Synopsis

Bio-inspired or biomimetic chemistry deals with the replication of the nature's fundamental processes, which can help in understanding the functioning of biological systems and develop novel applications. Although a large number of researchers worked towards the replication of natural synthetic pathways through biogenetic syntheses, enzyme mimicry by the small organic molecules and inorganic complexes emerged in leaps and bounds over the years. The development of biomimetic chemistry then continued in designing the molecules that can function like enzymes. And now, with the advent of nanotechnology, nanostructured materials have been shown to exhibit enzyme-like activities (nanozymes). Interestingly, the two distinct fields, biology and materials science, have been integrated to form an entirely new area of research that has captured a great attention. Along with the pronounced application of nanomaterials as drug delivery vehicles, anticancer agents, antimicrobials, etc., research is also focused on designing nanomaterials for the biomimetic applications.

The thesis consists of **five chapters**. The **first chapter** provides a general overview of the recently discovered nanozymes that mimic heme-peroxidase, oxidase, superoxide dismutase, catalase, haloperoxidase and phosphatase. This chapter also deals with the nanozymes' application in sensing and immunoassay, and as antioxidants, neuroprotective agents. The factors affecting the nanozymes' activity and the challenges associated with them is also covered in this chapter. **Chapter 2** is divided into two parts and it deals with the biomimetic properties of graphene-based materials. In **part A**, the remarkable peroxynitrite (PN) reductase and isomerase activities of hemin-functionalized reduced graphene oxide (rGO) is discussed. In **part B**, the activity of graphene oxide (GO) as peroxide substrate for the glutathione peroxidase (GPx) enzyme is discussed. In **chapter 3**, the oxidant material, V_2O_5, is shown to exhibit significant GPx-like antioxidant activity in its nano-form. **Chapter 4** deals with the oxidase-like activity of $MnFe_2O_4$ nanooctahedrons for the antibody-free detection of major oxidative stress biomarker, carbonylated proteins. In **chapter 5**, the phosphotriesterase mimetic role of vacancy engineered nanoceria is discussed.

The **part A** of **chapter 2** deals with the peroxynitrite (PN) scavenging activity of nanomaterials. PN is generally known as strong oxidizing and nitrating species produced *in vivo*. Organoselnium compounds and some metal porphyrins are some of the class of compounds that help in decomposition of PN. However, metal prophyrins tend to get deactivated due to dimerization and loose PN decomposition activity. We thought that the critical solution to this problem would be noncovalently functionalizing monomeric metal porphyrin such as hemin on the graphene sheets (basically, rGO) (Fig. 1a). The rGO prepared during the study was obtained by a novel GO reduction procedure using dithiothreitol (DTT) as a reductant. The hemin-reduced graphene oxide, when tested against PN-mediated oxidation of dihydrorhodamine, L-tyrosine and bovine serum albumin (BSA) nitration, exhibited significant antioxidant enzyme-like activity than that of hemin alone. A detailed mechanistic investigation indicated that the interaction of rGO with hemin resulted in strong synergistic effect in scavenging PN. It is also believed that the rGO sheets prevent cage-escape of generated $\bullet NO_2$ and helps in facile recombination of $\bullet NO_2$ with FeIV=O species to give nitrate as a PN isomerization product (Fig. 1b). In the presence of ascorbic acid (asc), PN reductase cycle is followed to give nitrite (Fig. 1b). This study is an excellent example of tuning the enzyme-like activity of small molecules at the nano-interface.

