifts of the reflected and incident waves. These two independent parameters allow determining the optical properties of the substrate, such a reflective index, or geometric properties, such as thickness of a transparent film deposited on a known substrate.<sup>203</sup>

#### **D.2.2 Instrument and experimental set-up**

Solid-state ellipsometry measurements were carried out at ILL/ESRF laboratories in Grenoble (France), using a fixed angle (73°) home-made instrument. The instrument consists of a red laser, a monochromator, and two polarizers. The first place between the source and the sample, necessary to send the linear polarized light to the sample, the second, instead, places between the sample and the monochromator to analyze the polarization of reflected light.

Measurements were first conducted on cleaned silicon supports to determine their thickness. After the NP film deposition procedure, measurements were carried out with at least 20 analysis points per substrate.

# References

- (1) Sudha, P. N.; Sangeetha, K.; Vijayalakshmi, K.; Barhoum, A. Chapter 12 Nanomaterials History, Classification, Unique Properties, Production and Market. In *Emerging Applications of Nanoparticles and Architecture Nanostructures*; Barhoum, A., Makhlouf, A. S. H., Eds.; Micro and Nano Technologies; Elsevier, 2018; pp 341–384. https://doi.org/10.1016/B978-0-323-51254-1.00012-9.
- (2) Harish, V.; Tewari, D.; Gaur, M.; Yadav, A. B.; Swaroop, S.; Bechelany, M.; Barhoum, A. Review on Nanoparticles and Nanostructured Materials: Bioimaging, Biosensing, Drug Delivery, Tissue Engineering, Antimicrobial, and Agro-Food Applications. *Nanomaterials* **2022**, *12* (3), 457. https://doi.org/10.3390/nano12030457.
- (3) A Comprehensive Review of Nanomaterials: Types, Synthesis, Characterization, and Applications. *Biointerface Res. Appl. Chem.* **2022**, *13* (1), 41. https://doi.org/10.33263/BRIAC131.041.
- Singh, J. P.; Kumar, M.; Sharma, A.; Pandey, G.; Chae, K. H.; Lee, S.; Singh, J. P.; Kumar, M.; Sharma, A.; Pandey, G.; Chae, K. H.; Lee, S. *Bottom-Up and Top-Down Approaches for MgO*; IntechOpen, 2020. https://doi.org/10.5772/intechopen.91182.
- (5) Environmental, Chemical and Medical Sensors; Bhattacharya, S., Agarwal, A. K., Chanda, N., Pandey, A., Sen, A. K., Eds.; Energy, Environment, and Sustainability; Springer Singapore: Singapore, 2018. https://doi.org/10.1007/978-981-10-7751-7.
- (6) T. Bromley, S.; R. Moreira, I. de P.; M. Neyman, K.; Illas, F. Approaching Nanoscale Oxides : Models and Theoretical Methods. *Chem. Soc. Rev.* 2009, 38 (9), 2657–2670. https://doi.org/10.1039/B806400H.
- (7) Immanuel, S.; Aparna, T. K.; Sivasubramanian, R. Chapter 5 Graphene–Metal Oxide Nanocomposite Modified Electrochemical Sensors. In *Graphene-Based Electrochemical Sensors for Biomolecules*; Pandikumar, A., Rameshkumar, P., Eds.; Micro and Nano Technologies; Elsevier, 2019; pp 113–138. https://doi.org/10.1016/B978-0-12-815394-9.00005-4.
- (8) Yamashita, M.; Kameyama, K.; Yabe, S.; Yoshida, S.; Fujishiro, Y.; Kawai, T.; Sato, T. Synthesis and Microstructure of Calcia Doped Ceria as UV Filters. J. Mater. Sci. 2002, 37 (4), 683–687. https://doi.org/10.1023/A:1013819310041.
- (9) Fundamentals and Catalytic Applications of CeO2-Based Materials / Chemical Reviews. https://pubs.acs.org/doi/full/10.1021/acs.chemrev.5b00603 (accessed 2023-01-30).
- (10) Armini, S.; Messemaeker, J. D.; Whelan, C. M.; Moinpour, M.; Maex, K. Composite Polymer Core–Ceria Shell Abrasive Particles during Oxide CMP: A Defectivity Study. J. Electrochem. Soc. 2008, 155 (9), H653. https://doi.org/10.1149/1.2949085.
- (11) Mei, Z.; Xidong, W.; Fuming, W.; Wenchao, L. Oxygen Sensitivity of Nano-CeO2 Coating TiO2 Materials. Sens. Actuators B Chem. 2003, 92 (1), 167–170. https://doi.org/10.1016/S0925-4005(03)00259-4.
- (12) Auffan, M.; Rose, J.; Orsiere, T.; De Meo, M.; Thill, A.; Zeyons, O.; Proux, O.; Masion, A.; Chaurand, P.; Spalla, O.; Botta, A.; Wiesner, M. R.; Bottero, J.-Y. CeO2 Nanoparticles Induce DNA Damage towards Human Dermal Fibroblasts in Vitro. *Nanotoxicology* **2009**, *3* (2), 161–171. https://doi.org/10.1080/17435390902788086.
- (13) Gao, Y.; Chen, K.; Ma, J.; Gao, F. Cerium Oxide Nanoparticles in Cancer. *OncoTargets Ther.* 2014, 7, 835–840. https://doi.org/10.2147/OTT.S62057.
- (14) Nyoka, M.; Choonara, Y. E.; Kumar, P.; Kondiah, P. P. D.; Pillay, V. Synthesis of Cerium Oxide Nanoparticles Using Various Methods: Implications for Biomedical Applications. *Nanomaterials* 2020, *10* (2), 242. https://doi.org/10.3390/nano10020242.
- (15) Scire, S.; Palmisano, L. Cerium Oxide (CeO2): Synthesis, Properties and Applications; Elsevier, 2019.
- (16) Zinc Oxide Nanoparticle as a Novel Class of Antifungal Agents: Current Advances and Future Perspectives / Journal of Agricultural and Food Chemistry. https://pubs.acs.org/doi/full/10.1021/acs.jafc.8b03210 (accessed 2023-01-24).
- (17) Shaba, E. Y.; Jacob, J. O.; Tijani, J. O.; Suleiman, M. A. T. A Critical Review of Synthesis Parameters Affecting the Properties of Zinc Oxide Nanoparticle and Its Application in Wastewater Treatment. *Appl. Water Sci.* 2021, *11* (2), 48. https://doi.org/10.1007/s13201-021-01370-z.
- (18) Pushpalatha, C.; Suresh, J.; Gayathri, V.; Sowmya, S.; Augustine, D.; Alamoudi, A.; Zidane, B.; Mohammad Albar, N. H.; Patil, S. Zinc Oxide Nanoparticles: A Review on Its Applications in Dentistry. *Front. Bioeng. Biotechnol.* 2022, *10*, 917990. https://doi.org/10.3389/fbioe.2022.917990.
- (19) Park, B. C.; Ko, M. J.; Kim, Y. K.; Kim, G. W.; Kim, M. S.; Koo, T. M.; Fu, H. E.; Kim, Y. K. Surface-Ligand-Induced Crystallographic Disorder–Order Transition in Oriented Attachment for the Tuneable Assembly of Mesocrystals. *Nat. Commun.* **2022**, *13* (1), 1144. https://doi.org/10.1038/s41467-022-28830-7.
- (20) Si, K. J.; Chen, Y.; Shi, Q.; Cheng, W. Nanoparticle Superlattices: The Roles of Soft Ligands. *Adv. Sci.* **2018**, *5* (1), 1700179. https://doi.org/10.1002/advs.201700179.
- (21) Metal Catalysts for Heterogeneous Catalysis: From Single Atoms to Nanoclusters and Nanoparticles / Chemical Reviews. https://pubs.acs.org/doi/full/10.1021/acs.chemrev.7b00776 (accessed 2023-01-30).
- (22) Stolarczyk, J. K.; Deak, A.; Brougham, D. F. Nanoparticle Clusters: Assembly and Control Over Internal Order, Current Capabilities, and Future Potential. *Adv. Mater.* **2016**, *28* (27), 5400–5424. https://doi.org/10.1002/adma.201505350.
- (23) Sorrenti, A.; Illa, O.; Ortuño, R. M. Amphiphiles in Aqueous Solution: Well beyond a Soap Bubble. *Chem. Soc. Rev.* 2013, 42 (21), 8200–8219. https://doi.org/10.1039/C3CS60151J.
- (24) Yue, K.; Huang, M.; Marson, R. L.; He, J.; Huang, J.; Zhou, Z.; Wang, J.; Liu, C.; Yan, X.; Wu, K.; Guo, Z.; Liu, H.; Zhang, W.; Ni, P.; Wesdemiotis, C.; Zhang, W.-B.; Glotzer, S. C.; Cheng, S. Z. D. Geometry Induced Sequence of Nanoscale Frank–Kasper and Quasicrystal Mesophases in Giant Surfactants. *Proc. Natl. Acad. Sci.* 2016, *113* (50), 14195–14200. https://doi.org/10.1073/pnas.1609422113.
