Introduction
1.1. Prologue

This dissertation is an attempt to explore various manifestations of static magnetic field, alternatively, spin, along the biological hierarchy. The study includes small metabolites, proteins, whole cells and complex clinical samples. The focus in this dissertation is on a few unexplored (or less studied) areas like nanoscale magnetism and magnetically inspired bio-imaging and/or spectroscopic techniques. The other independent perspectives of the study are some perspectives of quantum biology e.g., spin reorientation driven self-assembly, spin induced intracellular streaming.

The introductory chapter starts with brief description on reported emerging properties at nanoscale with emphasis on magnetic property of magnetic nanoparticles (NPs) and their biomimetic activity. Next, magnetic property of a naturally occurring magnetic protein (ferritin) and its relation with body oxidative stress is discussed. The residual part describes the reported effects of Static Magnetic Field (SMF) on biomolecules and live cells. In addition, spin mediated quantum coherent phenomenon and its functional role in living system has also been discussed. In last section the chapter wise outlines of the dissertation are provided.

1.2. Emerging properties at nanoscale and magnetic nanoparticles

In nano size regime apart from enhancement of catalytic activity due to enhanced surface to volume ratio, various interesting physical and optical properties also emerge. The emergences of these of properties are due to reordering
of electronic band gaps structure [1], surface lattice strain [2] and surface spin deficiency (spin canting) [3]. Discretization of band gap structure results in fluorescence property in nano size dimension due to quantum confinement (like fluorescent gold NPs and several semiconductor Quantum Dots) [4]. Another interesting optical property namely Surface Plasmon Resonance (SPR) is found in NPs of noble metal like Gold (Au) and Silver (Ag) [5,6]. SPR occurs due to resonance between collective oscillation of the surface plasmons [7] and incident light beam which further results in variety of color formation. This phenomenon is classically known as Mie resonance [8].

For magnetic materials in nano dimension, surface spin deficiency due to surface rupture results in emerging magnetic properties like Super Paramagnetism [9], reduced magnetic coercivity [10], etc. Generally magnetic elements (Fe, Co and Ni) and its oxides show this type of emerging properties in nano dimension. For example, in bulk scale magnetite (Fe₃O₄) shows ferrimagnetic properties due presence of two sub-lattices with unequal spin distribution [11]. The same material in nanoscale shows super Paramagnetism [9]. The magnetism in nanoscale critically depends on the competition between the magnetic anisotropy energy of an individual nanoparticle and the magnetic dipole-dipole interaction between the particles. If the former is higher, then the dynamic behaviour follows the Neel-Brown model [12,13] and the system is termed as super paramagnetic, exhibiting magnetic viscosity due to Neel relaxation [12]. If the dipole-dipole interaction is of the order of the particle anisotropy energy then the system can go to a magnetically
frustrated state, leading to a system termed as super spin glass (SSG) system [14,15]. Both the systems mentioned above have a characteristic temperature; $T_B$ or blocking temperature in the case of super paramagnetic NPs and $T_f$ or spin freezing temperature in the case of SSG NPs. Below this characteristic temperature, the magnetic moments are frozen and the system exhibits non-equilibrium properties like memory effect and magnetic hysteresis [16,17].

The oxides of iron with Super Paramagnetic behavior are known as Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) [9]. In application domain, SPIONs are used as MRI contrast agents [18]. SPIONs can also be used in targeted drug delivery through application external magnetic field [19,20]. The combination of SPIONs (local magnetic field) and External Static Magnetic Field (SMF) can be used in sorting of cells [21] and different desired analytes (small and macromolecules) [22]. SPIONs and Alternating Magnetic Field (AMF) are used in hyperthermia [23] for killing of cancer cells.

Apart from emergence of nanoscale magnetism iron oxide NPs shows intrinsic peroxidase-like activity. Surface defects create some local structures such that they function like oxidation-reduction cell, which is the main reason for observed peroxidase-like enzyme activity of colloidal SPIONs [24]. This type of biomimetic activity can be observed in other inorganic [25] and organic nanomaterials [26] due to same reason. For peroxidase activity in iron oxide NPs, the accessibility of ferrous ion solely determines the catalytic activity [27]. The peroxidase like activity of iron oxide NPs was first reported by Gao et al [27]. The authors extensively
studied the mechanism of catalysis and compared the activity with Horse Radish Peroxidase (HRP). The surface ferrous ions are responsible for the observed catalytic activity as increase and decrease in number of ferrous ions decreases and increases the catalytic activity respectively [27]. That is why the activity decreases upon increase in thickness of surface coating. Peroxidase-like activity of SPIONs can be used as peroxide sensor and due its reusability [27] it can replace HRP, a secondary antibody used in ELISA based assay. There are several reports where iron oxide NPs has been used as glucose sensor [27-29].