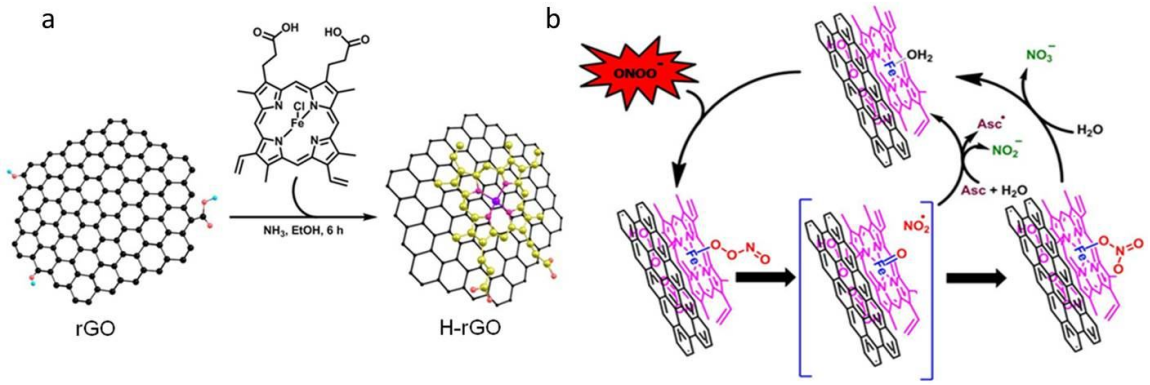

Figure 1. a) Functionalization of hemin on rGO sheets. b) PN reductase and isomerase cycles.

The **part B** of **chapter 2** highlights the novel observation during our investigation on the graphene-based materials for biological applications. Inspired by the oxidation chemistry of GO in organic reactions, we found that GO can act as a peroxide substrate

instead of H_2O_2 for glutathione peroxidase (GPx) enzyme. As partial reduction of GO was observed when treated with GPx enzyme due to the fact that large sheet-like structures cannot be accessible to the active site, we studied the reaction with some GPx mimetics (Fig. 2). Varying the concentration of cofactor glutathione (GSH) required for the reaction, GPx mimic, ditelluride**,** could accomplish the reduction of GO following Michaelis-Menten kinetics. As the structure of GO is elusive and under active investigation, our study highlights the presence of peroxide linkages as integral part of GO other than hydroxyl, epoxy and carboxylic groups. This study also highlights an important fact that the modification of GO by biologically relevant compounds such as redox proteins must be taken into account when using GO for biomedical applications because such modifications can alter the fundamental properties of GO.

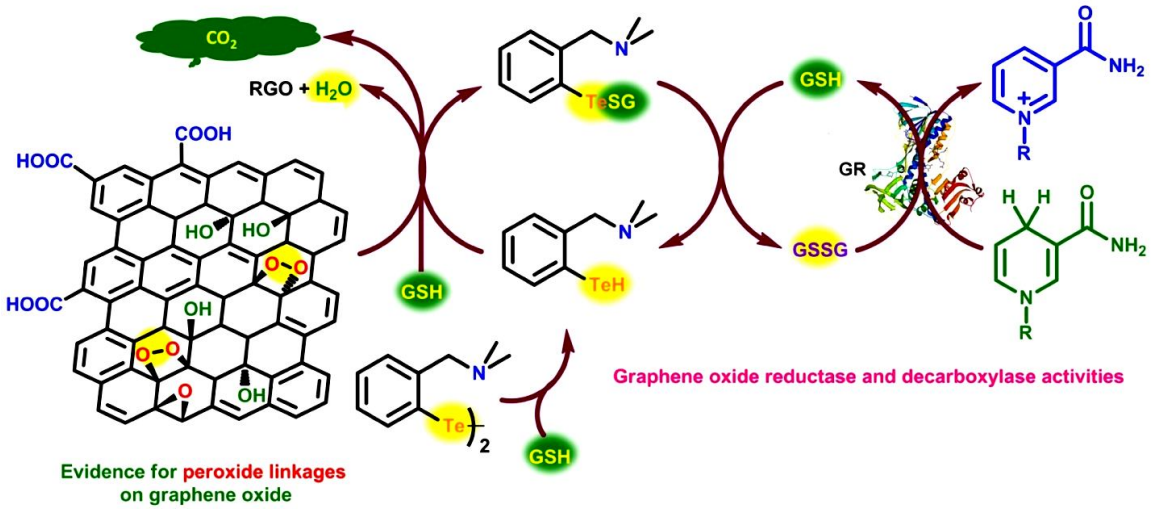

Figure 2. The GO reductase and decarboxylase activities of GPx mimetic ditelluride compound, suggesting the presence of peroxide linkages on GO.