- (25) Baillis, D.; Coquard, R.; Kaemmerlen, A. *Classification of Foam Structures*; Begel House Inc., 2012. https://doi.org/10.1615/thermopedia.000166.
- (26) Sánchez-Iglesias, A.; Grzelczak, M.; Altantzis, T.; Goris, B.; Pérez-Juste, J.; Bals, S.; Van Tendeloo, G.; Donaldson, S. H. Jr.; Chmelka, B. F.; Israelachvili, J. N.; Liz-Marzán, L. M. Hydrophobic Interactions Modulate Self-Assembly of Nanoparticles. ACS Nano 2012, 6 (12), 11059–11065. https://doi.org/10.1021/nn3047605.
- (27) Takagi, H.; Hashimoto, R.; Igarashi, N.; Kishimoto, S.; Yamamoto, K. Synchrotron SAXS Studies on Lattice Structure of Spherical Micelles in Binary Mixtures of Block Copolymers and Homopolymers. J. Fiber Sci. Technol. 2018, 74 (1), 10–16. https://doi.org/10.2115/fiberst.2018-0002.
- (28) The Role of Ligands in the Chemical Synthesis and Applications of Inorganic Nanoparticles / Chemical Reviews. https://pubs.acs.org/doi/10.1021/acs.chemrev.8b00733 (accessed 2023-01-24).
- (29) Shrestha, S.; Wang, B.; Dutta, P. Nanoparticle Processing: Understanding and Controlling Aggregation. *Adv. Colloid Interface Sci.* **2020**, *279*, 102162. https://doi.org/10.1016/j.cis.2020.102162.
- (30) Zhang, X.-J. Van Der Waals Forces. In *Encyclopedia of Tribology*; Wang, Q. J., Chung, Y.-W., Eds.; Springer US: Boston, MA, 2013; pp 3945–3947. https://doi.org/10.1007/978-0-387-92897-5\_457.
- (31) Israelachvili, J. N. Intermolecular and Surface Forces; Academic Press, 2011.
- (32) Casimir, H. B. G.; Polder, D. The Influence of Retardation on the London-van Der Waals Forces. *Phys. Rev.* **1948**, *73* (4), 360–372. https://doi.org/10.1103/PhysRev.73.360.
- (33) Hamaker, H. C. The London—van Der Waals Attraction between Spherical Particles. *Physica* **1937**, *4* (10), 1058–1072. https://doi.org/10.1016/S0031-8914(37)80203-7.
- (34) Bhattacharjee, S.; Elimelech, M. Surface Element Integration: A Novel Technique for Evaluation of DLVO Interaction between a Particle and a Flat Plate. J. Colloid Interface Sci. 1997, 193 (2), 273–285. https://doi.org/10.1006/jcis.1997.5076.
- (35) Dzyaloshinskii, I. E.; Lifshitz, E. M.; Pitaevskii, L. P. The General Theory of van Der Waals Forces. Adv. Phys. 1961, 10 (38), 165–209. https://doi.org/10.1080/00018736100101281.
- (36) Stenhammar, J.; Linse, P.; Wennerström, H.; Karlström, G. An Exact Calculation of the van Der Waals Interaction between Two Spheres of Classical Dipolar Fluid. J. Phys. Chem. B 2010, 114 (42), 13372–13380. https://doi.org/10.1021/jp105754t.
- (37) Bishop, K. J. M.; Wilmer, C. E.; Soh, S.; Grzybowski, B. A. Nanoscale Forces and Their Uses in Self-Assembly. *Small* 2009, 5 (14), 1600–1630. https://doi.org/10.1002/smll.200900358.
- (38) Belloni, J.; Mostafavi, M.; Remita, H.; Marignier, J.-L.; Delcourt, and M.-O. Radiation-Induced Synthesis of Mono- and Multi-Metallic Clusters and Nanocolloids. *New J. Chem.* **1998**, *22* (11), 1239–1255. https://doi.org/10.1039/A801445K.
- (39) Kalsin, A. M.; Pinchuk, A. O.; Smoukov, S. K.; Paszewski, M.; Schatz, G. C.; Grzybowski, B. A. Electrostatic Aggregation and Formation of Core–Shell Suprastructures in Binary Mixtures of Charged Metal Nanoparticles. *Nano Lett.* 2006, 6 (9), 1896–1903. https://doi.org/10.1021/nl060967m.
- (40) Fresnais, J.; Ishow, E.; Sandre, O.; Berret, J.-F. Electrostatic Co-Assembly of Magnetic Nanoparticles and Fluorescent Nanospheres: A Versatile Approach Towards Bimodal Nanorods. *Small* 2009, 5 (22), 2533–2536. https://doi.org/10.1002/smll.200900703.
- (41) Zhuang, J.; Wu, H.; Yang, Y.; Cao, Y. C. Supercrystalline Colloidal Particles from Artificial Atoms. J. Am. Chem. Soc. 2007, 129 (46), 14166–14167. https://doi.org/10.1021/ja076494i.
- (42) Donaldson, S. H. Jr.; Røyne, A.; Kristiansen, K.; Rapp, M. V.; Das, S.; Gebbie, M. A.; Lee, D. W.; Stock, P.; Valtiner, M.; Israelachvili, J. Developing a General Interaction Potential for Hydrophobic and Hydrophilic Interactions. *Langmuir* 2015, *31* (7), 2051–2064. https://doi.org/10.1021/la502115g.
- (43) Pansu, B.; Goldmann, C.; Constantin, D.; Impéror-Clerc, M.; Sadoc, J.-F. Softness-Driven Complexity in Supercrystals of Gold Nanoparticles. *Soft Matter* **2021**, *17* (26), 6461–6469. https://doi.org/10.1039/D1SM00617G.
- (44) Goodfellow, B. W.; Yu, Y.; Bosoy, C. A.; Smilgies, D.-M.; Korgel, B. A. The Role of Ligand Packing Frustration in Body-Centered Cubic (Bcc) Superlattices of Colloidal Nanocrystals. J. Phys. Chem. Lett. 2015, 6 (13), 2406–2412. https://doi.org/10.1021/acs.jpclett.5b00946.
- (45) Liu, Y.; Nie, H.; Bansil, R.; Steinhart, M.; Bang, J.; Lodge, T. P. Kinetics of Disorder-to-Fcc Phase Transition via an Intermediate Bcc State. *Phys. Rev. E* 2006, 73 (6), 061803. https://doi.org/10.1103/PhysRevE.73.061803.
- (46) Hajiw, S.; Pansu, B.; Sadoc, J.-F. Evidence for a C14 Frank–Kasper Phase in One-Size Gold Nanoparticle Superlattices. ACS Nano 2015, 9 (8), 8116–8121. https://doi.org/10.1021/acsnano.5b02216.
- (47) *Frank and Kasper phases*. Superlattices of nanoparticles. https://softqc.wordpress.com/frank-and-kasper-phases/ (accessed 2023-01-30).
- (48) Wei, W.; Bai, F.; Fan, H. Surfactant-Assisted Cooperative Self-Assembly of Nanoparticles into Active Nanostructures. *iScience* **2019**, *11*, 272–293. https://doi.org/10.1016/j.isci.2018.12.025.
- (49) Yadav, N. Cerium Oxide Nanostructures: Properties, Biomedical Applications and Surface Coatings. *3 Biotech* **2022**, *12* (5), 121. https://doi.org/10.1007/s13205-022-03186-3.
- (50) Scirè, S.; Palmisano, L. 1 Cerium and Cerium Oxide: A Brief Introduction. In *Cerium Oxide (CeO<sub>2</sub>): Synthesis, Properties and Applications*; Scirè, S., Palmisano, L., Eds.; Metal Oxides; Elsevier, 2020; pp 1–12. https://doi.org/10.1016/B978-0-12-815661-2.00001-3.
- (51) Mullins, D. R. The Surface Chemistry of Cerium Oxide. Surf. Sci. Rep. 2015, 70 (1), 42–85. https://doi.org/10.1016/j.surfrep.2014.12.001.
- (52) He, L.; Su, Y.; Lanhong, J.; Shi, S. Recent Advances of Cerium Oxide Nanoparticles in Synthesis, Luminescence and Biomedical Studies: A Review. J. Rare Earths 2015, 33 (8), 791–799. https://doi.org/10.1016/S1002-0721(14)60486-5.
- (53) Zikalala, N.; Matshetshe, K.; Parani, S.; Oluwafemi, O. S. Biosynthesis Protocols for Colloidal Metal Oxide Nanoparticles. Nano-Struct. Nano-Objects 2018, 16, 288–299. https://doi.org/10.1016/j.nanoso.2018.07.010.
