Chapter 1: Introduction

1.1 Introduction
1.2 Bio-nanomaterials
1.3 Semiconductor nanomaterials
1.4 Biopolymers
1.5 Catalase
1.6 Chlorophyll
1.7 Hydrilla verticillata
1.1 Introduction

The word “nano” in Nanotechnology comes from Greek word nanos which means dwarf. One nanometer is one billionth meter that is about 100,000 times smaller than the diameter of a single human hair. There are various definitions [1.1] on nanomaterials in the literature and it should be mentioned that the International Standards Organisation (ISO) has recently developed a technical specification on the terminology and definitions of nano-objects. [1.2] The specification does not define the term nanomaterial, but it describes the term nano-object as a material with one, two or three external dimensions in the nanoscale, with nanoscale being the size range from approximately 1 nm to 100 nm.

“Nanotechnology” refers to the design, production and application of structures, devices or systems at the incredibly small scale of atoms and molecules – the “nanoscale”. And “Nanoscience” is the study of phenomena and the manipulation of materials at this scale. The birth of this concept is usually linked to a speech by Richard Feynman at the December 1959 meeting of the American Physical Society [1.3] where he asked, “What would happen if we could arrange the atoms one by one the way we want them?.

Manufactured nanoparticles often exhibit special physico-chemical properties and reactivities due to their extremely small size and controlled composition, structure or surface characteristics which are not present at the larger scale. Nanoparticles possess a much higher specific surface area (SSA) than their larger counterparts of the same material, and the proportion of atoms on the surface versus the interior of the particle is also much larger. These factors can give rise to higher surface reactivity for the same mass of material [1.1]. Nanomaterials are “first generation” products of nanotechnology and have already entered wide-scale commercial use. There are over
720 products that contain nanomaterials on the global market [1.4]. These include transparent sunscreens and cosmetics, odour and wrinkle-repellent clothing, long-lasting paints, electronic and sports equipment, fuel catalysts, building equipment, a small number of medicines, and even some food products [1.5]. In coming years “next generation nanotechnology” is estimated to bring more complex nanosystems, nanodevices, nanomachines and nanobiotechnology [1.6] that will transform manufacturing, agriculture, healthcare, military, communications and energy production [1.7]. Because of their widespread use in consumer products it is expected that nanomaterials will find their way into aquatic, terrestrial and atmosphere environments, where their fate and behaviour are largely unknown. Therefore organisms and especially those that interact strongly with their immediate environments, are expected to be affected as a result to their exposition to nanomaterials. The toxicity of nanomaterials is due to their extremely small size, smaller particles have a greater reactive surface area than larger particles and are more chemically reactive and produce greater numbers of reactive oxygen species that include free radicals [1.8]. Reactive oxygen species production has been found in a diverse range of nanomaterials including carbon fullerenes, carbon nanotubes and metal oxides [1.9].

Released Nanoparticles are destined to eventually reach the aquatic systems, be it through atmospheric depositions, surface run-off, waste-water or direct injection [1.10]. Nanoparticles have multiple interactions with the aquatic environment and water organisms. How much these interactions will affect the aquatic biosphere is not known. Yet, the consequences might be considerable, and ultimately, these effects on the aquatic ecosystem might have repercussions on the overall biosphere, including humans, it is therefore indispensible to evaluate the effects of man-made Nanoparticles on the aquatic environment and prevent an irreversible impact by
shaping appropriate legislations and implementing mitigation measures. In this context, it is crucial to gain knowledge about the fate and behavior of Nanoparticles in aquatic systems as well as their interactions with organisms.

Template assisted fabrication of nanomaterials is an easy, versatile and low cost method. Using micro porous or nanoporous materials as template different nanostructures can be synthesized by depositing material of choice within templates nanochannels. ZnO, a wide band-gap semiconductor with a band gap of 3.36 eV, has received increased attention due to its unique optical, electrical and chemical properties [1.11]. It is a multifunctional material promising application in solar cells, sensors, displays, gas sensors, varistors, piezoelectric devices, electro-acoustic transducers, photodiodes and UV light emitting devices [1.12]. ZnO has been found to have several advantages, including marked antibacterial activity in the neutral region (pH = 7) without the presence of light, and being non-toxic to humans. ZnO is used extensively as a component of cements and periodontal dressing and as fillers in endodontic gutta percha cones, where its antibacterial effect is proved [1, 13]. ZnO nanoparticles embedded in polymer matrices like soluble starch are a good example of functional nanostructures with potential for applications such as UV-protection ability in textiles and sunscreens, and antibacterial finishes in medical textiles and inner wears. Despite the excellent advantages, the ecological risk arising from their release during the production and application process has received increasing attention in many reports. Nano-ZnO has been found to be toxic to algae [1.14, 1.15, and 1.16] crustaceans [1.17, 1.18, and 1.19] fish [1.16] bacteria [1.20-1.24] nematodes [1.25] and plants [1.26, 1.27] at various levels.
1.2 Bio-nanomaterials

Biomolecules such as proteins, biopolymers, antibodies, antigens and DNA exhibit dimensions comparable to metallic or semiconductor nanoparticles (NPs). Thus, by integrating biomolecules and nanoparticles into hybrid conjugates, new functional chemical entities that combine the unique electronic, optical, and catalytic properties of metallic or semiconductor nanoparticles with the unique recognition and catalytic properties of biomolecules might be envisaged which can be called as bio-nanomaterials.