In **chapter 3**, we have discussed about the novel antioxidant nanozyme that combats oxidative stress. During our attempts in the investigation of antioxidant nanozymes, we surprisingly noticed that the oxidant material, V_2O_5, shows significant GPx-like antioxidant activity in its nano-form. The Vn readily internalize in the cells and exhibit remarkable protective effects when challenged against reactive oxygen species (ROS). Although Vn has been shown to protect cells from ROS-induced damage, cells treated with bulk V_2O_5 and few vanadium complexes resulted in generation of ROS and severe toxicity. Detailed investigation on the mechanism of this interesting phenomenon

revealed that the protective effects result due to conservation of the oxidation state of vanadium in (V) throughout the catalytic cycle. While the vanadium center in bulk V_2O_5 and vanadium complexes undergo redox recycling during the catalysis, generation of ROS due to Fenton reaction leads to toxicity. The remarkable change in property from oxidant to antioxidant is due to the quantum size confinement of electrons that results in change in redox potential of a material. Based on our findings, we envision that biocompatible vanadia nanowires can provide tremendous future therapeutic potential to prevent ageing, cardiac disorders and several neurological conditions, including Parkinson's and Alzheimer's diseases.

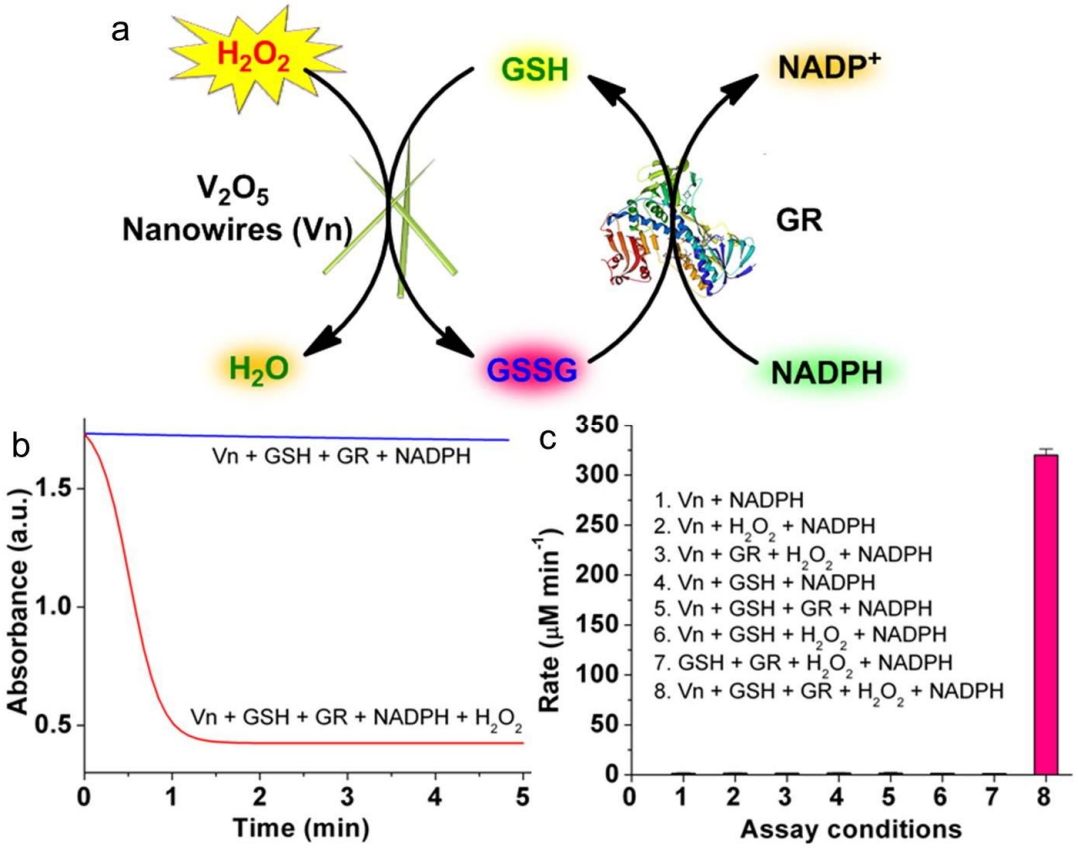

Figure 3.a) The GPx-like antioxidant activity of Vn and GSH recycling by glutathione reductase (GR). b)Plot of absorbance versus time (min) revealing the activity of Vn in the presence of Vn (0.020 mg mL-1), GSH (2 mM), NADPH (0.4 mM), GR (1.7 units), H_2O_2 (240 μM) in phosphate buffer (100 mM, pH 7.4) at 25 ^oC. When H_2O_2 was absent in control, no reactivity was obtained. c) Bar diagram showing the initial rates at different assay conditions.