- (54) Lee, Y.-J.; Kim, T.-G.; Sung, Y.-M. Lattice Distortion and Luminescence of CdSe/ZnSe Nanocrystals. Nanotechnology 2006, 17 (14), 3539. https://doi.org/10.1088/0957-4484/17/14/030.
- (55) Tok, A. I. Y.; Boey, F. Y. C.; Dong, Z.; Sun, X. L. Hydrothermal Synthesis of CeO2 Nano-Particles. J. Mater. Process. Technol. 2007, 190 (1), 217–222. https://doi.org/10.1016/j.jmatprotec.2007.02.042.
- (56) Farahmandjou, M.; Zarinkamar, M.; Firoozabadi, T. P.; Farahmandjou, M.; Zarinkamar, M.; Firoozabadi, T. P. Synthesis of Cerium Oxide (CeO2) Nanoparticles Using Simple CO-Precipitation Method. *Rev. Mex. Física* 2016, 62 (5), 496–499.
- (57) Chen, P. Crystal Sizes and Energy Gaps of Cerium Oxide Using Co-Precipitation Method. *Mater. Sci. Appl.* **2022**, *13* (4), 213–231. https://doi.org/10.4236/msa.2022.134012.

- (58) Samiee, S.; Goharshadi, E. K. Effects of Different Precursors on Size and Optical Properties of Ceria Nanoparticles Prepared by Microwave-Assisted Method. *Mater. Res. Bull.* **2012**, 47 (4), 1089–1095. https://doi.org/10.1016/j.materresbull.2011.12.058.
- (59) Hwang, C.-C.; Wu, T.-Y.; Wan, J.; Tsai, J.-S. Development of a Novel Combustion Synthesis Method for Synthesizing of Ceramic Oxide Powders. *Mater. Sci. Eng. B* **2004**, *111* (1), 49–56. https://doi.org/10.1016/j.mseb.2004.03.023.
- (60) Wang, G.; Mu, Q.; Chen, T.; Wang, Y. Synthesis, Characterization and Photoluminescence of CeO2 Nanoparticles by a Facile Method at Room Temperature. J. Alloys Compd. 2010, 493 (1), 202–207. https://doi.org/10.1016/j.jallcom.2009.12.053.
- (61) S. Chaudhary, Y.; Panigrahi, S.; Nayak, S.; Satpati, B.; Bhattacharjee, S.; Kulkarni, N. Facile Synthesis of Ultra-Small Monodisperse Ceria Nanocrystals at Room Temperature and Their Catalytic Activity under Visible Light. J. Mater. Chem. 2010, 20 (12), 2381–2385. https://doi.org/10.1039/B922914K.
- (62) Li, C. R.; Cui, M. Y.; Sun, Q. T.; Dong, W. J.; Zheng, Y. Y.; Tsukamoto, K.; Chen, B. Y.; Tang, W. H. Nanostructures and Optical Properties of Hydrothermal Synthesized CeOHCO3 and Calcined CeO2 with PVP Assistance. J. Alloys Compd. 2010, 504 (2), 498–502. https://doi.org/10.1016/j.jallcom.2010.05.151.
- (63) Yang, S.; Gao, L. Controlled Synthesis and Self-Assembly of CeO2 Nanocubes. J. Am. Chem. Soc. 2006, 128 (29), 9330– 9331. https://doi.org/10.1021/ja063359h.
- (64) Wang, D.; Kang, Y.; Doan-Nguyen, V.; Chen, J.; Küngas, R.; Wieder, N. L.; Bakhmutsky, K.; Gorte, R. J.; Murray, C. B. Synthesis and Oxygen Storage Capacity of Two-Dimensional Ceria Nanocrystals. *Angew. Chem.* 2011, *123* (19), 4470–4473. https://doi.org/10.1002/ange.201101043.
- Luchini, A.; Vitiello, G.; Rossi, F.; Ballesteros, O. R. D.; Radulescu, A.; D'Errico, G.; Montesarchio, D.; Fernández, C. de J.; Paduano, L. Developing Functionalized Fe 3 O 4 –Au Nanoparticles: A Physico-Chemical Insight. *Phys. Chem. Chem. Phys.* 2015, 17 (8), 6087–6097. https://doi.org/10.1039/C4CP05854B.
- (66) Kluenker, M.; Mondeshki, M.; Nawaz Tahir, M.; Tremel, W. Monitoring Thiol–Ligand Exchange on Au Nanoparticle Surfaces. *Langmuir* **2018**, *34* (4), 1700–1710. https://doi.org/10.1021/acs.langmuir.7b04015.
- (67) A. Bohara, R.; D. Thorat, N.; H. Pawar, S. Role of Functionalization: Strategies to Explore Potential Nano-Bio Applications of Magnetic Nanoparticles. *RSC Adv.* **2016**, *6* (50), 43989–44012. https://doi.org/10.1039/C6RA02129H.
- (68) Wei, H.; Insin, N.; Lee, J.; Han, H.-S.; Cordero, J. M.; Liu, W.; Bawendi, M. G. Compact Zwitterion-Coated Iron Oxide Nanoparticles for Biological Applications. *Nano Lett.* **2012**, *12* (1), 22–25. https://doi.org/10.1021/nl202721q.
- (69) Woehrle, G. H.; Brown, L. O.; Hutchison, J. E. Thiol-Functionalized, 1.5-Nm Gold Nanoparticles through Ligand Exchange Reactions: Scope and Mechanism of Ligand Exchange. J. Am. Chem. Soc. 2005, 127 (7), 2172–2183. https://doi.org/10.1021/ja0457718.
- (70) A review of defect structure and chemistry in ceria and its solid solutions Chemical Society Reviews (RSC Publishing) DOI:10.1039/C9CS00588A. https://pubs.rsc.org/en/content/articlehtml/2020/cs/c9cs00588a (accessed 2023-01-24).
- (71) A. Rodriguez, J.; C. Grinter, D.; Liu, Z.; M. Palomino, R.; D. Senanayake, S. Ceria-Based Model Catalysts: Fundamental Studies on the Importance of the Metal–Ceria Interface in CO Oxidation, the Water–Gas Shift, CO 2 Hydrogenation, and Methane and Alcohol Reforming. *Chem. Soc. Rev.* 2017, *46* (7), 1824–1841. https://doi.org/10.1039/C6CS00863A.
- (72) Fiorenza, R.; Spitaleri, L.; Gulino, A.; Scirè, S. Ru–Pd Bimetallic Catalysts Supported on CeO2-MnOX Oxides as Efficient Systems for H2 Purification through CO Preferential Oxidation. *Catalysts* 2018, 8 (5), 203. https://doi.org/10.3390/catal8050203.
- (73) Le Gal, A.; Abanades, S. Dopant Incorporation in Ceria for Enhanced Water-Splitting Activity during Solar Thermochemical Hydrogen Generation. *J. Phys. Chem. C* 2012, *116* (25), 13516–13523. https://doi.org/10.1021/jp302146c.
- (74) *Ceria-Based Solid Catalysts for Organic Chemistry Vivier 2010 ChemSusChem Wiley Online Library*. https://chemistryeurope.onlinelibrary.wiley.com/doi/full/10.1002/cssc.201000054 (accessed 2023-01-24).
- (75) Yang, C.; Yu, X.; Heißler, S.; Nefedov, A.; Colussi, S.; Llorca, J.; Trovarelli, A.; Wang, Y.; Wöll, C. Surface Faceting and Reconstruction of Ceria Nanoparticles. *Angew. Chem. Int. Ed.* 2017, 56 (1), 375–379. https://doi.org/10.1002/anie.201609179.
- (76) Cook, G. B.; Cooper, R. F.; Wu, T. Chemical Diffusion and Crystalline Nucleation during Oxidation of Ferrous Iron-Bearing Magnesium Aluminosilicate Glass. J. Non-Cryst. Solids 1990, 120 (1), 207–222. https://doi.org/10.1016/0022-3093(90)90205-Z.
- (77) Hedrick, J. B.; Sinha, S. P. Cerium-Based Polishing Compounds: Discovery to Manufacture. J. Alloys Compd. 1994, 207– 208, 377–382. https://doi.org/10.1016/0925-8388(94)90243-7.
- (78) Kilbourn, B. T. Cerium and Cerium Compounds. In *Kirk-Othmer Encyclopedia of Chemical Technology*; John Wiley & Sons, Ltd, 2011; pp 1–23. https://doi.org/10.1002/0471238961.0305180911091202.a01.pub3.
- (79) Montemor, M. F. Functional and Smart Coatings for Corrosion Protection: A Review of Recent Advances. Surf. Coat. Technol. 2014, 258, 17–37. https://doi.org/10.1016/j.surfcoat.2014.06.031.