Modification of the nanoparticle’s outer layer allows a large variety of chemical, molecular, and biological entities to be covalently or otherwise bound to it. Manipulation of this corona confers advantageous properties to the particle, such as increased solubility and biocompatibility. The expanding use of a variety of nanostructures with highly controlled properties in the nanometer size range has sparked widespread interest in their use in biotechnological systems. First it is necessary to define bionanoscience. Bionanoscience is a multi- and inter-disciplinary area of research that sits at the interface of chemistry, biology, physics, materials science, engineering and medicine. It involves the utilisation of biomaterials, devices or methodologies in which dimensions of the functional components are in the nanoscale.

The fact that nanoparticles are similar in size range to many common biomolecules makes them appear to be natural companions in hybrid systems. More importantly, however, are the new and unique properties that nanostructures bring to biotechnological applications. By controlling structure precisely at nanoscale dimensions, one can control and tailor properties of nanostructures. In addition, modifications can be done to nanostructures to better suit their integration with
biological systems; for example, modifying their surface layer for enhanced aqueous solubility, biocompatibility, or bio-recognition. With selected biomolecules bound to nanostructure surfaces, new ‘hybrid’ nanostructures or Bio-nanomaterials can be obtained for applications such as biosensing and imaging, or nanostructures can be embedded in other biocompatible materials to modify material properties or impart new functionality. Thus, integration of biological and non-biological systems at nanoscale gives rise to novel bio-nanomaterials. By considering peptidic or other bio-polymer molecules in the bottom-up materials self-assembly design process, one can take advantage of inherently biomolecular attributes; intramolecular folding events, secondary structure, and electrostatic interactions; in addition to more traditional self-assembling molecular attributes such as amphiphilicity, to define hierarchical material structure and consequent properties. In recent years there has been increasing interest in utilization of biological materials for application in nanotechnology.

1.3 Semiconductor nanomaterials

A semiconductor is a crystalline solid that in its pure form exhibits conductivity midway between that of metals and insulators. Elements in Groups II and VI and in Groups III and V are often combined to form compound semiconductors. The shape control of semiconductor nanostructures has been the topic of intensive investigation in recent materials chemistry. ZnO is one of the most important multifunctional II-VI semiconductor with a wide direct energy band gap of 3.37 eV; therefore, pure ZnO is colorless and transparent advantages associated with a large band gap include higher breakdown voltages, ability to sustain large electric fields, lower electronic noise, and high temperature and high-power operation and a large exciton binding energy of about 60 meV. It is called II-VI because zinc and oxygen belong to the 2nd and 6th groups of the periodic table respectively. This
A semiconductor has several favorable properties: high electron mobility, good transparency, strong room temperature luminescence and is also environmentally benign. ZnO is an inorganic compound it usually appears as a white powder nearly insoluble in water. Various ZnO nanostructures, including nanowires, [1.28] nanotubes, [1.29] nanobelts, [1.30] and nanodisks, [1.31] have been reported for potential applications [1.32–1.36]. Crystalline zinc oxide is thermo-chromic, changing from white to yellow when heated and in air reverting to white on cooling. This is caused by a very small loss of oxygen at high temperatures to form the non-stoichiometric \( \text{Zn}_{1+x} \text{O} \), where at 800 °C, \( x = 0.00007 \). Zinc oxide crystallizes in three forms: hexagonal wurtzite, cubic zincblende, and the rarely observed cubic rock salt. The wurtzite structure is most stable and thus most common at ambient conditions. The zincblende form can be stabilized by growing ZnO on substrates with cubic lattice structure. In both cases, the zinc and oxide are tetrahedral. The rock salt NaCl-type structure is only observed at fairly high pressures \(~10\) GPa. ZnO has advantageous properties promising manifold applications [1.37] in ultraviolet (UV) lasers with low threshold [1.37,1.38,1.39], as hydrogen storage material [1.40], for field emission displays [1.41], as UV-shielding material [1.42], for nanoscaled sensitive UV and gas sensors [1.43], for ultrasensitive DNA and bio-sensors in nanomedicine [1.44] etc. ZnO is an ideal candidate for fabricating UV light emitting diodes and lasers. ZnO is a phosphor material with the ability to retain a high efficiency and at low-voltage excitation [1.45].