Chapter 4 deals with the development of novel methodology for detection of biomarkers. Inspired by the use of antibodies and enzymes for detection of a specific antigen, we have shown for the first time that the nanozymes can entirely replace antibodies and enzymes in Enzyme-linked Immunosorbent Assays (ELISA). As a specific example, we focused on the antibody-free detection of chief oxidative stress biomarker, carbonylated proteins, as our target. To achieve this, we designed $MnFe_2O_4$ nanooctahedrons that can function as oxidase enzyme and form signaling point of detection. We functionalized $MnFe_2O_4$ nanooctahedrons with hydrazide terminating groups so that carbonylated proteins can be linked to nanozymes by hydrazone linkage (Fig. 4a). Treatment of various carbonylated proteins (hemoglobin (Hb), Myoglobin (Mb), Cytochrome c (Cyt c), RNase and BSA) coated in well plate with hydrazide-terminated $MnFe_2O_4$ nanooctahedrons and then with 3,3',5,5'-tetramethylbenzidine substrate, resulted in instantaneous detection by well plate reader (Fig. 4b). Considering the challenges and difficulties associated with the conventional methods used to detect such modified proteins, this methodology opens up a new avenue for the simple, cost-effective, instantaneous and entirely antibody-free ELISA-type detection of carbonylated proteins. Our results provide a cumulative application of nanozymes' technology in oxidative stress associated areas and pave a new way for direct early detection of post translational modification (PTM) related diseases.

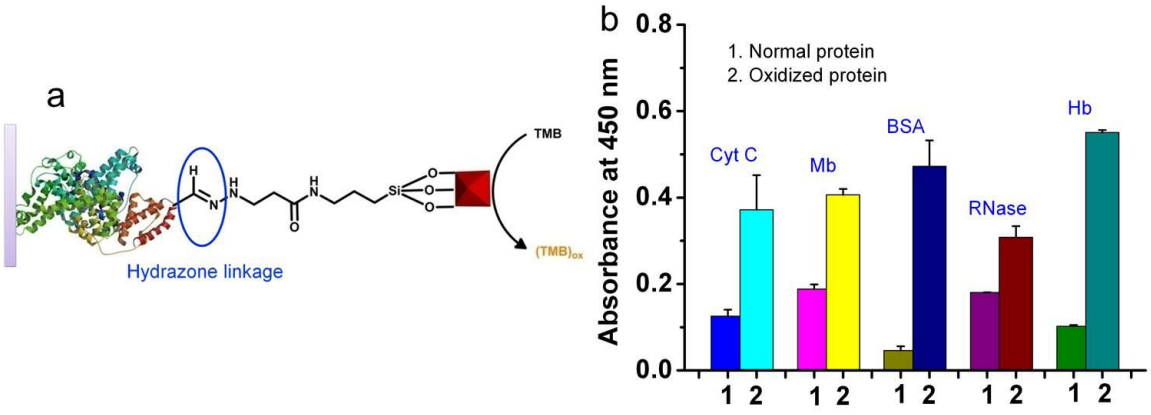

Figure 4. a) Nanozyme linked to the carbonylated protein coated on a plate through hydrazone linkage. b) General bar diagram showing detection of oxidized (carbonylated) proteins by nanozymes.