- (80) Rajeshkumar, S.; Naik, P. Synthesis and Biomedical Applications of Cerium Oxide Nanoparticles A Review. *Biotechnol. Rep.* 2018, 17, 1–5. https://doi.org/10.1016/j.btre.2017.11.008.
- (81) Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. Nanomaterials with Enzyme-like Characteristics (Nanozymes): Next-Generation Artificial Enzymes (II). *Chem. Soc. Rev.* 2019, 48 (4), 1004–1076. https://doi.org/10.1039/C8CS00457A.
- (82) Walkey, C.; Das, S.; Seal, S.; Erlichman, J.; Heckman, K.; Ghibelli, L.; Traversa, E.; F. McGinnis, J.; T. Self, W. Catalytic Properties and Biomedical Applications of Cerium Oxide Nanoparticles. *Environ. Sci. Nano* 2015, 2 (1), 33–53. https://doi.org/10.1039/C4EN00138A.
- (83) Dhall, A.; Self, W. Cerium Oxide Nanoparticles: A Brief Review of Their Synthesis Methods and Biomedical Applications. *Antioxidants* **2018**, 7 (8), 97. https://doi.org/10.3390/antiox7080097.
- (84) Antioxidant Properties of Cerium Oxide Nanocrystals as a Function of Nanocrystal Diameter and Surface Coating / ACS Nano. https://pubs.acs.org/doi/full/10.1021/nn4026806 (accessed 2023-01-24).
- (85) Luchini, A.; Gerelli, Y.; Fragneto, G.; Nylander, T.; Pálsson, G. K.; Appavou, M.-S.; Paduano, L. Neutron Reflectometry Reveals the Interaction between Functionalized SPIONs and the Surface of Lipid Bilayers. *Colloids Surf. B Biointerfaces* 2017, 151, 76–87. https://doi.org/10.1016/j.colsurfb.2016.12.005.

- (86) Russo Krauss, I.; Picariello, A.; Vitiello, G.; De Santis, A.; Koutsioubas, A.; Houston, J. E.; Fragneto, G.; Paduano, L. Interaction with Human Serum Proteins Reveals Biocompatibility of Phosphocholine-Functionalized SPIONs and Formation of Albumin-Decorated Nanoparticles. *Langmuir* 2020, *36* (30), 8777–8791. https://doi.org/10.1021/acs.langmuir.0c01083.
- (87) Girija, D. K.; Naik, H.; Sudhamani, cn; Kumar, B. V. Cerium Oxide Nanoparticles-a Green, Reusable, and Highly Efficient Heterogeneous Catalyst for the Synthesis of Polyhydroquinolines Under Solvent-Free Conditions. Arch Appl Sci Res 2011, 3.
- (88) Okuda, M.; Suzumoto, Y.; Yamashita, I. Bioinspired Synthesis of Homogenous Cerium Oxide Nanoparticles and Two- or Three-Dimensional Nanoparticle Arrays Using Protein Supramolecules. *Cryst. Growth Des.* 2011, 11 (6), 2540–2545. https://doi.org/10.1021/cg200299y.
- (89) Jayakumar, G.; Irudayaraj, A. A.; Raj, A. D. Particle Size Effect on the Properties of Cerium Oxide (CeO2) Nanoparticles Synthesized by Hydrothermal Method. *Mech. Mater. Sci. Eng. J.* **2017**, *9* (1). https://doi.org/10.2412/mmse.3.4.481.
- (90) Prieur, D.; Bonani, W.; Popa, K.; Walter, O.; Kriegsman, K. W.; Engelhard, M. H.; Guo, X.; Eloirdi, R.; Gouder, T.; Beck, A.; Vitova, T.; Scheinost, A. C.; Kvashnina, K.; Martin, P. Size Dependence of Lattice Parameter and Electronic Structure in CeO2 Nanoparticles. *Inorg. Chem.* 2020, 59 (8), 5760–5767. https://doi.org/10.1021/acs.inorgchem.0c00506.
- (91) Mokkelbost, T.; Kaus, I.; Grande, T.; Einarsrud, M.-A. Combustion Synthesis and Characterization of Nanocrystalline CeO2-Based Powders. *Chem. Mater.* 2004, *16* (25), 5489–5494. https://doi.org/10.1021/cm048583p.
- (92) Rahdar, A.; Beyzaei, H.; Askari, F.; Kyzas, G. Z. Gum-Based Cerium Oxide Nanoparticles for Antimicrobial Assay. Appl. Phys. A 2020, 126 (5), 324. https://doi.org/10.1007/s00339-020-03507-4.
- (93) Helman, A.; Borgström, M. T.; van Weert, M.; Verheijen, M. A.; Bakkers, E. P. A. M. Synthesis and Electronic Devices of III–V Nanowires. In *Encyclopedia of Materials: Science and Technology*; Buschow, K. H. J., Cahn, R. W., Flemings, M. C., Ilschner, B., Kramer, E. J., Mahajan, S., Veyssière, P., Eds.; Elsevier: Oxford, 2008; pp 1–6. https://doi.org/10.1016/B978-008043152-9.02178-3.
- (94) Chen, X.; Chi, L. Chapter 8 Interfacial Assembly of Nanoparticles into Higher-Order Patterned Structures. In *Frontiers of Nanoscience*; Wilde, G., Ed.; Nanostructured Materials; Elsevier, 2009; Vol. 1, pp 326–365. https://doi.org/10.1016/B978-0-08-044965-4.50011-0.
- (95) Bloemen, M.; Brullot, W.; Luong, T. T.; Geukens, N.; Gils, A.; Verbiest, T. Improved Functionalization of Oleic Acid-Coated Iron Oxide Nanoparticles for Biomedical Applications. J. Nanoparticle Res. 2012, 14 (9), 1100. https://doi.org/10.1007/s11051-012-1100-5.
- (96) Parvizian, F.; Ansari, F.; Bandehali, S. Oleic Acid-Functionalized TiO2 Nanoparticles for Fabrication of PES-Based Nanofiltration Membranes. *Chem. Eng. Res. Des.* 2020, 156, 433–441. https://doi.org/10.1016/j.cherd.2020.02.019.
- (97) Jadhav, N. V.; Prasad, A. I.; Kumar, A.; Mishra, R.; Dhara, S.; Babu, K. R.; Prajapat, C. L.; Misra, N. L.; Ningthoujam, R. S.; Pandey, B. N.; Vatsa, R. K. Synthesis of Oleic Acid Functionalized Fe3O4 Magnetic Nanoparticles and Studying Their Interaction with Tumor Cells for Potential Hyperthermia Applications. *Colloids Surf. B Biointerfaces* 2013, 108, 158–168. https://doi.org/10.1016/j.colsurfb.2013.02.035.
- (98) Garcia, P. R. A. F.; Prymak, O.; Grasmik, V.; Pappert, K.; Wlysses, W.; Otubo, L.; Epple, M.; Oliveira, C. L. P. An in Situ SAXS Investigation of the Formation of Silver Nanoparticles and Bimetallic Silver–Gold Nanoparticles in Controlled Wet-Chemical Reduction Synthesis. *Nanoscale Adv.* 2020, 2 (1), 225–238. https://doi.org/10.1039/C9NA00569B.
- (99) Tanford, C. Micelle Shape and Size. J. Phys. Chem. 1972, 76 (21), 3020–3024. https://doi.org/10.1021/j100665a018.
- (100) Sanchez-Burgos, I.; Sanz, E.; Vega, C.; R. Espinosa, J. Fcc vs. Hcp Competition in Colloidal Hard-Sphere Nucleation: On Their Relative Stability, Interfacial Free Energy and Nucleation Rate. *Phys. Chem. Chem. Phys.* 2021, 23 (35), 19611–19626. https://doi.org/10.1039/D1CP01784E.
- (101) Lindvig, T.; Michelsen, M. L.; Kontogeorgis, G. M. A Flory–Huggins Model Based on the Hansen Solubility Parameters. *Fluid Phase Equilibria* 2002, 203 (1), 247–260. https://doi.org/10.1016/S0378-3812(02)00184-X.
- (102) Luo, J.; Xie, Z.; Y. Lam, J. W.; Cheng, L.; Chen, H.; Qiu, C.; Sing Kwok, H.; Zhan, X.; Liu, Y.; Zhu, D.; Zhong Tang, B. Aggregation-Induced Emission of 1-Methyl-1,2,3,4,5-Pentaphenylsilole. *Chem. Commun.* 2001, 0 (18), 1740–1741. https://doi.org/10.1039/B105159H.
- (103) Chen, J.; Law, C. C. W.; Lam, J. W. Y.; Dong, Y.; Lo, S. M. F.; Williams, I. D.; Zhu, D.; Tang, B. Z. Synthesis, Light Emission, Nanoaggregation, and Restricted Intramolecular Rotation of 1,1-Substituted 2,3,4,5-Tetraphenylsiloles. *Chem. Mater.* 2003, 15 (7), 1535–1546. https://doi.org/10.1021/cm021715z.