### 1.4 Biopolymer

Bio-templated and bio-mediated control of crystallization is a relatively recent area of investigation in nanotechnology. Morphology of functional materials can be controlled using templated growth mediated by a biopolymer. By involving a
biopolymer in the synthetic protocol, nanoparticles and assemblages of even quite complex materials can be generated in synthesis that are simple, elegant and highly specific. Biopolymer-mediated synthesis offers some advantages such as: (a) The majority of biopolymers, particularly the polysaccharides and some proteins, are composed of long molecular chains, which leads naturally to fibrous morphologies on the macro scale. The presence of an anisotropic motif at all length scales in the biopolymer can direct crystal growth naturally in this morphology. (b) Many biopolymers have an extremely strong affinity for metal cations. By chelating metal ions, the biopolymer can act effectively as a ‘cocoon’ for the inorganic phase, thus ensuring that the inorganic phase will remain nano-sized. (c) The biopolymer can provide a ready source of carbon during the synthesis, either for use in the formation of carbide phases or as a means of ensuring a localized reducing environment by carbothermal reduction of the inorganic phase. (d) Biopolymers can be functionalized with, a number of reactive functional groups such as carboxylates, sulphates, nitrates and sulphides, leading to the incorporation of the functional group. (e) Biopolymers are cheap and readily available. Biopolymers used in synthesis of various nanostructures of zinc oxide are as follows:

(a) **Starch** Starch is a polymer of \( \alpha \)-D-glucose it consists of two main components: a linear compound called amylase and a branched compound called amylopectin. Amylose is a linear polymer that typically consists of up to 3000 monomer units of glucose molecules interconnected by \( \alpha \)-1, 4 glycosidic bonds with virtually no 1,6 links \([1.46,1.47,1.48]\). Amylose generally tends to form a relatively stiff parallel left handed single helix or a stiffer left-handed double helix. The oxygen atoms in positions 2 and 6 of the glucose monomer lie on the outside surface of the helix, with the ring oxygen pointing inward. Under some conditions, perfectly aligned
chains may form double stranded crystallites that are resistant to amylases [1.49]. The second and most abundant component of starch, amylopectin, consists of a more highly branched polycarbohydrate with α1, 4 bonds that serve as the backbone and α–1, 6 branching points which occur approximately every 20-25 glucose units [1.46,1.50]. Starch is a ubiquitous, complex glucose polymer, a mixture of amylose and amylopectin, in a 1:4 ratio. Starch possesses an inherent molecular anisotropy in that it is composed of long polysaccharide chains. This motif can be easily transferred to other materials introduced into starch solutions. Nanowires of polypyrrole are one such example [1.51]. Polypyrrole is a simple polymer constructed from pyrrole heterocycles that shows particularly high conductivity [1.52, 1.53]. In the nanowire study, it was found that, in a starch solution, the addition of pyrrole monomers resulted in the adsorption of the pyrrole onto the starch via hydrogen bonding. The adsorbed monomers were then able to be polymerized, the polymerization following the chains of starch molecules to form Polypyrrole nanowires. SEM and TEM images of the Polypyrrole materials showed a uniform wire-like Polypyrrole nanostructure with an average diameter of approximately 100 nm, with lengths varying from hundreds of nanometers to several micrometers. Formation of Polypyrrole in the absence of starch resulted in the uncontrolled polymerization and growth of Polypyrrole aggregates rather than nanowires, either with or without a conductive substrate.

(b) **Agarose** Agarose is an oxygen rich naturally occurring polysaccharide which is extracted from seaweed. It consists of alternating 1,3 linked β-D-galactose and 1,4 linked 3,6- anhydro-α-L-galactose it can form transparent gel and films which can be used for packaging and finds application in gel electrophoresis [1.54]. When hot aqueous agarose solution is cooled below gelling temperature (31- 36 ° C)
thermally reversible hydrogel is formed which is stable over a wide range of pH from 3 to 9. Thermal gelation results from the formation of helicoidal structures which is responsible for a three-dimensional network in which large amounts of water can be entrapped. The hydrogel, being hydrophilic, inert, and biocompatible, forms a suitable matrix for proteins and peptides that can be entrapped in the gel during formation [1.55].

1.5 Catalase

Catalase is an antioxidant enzyme which is ubiquitously present in all aerobic cells. It catalyses the decomposition of hydrogen peroxide (H₂O₂) by dismutation reaction to water and oxygen and also shows peroxidatic activity. Hydrogen peroxide is formed in cells by controlled pathways. H₂O₂ shows a broad spectrum of cellular response ranging from mitogenic growth stimulation to apoptosis to necrosis at different concentration levels. Locally intense amount of hydrogen peroxide is produced by inflammatory cells to kill pathogens. Hydrogen peroxide at high concentration is deleterious to cells and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death. Removal of the H₂O₂ from the cell by catalase provides protection against oxidative damage to the cell. The role of catalase in oxidative stress related diseases has been widely known. Catalase activity varies greatly between tissues. The activity is highest in the liver, kidney and erythrocyte, and lowest in connective tissues. In eukaryotic cells the enzyme is concentrated in the sub cellular organelles called peroxisomes. The enzyme consists of 4 subunits of the same size, each of which contains a heme active site to accelerate decomposition of hydrogen peroxide.

