Summary

The field of nanobiotechnology combines the ability to prepare materials that typically measure between 1 and 100 nm with the ability to exert precise interventions in biological systems. Nanobioconstructs aspire to synergize the function and characteristics of nanomaterials with biomacromolecules. In this thesis I describe the development and investigation of various nanobioconstructs towards applications that can be broadly categorized under catalysis and sensing. The thesis is divided into two sections; in the first section I discuss reusable nanobiocatalysts for the efficient extraction of bio-active compounds. The first chapter provides a broad introduction of concepts and topics covered in the thesis.

In chapter 2, I examine the effect of immobilizing hydrolytic enzymes on nanoparticles. Hydrolytic enzymes cellulase and pectinase are immobilized on the magnetic iron oxide nano particles via established conjugation strategies. The size and morphology of the enzyme-nanoparticle constructs are characterized by Atomic Force Microscopy (AFM), the composition of the enzyme-nanoparticle constructs are characterized by X-Ray Diffraction (XRD) and Transmission Electron Microscopy (TEM) is used to assess the enzyme immobilization. The enzyme immobilized magnetic iron oxide nano particles are found to improve the yield of extraction of bioactive compounds by nearly 8-9 fold. In addition, the improved extraction of carotenoidic bioactive compounds bears a strong correlation with the extent of peel hydrolysis. There are relatively few approaches which address the issue of reusability of the biocatalyst in such extraction processes. In this work, the reusability of the enzyme-MNP constructs demonstrated towards the extraction of bioactive compounds from separate source batches of orange peel. The reusability of the nanoparticle-immobilized

catalyst along with their improved stability compared to free enzymes bodes well for their active use. The elucidation of conditions for the extraction of bioactive compounds from a source like orange peel further enhances the value of the nanobiocatalyst-mediated process.

In chapter 3, I scrutinize the construction and application of nanoparticle-immobilized hydrolytic enzymes. There are dearths of reports on the systematic examination of factors influencing nanoparticle-immobilized hydrolytic enzyme activity. First the effect of nanoparticle composition and chemical linkage has been investigated on the immobilized cellulase activity on onion skins. Our choice of onion skins as source material is aligned with our efforts in rendering value from waste. Onion skins are known to bear polyphenols such as quercetin. The products of hydrolysis of onion skin are assesed with respect to glycosidic and aglycosidic forms of polyphenols. Application of the cellulase-immobilized nanoparticles on onion skins results in release of a distinctive composition of polyphenols. The aglycosidic form of quercetin is the dominant product of onion skin hydrolysis affected by cellulase nanobiocatalysts. The nanoparticles with different composition (such as IOMNPs, SNPs. S-IOMNPs and C-IOMNPs) and with different chemical linkers (C-5, C-6, C-14 and C-22) are synthesized and characterized. I assess these parameters towards achieving optimal hydrolytic activity of the immobilized enzyme. Chitosan-coated iron oxide nanoparticles with APTES-conjugated cellulase are found to be most effective for polyphenol release and for transformation of glycosidic to aglycosidic form of quercetin. Further, the quercetin-rich extracts exhibit a distinctive inhibitory activity on tau-fibril aggregation. In contrast, quercetin-diglucoside does not have an inhibitory effect.

The second section of the thesis is devoted to two different aspects of nanobiosensors.

In chapter 4, a novel immobilized-enzyme based strategy has been developed for the colorimetric detection of herbicide glyphosate. The latter is the most widely used herbicide in

the world and has been at the center of an intense debate pertaining to its long-term effects on human health. Our method relies on concerted activity of laccase mediator system with syringaldazine as specific mediator. These oxidative systems catalyze the breakdown of glyphosate and simultaneously provide visual indication of the same. A combination of spectro-electrochemistry, NMR spectroscopy and glyphosate-derivatization based chromatography have been used to investigate and validate the assay. Presence of glyphosate enhances kinetics of the laccase-mediator by up to 30% and 10-200 ppm of glyphosate can be easily detected. These concentrations are well within the range of glyphosate concentrations that are likely to cause long-term harm to human populations. Reusability and superior sensitivity of our assay is enabled by use of silica-coated magnetic iron oxide nanoparticles with immobilized laccase.

In chapter 5, I deploy nucleic acid enabled biosensors for the detection of target DNA. Target DNA is detected by exploiting the response of pNIPAm-co-AAc microgel-based etalons to NaCl. NaCl in solution is capable of penetrating pores in the top Au layer of an etalon resulting in easily quantifiable color change. Probe DNA is immobilized on the top Au layer of an etalon that is capable of binding complementary target sequences. Upon binding of the complementary sequence to the surface (forming a double strand), the ability of the NaCl to penetrate the etalon's Au layer is hindered, while its response can be restored by removal of the DNA from the surface. Target DNA that has varying degrees of mismatches namely 3 base pair (BP), 10 BP and completely mismatched can all be distinguished by our approach. I validate the response of etalons through the removal of only hybridized nucleic acid sequences using specific enzymes (nuclease Bal-31). This system demonstrates a straight-forward detection method to sense target DNA without any modification or the use of labels.

My thesis highlights (1) the emergent behavior of nano-bioconjugates and (2) ability of nano-conjugates to expand the scope of application of distinctive biomolecular behavior.