- (104) Zhao, Z.; Zhang, H.; Lam, J. W. Y.; Tang, B. Z. Aggregation-Induced Emission: New Vistas at the Aggregate Level. *Angew. Chem. Int. Ed.* **2020**, *59* (25), 9888–9907. https://doi.org/10.1002/anie.201916729.
- (105) Reddy, D. S.; Kang, B.; Yu, S. C.; Gunasekhar, K. R.; Rao, K. N.; Reddy, P. S. Strain, Luminescence, and Electrical Properties of Zn1-xMnxS Nanocrystalline Films Prepared on Silicon Wafers. J. Appl. Phys. 2008, 104 (1), 013532. https://doi.org/10.1063/1.2955270.
- (106) Mayeshiba, T.; Morgan, D. Strain Effects on Oxygen Vacancy Formation Energy in Perovskites. Solid State Ion. 2017, 311, 105–117. https://doi.org/10.1016/j.ssi.2017.09.021.
- (107) Jassby, D.; Wiesner, M. Characterization of ZnS Nanoparticle Aggregation Using Photoluminescence. Langmuir ACS J. Surf. Colloids 2011, 27 (3), 902–908. https://doi.org/10.1021/la103470r.
- (108) Hildebrandt, N.; Wegner, K. D.; Algar, W. R. Luminescent Terbium Complexes: Superior Förster Resonance Energy Transfer Donors for Flexible and Sensitive Multiplexed Biosensing. *Coord. Chem. Rev.* 2014, 273–274, 125–138. https://doi.org/10.1016/j.ccr.2014.01.020.
- (109) Geißler, D.; Linden, S.; Liermann, K.; Wegner, K. D.; Charbonnière, L. J.; Hildebrandt, N. Lanthanides and Quantum Dots as Förster Resonance Energy Transfer Agents for Diagnostics and Cellular Imaging. *Inorg. Chem.* 2014, 53 (4), 1824–1838. https://doi.org/10.1021/ic4017883.
- (110) Ifijen, I. H.; Maliki, M.; Anegbe, B. Synthesis, Photocatalytic Degradation and Antibacterial Properties of Selenium or Silver Doped Zinc Oxide Nanoparticles: A Detailed Review. OpenNano 2022, 8, 100082. https://doi.org/10.1016/j.onano.2022.100082.
- (111) Zinc Oxide Materials and Devices Grown by Molecular Beam Epitaxy ScienceDirect. https://www.sciencedirect.com/science/article/pii/B9780128121368000165 (accessed 2023-01-26).

- (112) Arya, S. K.; Saha, S.; Ramirez-Vick, J. E.; Gupta, V.; Bhansali, S.; Singh, S. P. Recent Advances in ZnO Nanostructures and Thin Films for Biosensor Applications: Review. Anal. Chim. Acta 2012, 737, 1–21. https://doi.org/10.1016/j.aca.2012.05.048.
- (113) Gurylev, V.; Perng, T. P. Defect Engineering of ZnO: Review on Oxygen and Zinc Vacancies. J. Eur. Ceram. Soc. 2021, 41 (10), 4977–4996. https://doi.org/10.1016/j.jeurceramsoc.2021.03.031.
- (114) Schmidt-Mende, L.; MacManus-Driscoll, J. L. ZnO Nanostructures, Defects, and Devices. *Mater. Today* 2007, *10* (5), 40– 48. https://doi.org/10.1016/S1369-7021(07)70078-0.
- (115) Schilling, W. Properties of Frenkel Defects. J. Nucl. Mater. **1994**, 216, 45–48. https://doi.org/10.1016/0022-3115(94)90005-1.
- (116) Ismail, A. M.; Menazea, A. A.; Kabary, H. A.; El-Sherbiny, A. E.; Samy, A. The Influence of Calcination Temperature on Structural and Antimicrobial Characteristics of Zinc Oxide Nanoparticles Synthesized by Sol–Gel Method. J. Mol. Struct. 2019, 1196, 332–337. https://doi.org/10.1016/j.molstruc.2019.06.084.
- (117) Chandekar, K. V.; Shkir, Mohd.; Khan, A.; Al-Shehri, B. M.; Hamdy, M. S.; AlFaify, S.; El-Toni, M. A.; Aldalbahi, A.; Ansari, A. A.; Ghaithan, H. A Facile One-Pot Flash Combustion Synthesis of La@ZnO Nanoparticles and Their Characterizations for Optoelectronic and Photocatalysis Applications. J. Photochem. Photobiol. Chem. 2020, 395, 112465. https://doi.org/10.1016/j.jphotochem.2020.112465.
- (118) Wasly, H. S.; El-Sadek, M. S. A.; Henini, M. Influence of Reaction Time and Synthesis Temperature on the Physical Properties of ZnO Nanoparticles Synthesized by the Hydrothermal Method. *Appl. Phys. A* 2018, *124* (1), 76. https://doi.org/10.1007/s00339-017-1482-4.
- (119) Karakaya, S. Effect of Fluorine and Boron Co-Doping on ZnO Thin Films: Structural, Luminescence Properties and Hall Effect Measurements. J. Mater. Sci. Mater. Electron. 2018, 29 (5), 4080–4088. https://doi.org/10.1007/s10854-017-8352-x.
- (120) Janotti, A.; Walle, C. G. V. de. Fundamentals of Zinc Oxide as a Semiconductor. *Rep. Prog. Phys.* 2009, 72 (12), 126501. https://doi.org/10.1088/0034-4885/72/12/126501.
- (121) A comprehensive review of ZnO materials and devices: Journal of Applied Physics: Vol 98, No 4. https://aip.scitation.org/doi/full/10.1063/1.1992666 (accessed 2023-01-26).
- (122) Papari, G. P.; Silvestri, B.; Vitiello, G.; De Stefano, L.; Rea, I.; Luciani, G.; Aronne, A.; Andreone, A. Morphological, Structural, and Charge Transfer Properties of F-Doped ZnO: A Spectroscopic Investigation. J. Phys. Chem. C 2017, 121 (29), 16012–16020. https://doi.org/10.1021/acs.jpcc.7b04821.
- (123) Salameh, B.; Alsmadi, A. M.; El Akkad, F. Physicochemistry of Point Defects in Fluorine Doped Zinc Tin Oxide Thin Films. *Thin Solid Films* 2017, 626, 76–84. https://doi.org/10.1016/j.tsf.2017.02.021.
- (124) Anusha, M.; Arivuoli, D. High Intense Violet Luminescence in Fluorine Doped Zinc Oxide (FZO) Thin Films Deposited by Aerosol Assisted CVD. J. Alloys Compd. 2013, 580, 131–136. https://doi.org/10.1016/j.jallcom.2013.05.073.
- (125) Sivakumar, P.; Lee, M.; Kim, Y.-S.; Suk Shim, M. Photo-Triggered Antibacterial and Anticancer Activities of Zinc Oxide Nanoparticles. J. Mater. Chem. B 2018, 6 (30), 4852–4871. https://doi.org/10.1039/C8TB00948A.
- (126) Frizzo, M. S.; Feuser, P. E.; Berres, P. H.; Ricci-Júnior, E.; Campos, C. E. M.; Costa, C.; de Araújo, P. H. H.; Sayer, C. Simultaneous Encapsulation of Zinc Oxide and Octocrylene in Poly (Methyl Methacrylate-Co-Styrene) Nanoparticles Obtained by Miniemulsion Polymerization for Use in Sunscreen Formulations. *Colloids Surf. Physicochem. Eng. Asp.* 2019, 561, 39–46. https://doi.org/10.1016/j.colsurfa.2018.10.062.
- (127) Wibowo, A.; Agung Marsudi, M.; Ikhlasul Amal, M.; Bagas Ananda, M.; Stephanie, R.; Ardy, H.; Jaya Diguna, L. ZnO Nanostructured Materials for Emerging Solar Cell Applications. *RSC Adv.* 2020, *10* (70), 42838–42859. https://doi.org/10.1039/D0RA07689A.
- (128) Qin, X.; Xu, H.; Zhang, G.; Wang, J.; Wang, Z.; Zhao, Y.; Wang, Z.; Tan, T.; Bockstaller, M. R.; Zhang, L.; Matyjaszewski, K. Enhancing the Performance of Rubber with Nano ZnO as Activators. ACS Appl. Mater. Interfaces 2020, 12 (42), 48007– 48015. https://doi.org/10.1021/acsami.0c15114.
- (129) Shetti, N. P.; Bukkitgar, S. D.; Reddy, K. R.; Reddy, Ch. V.; Aminabhavi, T. M. ZnO-Based Nanostructured Electrodes for Electrochemical Sensors and Biosensors in Biomedical Applications. *Biosens. Bioelectron.* 2019, 141, 111417. https://doi.org/10.1016/j.bios.2019.111417.