The principal H₂O₂ scavenging enzyme in plants is catalase, which is located in peroxisomes or glyoxysomes and at least in maize in mitochondria and ascorbate...
peroxidase (APx), which is primarily found in the cytosol and chloroplasts [1.55, 1.56]. H$_2$O$_2$ is not strictly compartmentalized, and is able to diffuse freely through membranes. Catalase and ascorbate peroxidase have distinct catalytic properties, catalase does not consume reducing power and has a very high reaction rate, but only poor affinity for H$_2$O$_2$ whereas ascorbate peroxidase requires a source of reductant ascorbate and has higher affinity for H$_2$O$_2$ than catalase.

The function of catalase in the cell is therefore to remove the bulk of the H$_2$O$_2$, whereas peroxidases (apart from their biosynthetic function) are mainly involved in scavenging H$_2$O$_2$ that is not taken by catalase [1.57]. The catalase/peroxidase system may thus act cooperatively to remove H$_2$O$_2$ at a minimal expense of reducing power and yet at a maximal rate. Plant catalases are mostly found in peroxisomes, where many H$_2$O$_2$ producing oxidases are also located, this compartmentalization does not prevent catalase from operating as a general sink for H$_2$O$_2$ within the cell. Catalase is therefore essential for the antioxidant defense during biotic and abiotic stresses that generate Active Oxygen Species in cellular compartments other than the peroxisomes.

Catalase exhibits an unusual kinetic behaviour i.e., it is not possible to saturate the enzyme with substrate H$_2$O$_2$ up to 5 M concentration but there is a rapid inactivation of the enzyme above 0.1 M H$_2$O$_2$. Therefore, its activity assay is typically carried out at 10 - 50 mM H$_2$O$_2$. Because substantially lower concentration than saturated substrate is used, the enzyme activity is dependent on precise concentration of H$_2$O$_2$. The most common definition of one catalase unit is the amount of catalase decomposing 1.0 mM of hydrogen peroxide per minute at pH 7.0 at 25°C, with initial H$_2$O$_2$ concentration of 10.3 mM.
1.8 Chlorophyll

Chlorophyll is a natural green pigment found in leaves and stems of green plants. It is the main pigment which absorbs light energy from the sun for photosynthesis. This energy is then used by the plant to synthesize glucose from carbon dioxide and water in presence of chlorophyll. The structure of the chlorophyll molecule consists of several conjugated nitrogen-containing rings surrounding a magnesium ion by coordinate covalent bonds. Molecules such as this with a metal ion coordinated to an organic compound are called coordination compounds. Coordination compounds are found elsewhere in nature, and generally have distinct spectral characteristics that account for their energy-transfer function in metabolic reactions. This chemical behavior is also evident in the photo response of these compounds. For example, hemoglobin changes from a deep purplish red to a bright red upon the binding of oxygen. In the case of chlorophyll, a spectral analysis shows the wavelengths of sunlight absorbed, which is actually the combined absorption of two different chlorophylls, a and b. The maximum absorbance of chlorophyll a is at 420 and 660 nm and the maximum absorbance of chlorophyll b is at 435 and 643 nm. In leaves, chlorophyll is bound to thylakoid membranes in the chloroplasts, and absorbed wavelengths of light are converted to chemical energy. When chlorophyll is extracted from leaves, light energy cannot be transferred to the chloroplasts. Instead, the light is re-emitted and or absorbed as heat [1.58]. Krupa et al. [1.59] investigated the changes in the content of total chlorophyll in the first leaves of rye seedlings treated with cadmium and concluded that the determination of total chlorophyll is the reliable marker of cadmium toxicity in higher plants. When plants are subjected to harmful stress conditions, one of the possible biochemical changes is the production of activated oxygen species. The chloroplasts and mitochondria of plant cells are
important intracellular generators of activated oxygen species. These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage of lipids, proteins and nucleic acids. Decrease in chlorophyll content in green plants as a response to environmental stress has been reported. [1.60].

1.7 Hydrilla

Hydrilla verticillata is a rooted submerged species. It has strong adaptability and grows fast, and is also a common submerged macrophyte that is used for the ecological restoration of eutrophication shallow lakes. H. verticillata not only has high growth rates but also can potentially be used as candidate plant for phytoremediation of heavy metals contaminated water columns [1.61]