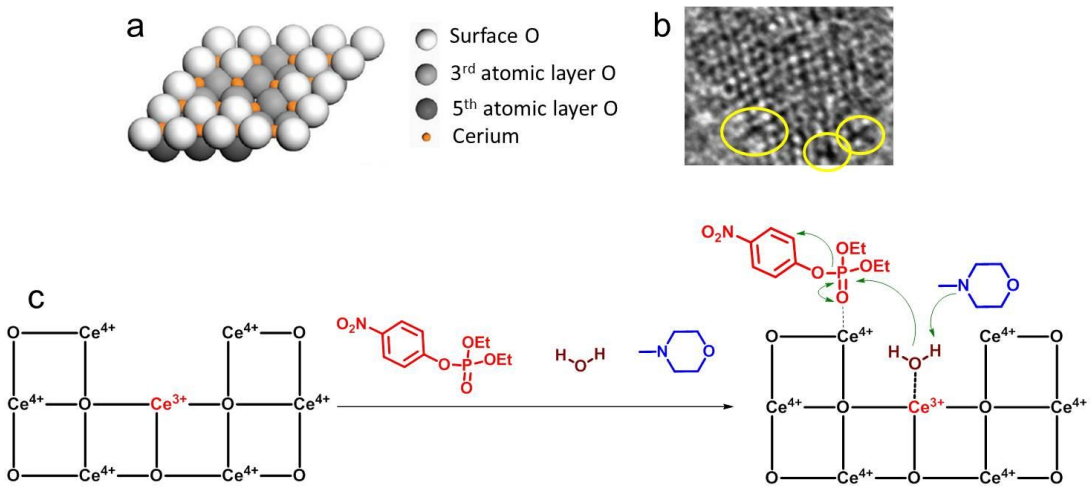

Figure 5. a) A cartoon view of surface of ceria showing vacancy. b) Zoomed portion of high resolution transmission electron microscopic image showing few vacancies on the surface of nanoceria. c) Catalytic mechanism of detoxification of paraoxon at the defect site.

In the final chapter, **chapter 5**, we have discussed about the nanomaterial that can function as phosphotriesterase enzyme. Phosphotriesterase enzyme is a bacterial enzyme that is involved in the rapid hydrolysis of sarin gas-related deadly nerve agents such as paraoxon, parathion and malathion. When encountered with these orgnaophospatetriesters, living beings tend to undergo nerve shock to cause paralysis by inhibiting an extremely important enzyme called acetylcholine esterase. They are also known to cause severe oxidative stress problems and are associated with neurodegenerative disorders. Therefore, curbing the toxic effects and detoxification of these nerve agents is a world-wide concern and many research teams have focused their attention to address this important problem. Working on the development of nanozymes for important problems, we found that nanoceria, especially the vacancy engineered one (Fig. 5a,b), can serve as active mimic of phosphotriesterase enzyme in the presence of *N*-methylmorpholine (acting as a distal base histidine). Vacancy engineered nanoceria has been shown to catalyze the hydrolysis of high amounts of paraoxon quiet efficiently and within few minutes with very low activation energy and high k_{cat}. Detailed mechanistic investigation revealed that the presence of both Ce(III) and Ce(IV) is very essential for detoxification activity (Fig. 5b). The vacancies on the surface of nanoceria, where the

buried Ce(III) ions are directly exposed to the reaction environment, behave as hotspots or enzyme active sites for detoxification reaction (Fig. 5b).