- (130) Napi, M. L. M.; Sultan, S. M.; Ismail, R.; How, K. W.; Ahmad, M. K. Electrochemical-Based Biosensors on Different Zinc Oxide Nanostructures: A Review. *Materials* 2019, *12* (18), 2985. https://doi.org/10.3390/ma12182985.
- (131) Biosensor Technologies for Detection of Biomolecules; Bhalla, N., Jolly, P., Formisano, N., Estrela, P., Series Eds.; Essays in Biochemistry; Portland Press, 2016.
- (132) Alhadrami, H. A. Biosensors: Classifications, Medical Applications, and Future Prospective. *Biotechnol. Appl. Biochem.* 2018, 65 (3), 497–508. https://doi.org/10.1002/bab.1621.
- (133) Bhattarai, P.; Hameed, S. Basics of Biosensors and Nanobiosensors. In *Nanobiosensors*; John Wiley & Sons, Ltd, 2020; pp 1–22. https://doi.org/10.1002/9783527345137.ch1.
- (134) Kawamura, A.; Miyata, T. 4.2 Biosensors. In *Biomaterials Nanoarchitectonics*; Ebara, M., Ed.; William Andrew Publishing, 2016; pp 157–176. https://doi.org/10.1016/B978-0-323-37127-8.00010-8.
- (135) Ali, J.; Najeeb, J.; Asim Ali, M.; Farhan Aslam, M.; Raza, A. Biosensors: Their Fundamentals, Designs, Types and Most Recent Impactful Applications: A Review. J. Biosens. Bioelectron. 2017, 08 (01). https://doi.org/10.4172/2155-6210.1000235.
- (136) Mehrotra, P. Biosensors and Their Applications A Review. J. Oral Biol. Craniofacial Res. 2016, 6 (2), 153–159. https://doi.org/10.1016/j.jobcr.2015.12.002.
- (137) Marquette, C. A.; Blum, L. J. Luminol Electrochemiluminescence-Based Fibre Optic Biosensors for Flow Injection Analysis of Glucose and Lactate in Natural Samples. *Anal. Chim. Acta* **1999**, *381* (1), 1–10. https://doi.org/10.1016/S0003-2670(98)00703-X.
- (138) Malinee, M.; Kumar, A.; Dhiman, A.; Sharma, T. K. Chapter 8 Aptamer-Mediated Nanobiosensing for Health Monitoring. In Advanced Biosensors for Health Care Applications; Inamuddin, Khan, R., Mohammad, A., Asiri, A. M., Eds.; Elsevier, 2019; pp 227–248. https://doi.org/10.1016/B978-0-12-815743-5.00008-1.
- (139) Haleem, A.; Javaid, M.; Singh, R. P.; Suman, R.; Rab, S. Biosensors Applications in Medical Field: A Brief Review. Sens. Int. 2021, 2, 100100. https://doi.org/10.1016/j.sintl.2021.100100.

- (140) Thevenot, D. R.; Tóth, K.; Durst, R. A.; Wilson, G. S. Electrochemical Biosensors: Recommended Definitions and Classification. *Pure Appl. Chem.* **1999**, *71* (12), 2333–2348. https://doi.org/10.1351/pac199971122333.
- (141) Yang, F.; Ma, Y.; Stanciu, S. G.; Wu, A. Transduction Process-Based Classification of Biosensors. In *Nanobiosensors*; John Wiley & Sons, Ltd, 2020; pp 23–44. https://doi.org/10.1002/9783527345137.ch2.
- (142) Sawant, S. N. 13 Development of Biosensors From Biopolymer Composites. In *Biopolymer Composites in Electronics*; Sadasivuni, K. K., Ponnamma, D., Kim, J., Cabibihan, J.-J., AlMaadeed, M. A., Eds.; Elsevier, 2017; pp 353–383. https://doi.org/10.1016/B978-0-12-809261-3.00013-9.
- (143) Encyclopedia of Microbiology; Academic Press, 2009.
- (144) Smith, S.; Goodge, K.; Delaney, M.; Struzyk, A.; Tansey, N.; Frey, M. A Comprehensive Review of the Covalent Immobilization of Biomolecules onto Electrospun Nanofibers. *Nanomaterials* 2020, 10 (11), 2142. https://doi.org/10.3390/nano10112142.
- (145) Chianese, G.; Terracciano, M.; Moretta, R.; Cappiello, P.; Vitiello, G.; Aronne, A.; Schiattarella, C.; De Stefano, L.; Rea, I. Synthesis and Surface Modification of Nanostructured F-Doped ZnO: Toward a Transducer for Label-Free Optical Biosensing. *Appl. Sci.* 2019, 9 (16), 3380. https://doi.org/10.3390/app9163380.
- (146) Bashir, N.; Sood, M.; Bandral, J. D. Enzyme Immobilization and Its Applications in Food Processing: A Review. Int. J. Chem. Stud. 2020, 8 (2), 254–261. https://doi.org/10.22271/chemi.2020.v8.i2d.8779.
- (147) Lim, S. A.; Ahmed, M. U. Introduction to Food Biosensors. 2016. https://doi.org/10.1039/9781782623908-00001.
- (148) Gavrilaş, S.; Ursachi, C. Ştefan; Perţa-Crişan, S.; Munteanu, F.-D. Recent Trends in Biosensors for Environmental Quality Monitoring. Sensors 2022, 22 (4), 1513. https://doi.org/10.3390/s22041513.
- (149) Beek, W. J. E.; Wienk, M. M.; Kemerink, M.; Yang, X.; Janssen, R. A. J. Hybrid Zinc Oxide Conjugated Polymer Bulk Heterojunction Solar Cells. J. Phys. Chem. B 2005, 109 (19), 9505–9516. https://doi.org/10.1021/jp050745x.
- (150) Mammeri, F. Chapter 3 Nanostructured Flexible PVDF and Fluoropolymer-Based Hybrid Films. In Frontiers of Nanoscience; Benelmekki, M., Erbe, A., Eds.; Nanostructured Thin Films; Elsevier, 2019; Vol. 14, pp 67–101. https://doi.org/10.1016/B978-0-08-102572-7.00003-9.
- (151) Yilbas, B.; Al-Sharafi, A.; Ali, H. Self-Cleaning of Surfaces and Water Droplet Mobility; Elsevier, 2019.
- (152) Mishra, A.; Bhatt, N.; Bajpai, A. K. Chapter 12 Nanostructured Superhydrophobic Coatings for Solar Panel Applications. In *Nanomaterials-Based Coatings*; Nguyen Tri, P., Rtimi, S., Ouellet Plamondon, C. M., Eds.; Micro and Nano Technologies; Elsevier, 2019; pp 397–424. https://doi.org/10.1016/B978-0-12-815884-5.00012-0.
- (153) Kaliyaraj Selva Kumar, A.; Zhang, Y.; Li, D.; Compton, R. G. A Mini-Review: How Reliable Is the Drop Casting Technique? *Electrochem. Commun.* **2020**, *121*, 106867. https://doi.org/10.1016/j.elecom.2020.106867.
- (154) Al-Hada, N. M.; Saion, E.; Shaari, A.; Ahmad Kamarudin, M.; Gene, S. The Influence of Calcination Temperature on the Formation of Zinc Oxide Nanoparticles by Thermal-Treatment. *Appl. Mech. Mater.* 2013, 2014. https://doi.org/10.4028/www.scientific.net/AMM.446-447.181.
- (155) Guo, Y.; Wuttke, S.; Vimont, A.; Daturi, M.; Lavalley, J.-C.; Teinz, K.; Kemnitz, E. Novel Sol–Gel Prepared Zinc Fluoride : Synthesis, Characterisation and Acid–Base Sites Analysis. J. Mater. Chem. 2012, 22 (29), 14587–14593. https://doi.org/10.1039/C2JM31357J.
- (156) Goh, E. G.; Xu, X.; McCormick, P. G. Effect of Particle Size on the UV Absorbance of Zinc Oxide Nanoparticles. *Scr. Mater.* 2014, 78–79, 49–52. https://doi.org/10.1016/j.scriptamat.2014.01.033.
- (157) Singh, J.; Rathi, A.; Rawat, M.; Kumar, V.; Kim, K.-H. The Effect of Manganese Doping on Structural, Optical, and Photocatalytic Activity of Zinc Oxide Nanoparticles. *Compos. Part B Eng.* 2019, 166, 361–370. https://doi.org/10.1016/j.compositesb.2018.12.006.
- (158) Hammad, T. M.; Salem, J. K.; Harrison, R. G. Structure, Optical Properties and Synthesis of Co-Doped ZnO Superstructures. *Appl. Nanosci.* **2013**, *3* (2), 133–139. https://doi.org/10.1007/s13204-012-0077-9.
- (159) Oliva, J.; Diaz-Torres, L.; Torres-Castro, A.; Salas, P.; Perez-Mayen, L.; Rosa, E. D. la. Effect of TEA on the Blue Emission of ZnO Quantum Dots with High Quantum Yield. *Opt. Mater. Express* 2015, 5 (5), 1109–1121. https://doi.org/10.1364/OME.5.001109.
- (160) Sahai, A.; Goswami, N. Probing the Dominance of Interstitial Oxygen Defects in ZnO Nanoparticles through Structural and Optical Characterizations. *Ceram. Int.* 2014, 40 (9, Part B), 14569–14578. https://doi.org/10.1016/j.ceramint.2014.06.041.
- (161) Raji, R.; Gopchandran, K. G. ZnO Nanostructures with Tunable Visible Luminescence: Effects of Kinetics of Chemical Reduction and Annealing. J. Sci. Adv. Mater. Devices 2017, 2 (1), 51–58. https://doi.org/10.1016/j.jsamd.2017.02.002.
- (162) Djurišić, A. B.; Leung, Y. H.; Tam, K. H.; Ding, L.; Ge, W. K.; Chen, H. Y.; Gwo, S. Green, Yellow, and Orange Defect Emission from ZnO Nanostructures: Influence of Excitation Wavelength. *Appl. Phys. Lett.* 2006, 88 (10), 103107. https://doi.org/10.1063/1.2182096.
- (163) Kennedy, O. W.; White, E. R.; Howkins, A.; Williams, C. K.; Boyd, I. W.; Warburton, P. A.; Shaffer, M. S. P. Mapping the Origins of Luminescence in ZnO Nanowires by STEM-CL. J. Phys. Chem. Lett. 2019, 10 (3), 386–392. https://doi.org/10.1021/acs.jpclett.8b03286.
- (164) Fery-Forgues, S.; Lavabre, D. Are Fluorescence Quantum Yields So Tricky to Measure? A Demonstration Using Familiar Stationery Products. J. Chem. Educ. 1999, 76 (9), 1260. https://doi.org/10.1021/ed076p1260.
- (165) Samadi, M.; Zirak, M.; Naseri, A.; Khorashadizade, E.; Moshfegh, A. Z. Recent Progress on Doped ZnO Nanostructures for Visible-Light Photocatalysis. *Thin Solid Films* **2016**, 605, 2–19. https://doi.org/10.1016/j.tsf.2015.12.064.
- (166) Sharma, A.; Hayat, A.; Mishra, R. K.; Catanante, G.; Bhand, S.; Marty, J. L. Titanium Dioxide Nanoparticles (TiO2) Quenching Based Aptasensing Platform: Application to Ochratoxin A Detection. *Toxins* 2015, 7 (9), 3771–3784. https://doi.org/10.3390/toxins7093771.
- (167) Wang, S.; Li, W.; Chang, K.; Liu, J.; Guo, Q.; Sun, H.; Jiang, M.; Zhang, H.; Chen, J.; Hu, J. Localized Surface Plasmon Resonance-Based Abscisic Acid Biosensor Using Aptamer-Functionalized Gold Nanoparticles. *PLOS ONE* 2017, *12* (9), e0185530. https://doi.org/10.1371/journal.pone.0185530.
- (168) Katiyar, N.; Selvakumar, L. S.; Patra, S.; Thakur, M. S. Gold Nanoparticles Based Colorimetric Aptasensor for Theophylline. *Anal. Methods* 2013, 5 (3), 653–659. https://doi.org/10.1039/C2AY26133B.

- (169) Xia, Y.; Tang, D.; Wu, H.; Wang, X.; Cao, M.; He, H.; Wang, S. Cell Attachment/Detachment Behavior on Poly(N-Isopropylacrylamide)-Based Microgel Films: The Effect of Microgel Structure and Swelling Ratio. J. Mater. Sci. 2018, 53 (12), 8795–8806. https://doi.org/10.1007/s10853-018-2217-4.
- (170) Encyclopedia Britannica / Britannica. https://www.britannica.com/ (accessed 2023-01-30).
- (171) Chu, B.; Liu, T. Characterization of Nanoparticles by Scattering Techniques. J. Nanoparticle Res. 2000, 2 (1), 29–41. https://doi.org/10.1023/A:1010001822699.
- (172) Goyal, P. S.; Aswal, V. K. Use of sans and Saxs in Study of Nanoparticles. *Int. J. Nanosci.* **2005**, *04* (05n06), 987–994. https://doi.org/10.1142/S0219581X05003954.
- (173) Neutrons, X-rays and Light: Scattering Methods Applied to Soft Condensed Matter 1st Edition. https://www.elsevier.com/books/neutrons-x-rays-and-light-scattering-methods-applied-to-soft-condensedmatter/lindner/978-0-444-51122-5 (accessed 2023-01-30).
- (174) Hammouda, B. Probing Nanoscale Structures the sans Toolbox; 2008.
- (175) Lomakin, A.; Teplow, D. B.; Benedek, G. B. Quasielastic Light Scattering for Protein Assembly Studies. *Methods Mol. Biol. Clifton NJ* 2005, 299, 153–174. https://doi.org/10.1385/1-59259-874-9:153.
- (176) Johnson, C. S.; Gabriel, D. A. Laser Light Scattering; Dover Publications, 2015.
- (177) Brumberger, H. Modern Aspects of Small-Angle Scattering; Springer Netherlands, 2010.
- (178)Chen, S.; Avakian, H.; Burkert, V. D.; Eugenio, P.; Adams, G.; Amarian, M.; Ambrozewicz, P.; Anghinolfi, M.; Asryan, G.; Bagdasaryan, H.; Baillie, N.; Ball, J. P.; Baltzell, N. A.; Barrow, S.; Batourine, V.; Battaglieri, M.; Beard, K.; Bedlinskiy, I.; Bektasoglu, M.; Bellis, M.; Benmouna, N.; Berman, B. L.; Biselli, A. S.; Bonner, B. E.; Bouchigny, S.; Boiarinov, S.; Bosted, P.; Bradford, R.; Branford, D.; Briscoe, W. J.; Brooks, W. K.; Bültmann, S.; Butuceanu, C.; Calarco, J. R.; Careccia, S. L.; Carman, D. S.; Carnahan, B.; Cazes, A.; Cole, P. L.; Collins, P.; Coltharp, P.; Cords, D.; Corvisiero, P.; Crabb, D.; Crannell, H.; Crede, V.; Cummings, J. P.; Masi, R. D.; DeVita, R.; Sanctis, E. D.; Degtyarenko, P. V.; Denizli, H.; Dennis, L.; Deur, A.; Dharmawardane, K. V.; Dhuga, K. S.; Djalali, C.; Dodge, G. E.; Donnelly, J.; Doughty, D.; Dugger, M.; Dytman, S.; Dzyubak, O. P.; Egiyan, H.; Egiyan, K. S.; Fassi, L. E.; Elouadrhiri, L.; Fatemi, R.; Fedotov, G.; Feldman, G.; Feuerbach, R. J.; Forest, T. A.; Funsten, H.; Garçon, M.; Gavalian, G.; Gilfoyle, G. P.; Giovanetti, K. L.; Girod, F. X.; Goetz, J. T.; Golovatch, E.; Gonenc, A.; Gothe, R. W.; Griffioen, K. A.; Guidal, M.; Guillo, M.; Guler, N.; Guo, L.; Gyurjyan, V.; Hadjidakis, C.; Hafidi, K.; Hakobyan, H.; Hakobyan, R. S.; Hardie, J.; Heddle, D.; Hersman, F. W.; Hicks, K.; Hleiqawi, I.; Holtrop, M.; Huertas, M.; Hyde-Wright, C. E.; Ilieva, Y.; Ireland, D. G.; Ishkhanov, B. S.; Isupov, E. L.; Ito, M. M.; Jenkins, D.; Jo, H. S.; Joo, K.; Juengst, H. G.; Keith, C.; Kellie, J. D.; Khandaker, M.; Kim, K. Y.; Kim, K.; Kim, W.; Klein, A.; Klein, F. J.; Klusman, M.; Kossov, M.; Kramer, L. H.; Kubarovsky, V.; Kuhn, J.; Kuhn, S. E.; Kuleshov, S. V.; Lachniet, J.; Laget, J. M.; Langheinrich, J.; Lawrence, D.; Li, J.; Lima, A. C. S.; Livingston, K.; Lu, H.; Lukashin, K.; MacCormick, M.; Markov, N.; McAleer, S.; McKinnon, B.; McNabb, J. W. C.; Mecking, B. A.; Mestayer, M. D.; Meyer, C. A.; Mibe, T.; Mikhailov, K.; Minehart, R.; Mirazita, M.; Miskimen, R.; Mokeev, V.; Morand, L.; Morrow, S. A.; Moteabbed, M.; Mueller, J.; Mutchler, G. S.; Nadel-Turonski, P.; Napolitano, J.; Nasseripour, R.; Natasha, N.; Niccolai, S.; Niculescu, G.; Niculescu, I.; Niczyporuk, B. B.; Niroula, M. R.; Niyazov, R. A.; Nozar, M.; O'Rielly, G. V.; Osipenko, M.; Ostrovidov, A. I.; Park, K.; Pasyuk, E.; Paterson, C.; Philips, S. A.; Pierce, J.; Pivnyuk, N.; Pocanic, D.; Pogorelko, O.; Polli, E.; Popa, I.; Pozdniakov, S.; Preedom, B. M.; Price, J. W.; Prok, Y.; Protopopescu, D.; Qin, L. M.; Raue, B. A.; Riccardi, G.; Ricco, G.; Ripani, M.; Ritchie, B. G.; Ronchetti, F.; Rosner, G.; Rossi, P.; Rowntree, D.; Rubin, P. D.; Sabatié, F.; Salgado, C.; Santoro, J. P.; Sapunenko, V.; Schumacher, R. A.; Serov, V. S.; Sharabian, Y. G.; Shaw, J.; Shvedunov, N. V.; Skabelin, A. V.; Smith, E. S.; Smith, L. C.; Sober, D. I.; Stavinsky, A.; Stepanyan, S. S.; Stepanyan, S.; Stokes, B. E.; Stoler, P.; Strakovsky, I. I.; Strauch, S.; Suleiman, R.; Taiuti, M.; Tedeschi, D. J.; Thoma, U.; Tkabladze, A.; Tkachenko, S.; Todor, L.; Tur, C.; Ungaro, M.; Vanderhaeghen, M.; Vineyard, M. F.; Vlassov, A. V.; Watts, D. P.; Weinstein, L. B.; Weygand, D. P.; Williams, M.; Wolin, E.; Wood, M. H.; Yegneswaran, A.; Yun, J.; Zana, L.; Zhang, J.; Zhao, B.; Zhao, Z. Measurement of Deeply Virtual Compton Scattering with a Polarized-Proton Target. Phys. Rev. Lett. 2006, 97 (7), 072002. https://doi.org/10.1103/PhysRevLett.97.072002.
- (179) *Progress in Rubber Nanocomposites 1st Edition*. https://www.elsevier.com/books/progress-in-rubbernanocomposites/thomas/978-0-08-100409-8 (accessed 2023-01-30).
- (180) *What is Microscopy?*. The University of Edinburgh. https://www.ed.ac.uk/clinical-sciences/edinburgh-imaging/for-patients-study-participants/tell-me-more-about-my-scan/what-is-microscopy (accessed 2023-01-30).
- (181) *Treccani La cultura Italiana Enciclopedia della scienza e della tecnica*. https://www.treccani.it/enciclopedia/elenco-opere/Enciclopedia\_della\_Scienza\_e\_della\_Tecnica (accessed 2023-01-30).
- (182) Optical nanoscopy / SpringerLink. https://link.springer.com/article/10.1007/s40766-020-00008-1 (accessed 2023-01-30).
- (183) Bell, S.; Morris, K. An Introduction to Microscopy; CRC Press, 2009.
- (184) Transmission Electron Microscopy: Physics of Image Formation; Springer Series in Optical Sciences; Springer: New York, NY, 2008; Vol. 36. https://doi.org/10.1007/978-0-387-40093-8.
- (185) Cryo-TEM Imaging the Flow-Induced Transition from Vesicles to Threadlike Micelles / The Journal of Physical Chemistry B. https://pubs.acs.org/doi/full/10.1021/jp0002998 (accessed 2023-01-30).
- (186) *AFM* imaging of extracellular vesicles / Encyclopedia MDPI. https://encyclopedia.pub/item/revision/4ba0443f27d17d0211e287b4435486ee (accessed 2023-01-30).
- (187) Farré, M.; Barceló, D. Introduction to the Analysis and Risk of Nanomaterials in Environmental and Food Samples. In *Comprehensive Analytical Chemistry*, 2012; Vol. 59, pp 1–32. https://doi.org/10.1016/B978-0-444-56328-6.00001-3.
- (188) Hofmann, A. Spectroscopic Techniques: I Spectrophotometric Techniques. In Principles and Techniques of Biochemistry and Molecular Biology; Wilson, K., Walker, J., Eds.; Cambridge University Press, 2010; pp 477–521. https://doi.org/10.1017/CBO9780511841477.013.
- (189) Principles of Fluorescence Spectroscopy; Lakowicz, J. R., Ed.; Springer US: Boston, MA, 2006. https://doi.org/10.1007/978-0-387-46312-4.
- (190) Solar absorber material and system designs for photothermal water vaporization towards clean water and energy production
  Energy & Environmental Science (RSC Publishing). https://pubs.rsc.org/en/content/articlelanding/2019/ee/c8ee01146j
  (accessed 2023-01-30).

- (191) Tauc, J.; Grigorovici, R.; Vancu, A. Optical Properties and Electronic Structure of Amorphous Germanium. *Phys. Status Solidi* B 1966, 15 (2), 627–637. https://doi.org/10.1002/pssb.19660150224.
- (192) Makuła, P.; Pacia, M.; Macyk, W. How To Correctly Determine the Band Gap Energy of Modified Semiconductor Photocatalysts Based on UV-Vis Spectra. J. Phys. Chem. Lett. 2018, 9 (23), 6814–6817. https://doi.org/10.1021/acs.jpclett.8b02892.
- (193) Pankove, J. I.; Kiewit, D. A. Optical Processes in Semiconductors. J. Electrochem. Soc. **1972**, 119 (5), 156Ca. https://doi.org/10.1149/1.2404256.
- (194) López, R.; Gómez, R. Band-Gap Energy Estimation from Diffuse Reflectance Measurements on Sol–Gel and Commercial TiO2: A Comparative Study. *J. Sol-Gel Sci. Technol.* **2012**, *61* (1), 1–7. https://doi.org/10.1007/s10971-011-2582-9.
- (195) Yates, K.; West, R. H. Small Area X-Ray Photoelectron Spectroscopy. Surf. Interface Anal. 1983, 5 (5), 217–221. https://doi.org/10.1002/sia.740050508.
- (196) Huong, P. V.; Verma, A. L.; Chaminade, J.-P.; Nganga, L.; Frison, J.-C. Characterization of Materials by Micro-Raman Spectroscopy. *Mater. Sci. Eng. B* 1990, 5 (2), 255–260. https://doi.org/10.1016/0921-5107(90)90064-I.
- (197) Bergwerff, J. A.; Visser, T.; Leliveld, G.; Rossenaar, B. D.; de Jong, K. P.; Weckhuysen, B. M. Envisaging the Physicochemical Processes during the Preparation of Supported Catalysts: Raman Microscopy on the Impregnation of Mo onto Al2O3 Extrudates. J. Am. Chem. Soc. 2004, 126 (44), 14548–14556. https://doi.org/10.1021/ja040107c.
- (198) Fragneto-Cusani, G. Neutron Reflectivity at the Solid/Liquid Interface: Examples of Applications in Biophysics. J. Phys. Condens. Matter 2001, 13 (21), 4973–4989. https://doi.org/10.1088/0953-8984/13/21/322.
- (199) Pethrick, R. A. Polymers and Neutron Scattering, Edited by J. S. Higgins and H. C. Benoit. Oxford University Press, Oxford, 1994. Pp. Xix + 436, Price £65.00. ISBN 0-19-85-1003-9. *Polym. Int.* 1995, 37 (2), 149–150. https://doi.org/10.1002/pi.1995.210370212.
- (200) Theis-Bröhl, K.; Gutfreund, P.; Vorobiev, A.; Wolff, M.; P. Toperverg, B.; A. Dura, J.; A. Borchers, J. Self Assembly of Magnetic Nanoparticles at Silicon Surfaces. *Soft Matter* 2015, *11* (23), 4695–4704. https://doi.org/10.1039/C5SM00484E.
- (201) Gibaud, A.; Chebil, M. S.; Beuvier, T. X-Ray Reflectivity. In Surface Science Techniques; Bracco, G., Holst, B., Eds.; Springer Series in Surface Sciences; Springer: Berlin, Heidelberg, 2013; pp 191–216. https://doi.org/10.1007/978-3-642-34243-1\_7.
- (202) X-Rays and Their Interaction with Matter. In *Elements of Modern X-ray Physics*; John Wiley & Sons, Ltd, 2011; pp 1–28. https://doi.org/10.1002/9781119998365.ch1.
- (203) Stracci, G. Libello Di Ellissometria e Spettrofotometria G.Stracci.