1.3. Emergence of magnetism due to interfacial effects

Due to presence of surface defects several diamagnetic and nonmetallic materials show magnetism in nanoscale. The reduction of diamagnetic moment in NPs of diamagnetic materials like copper (Cu), silver (Ag), gold (Au), antimony (Sb), bismuth (Bi) and graphite [30-33] were observed earlier. The existence of orbital moments at the defect sites was suggested to be the reason of such observed magnetism [34]. Ferromagnetic hysteresis in graphite and nonmetallic oxides was also due to orbital moment [35-37]. Polymer stabilized NPs of Pd and Au and Thiol capped Au also show magnetic moment [38,39].

In all the cases there is a common factor namely, the presence of an interface between particle surface and surface coating ligands. Orbital moment is generated due to redistribution of spins at the interface. For example, in thiol or polymer coated Au NPs a huge charge transfer from Au to polymer is occurred. As a result holes at ‘d-orbital’ are created at Au surface [31,40]. The holes are somehow spin
polarized and results in finite magnetic moment [38,41]. Alternative mechanisms are also proposed by several groups [42]. Recently it has been shown [42] that the interfacial effects can results in enhanced magnetism in magnetic NPs.

The functional role of such enhanced magnetism is enhancement of T1 image contrast in Magnetic Resonance Imaging (MRI) platform as T1 image contrast is directly proportional to the magnetic moment of the contrast agent [43].

1.4. Ferritin: a naturally occurring magnetic nanoparticle

Functional requirement of nanoscale magnetism remains an open question at the living system. The magnetotactic bacteria synthesize magnetic nanoclusters (stored in siderophores [44,45]) to overcome the barrier of cells inherent diamagnetic susceptibility during magnetotaxis [46]. The siderophores also control the accumulation of iron within bacterioferritin, which plays crucial role in bacterial growth [47]. The Eukaryotic counterpart of bacterioferritin is known as ferritin, which is a spherical, non-heme iron storage protein [48]. The holo-ferritin is polypeptide shell with an empty core of diameter of 8 nm (apo-ferritin). Ferritin sequesters ferrous ion and stores within core after oxidation to ferric ion [49]. The chemical composition of the core is similar with ferrihydrite mineral [50]. Ferritin molecule forms through self-assembly of 24 subunits guided by hydrophobic interaction [51], which disassemble at low pH (~pH 2). The ferritin subunits again reassemble upon increase of pH. This dynamic assembly and disassembly process offers a template for synthesizing monodispersed nanomaterials [52]. Spatial confinement of core results in nanomaterials with diameter of 8 nm.
Now confinement of iron complex in nanoscale makes ferritin a magnetic nanoparticle, functionalized with polypeptide shell. Ferritin shows super Paramagnetism with characteristic blocking temperature ($T_B$). Horse spleen ferritin shows magnetic birefringence [53] and several anomalous magnetic properties [54-56]. Generally, $T_B$ decreases with increasing field strength. But $T_B$ of horse spleen ferritin increases up to 0.3 Tesla and decreases beyond that field strength (normal behavior) [57]. Increase of $T_B$ with field strength suggests that the particles are non-interacting in nature and indicates presence of magnetic mixed population [57]. Dynamic nature of magnetic core of ferritin [54] along with anti-ferromagnetic transition [58] upon increasing iron loading was studied earlier.

The functional role of anti-ferromagnetism is little known; the shift from ferromagnetic to anti-ferromagnetic with increase in iron loading throws up the possibility of different magnetic interaction in subjects with iron overloading. The stronger and weaker inter-protein interaction at moderate and higher iron level may have intriguing implications.

To my knowledge there is no report on functional requirement of such observed dynamic magnetic properties of ferritin core in body iron homeostasis. In this context, the differential magnetic behavior of human serum with increasing ferritin level has been discussed (see Part-2 of Chapter 5).

1.5. Oxidative stress and its relation with serum ferritin

Ferritin is an acute phase protein, over expressed during inflammation [59,60] and iron overloading [61]. Ferritin is an important factor for body iron homeostasis.
Apart from occurrence in serum, ferritin has wide cell specific distribution [62,63]. Excess irons are stored within ferritin. During requirement of iron transferrin binds to ferritin and releases iron from the core of ferritin in ferrous form [59]. Then the iron bound transferrin binds to transferrin receptor on the cell surface and iron goes into the cells through receptor mediated endocytosis [59,64]. Ferritin level is found to be very high for thalassemic patients due to transfusion of blood [65,66]. With increasing iron overloading within ferritin core the susceptibility towards iron decreases, which results in decreased iron/protein ratio [67]. At high ferritin level the free iron increases due to inadequacy of iron storing [67]. Free iron results in oxidative stress through formation of Reactive Oxygen Species (ROS) [68]. Assessment of body oxidative stress and measurement of serum ferritin is required for assessment of body iron homeostasis of thalassemic patients in regular basis.

The tryptophan/kynurenine ratio of serum is a known indicator of oxidative stress [69,70]. The existing methods, used to measure the ratio are either High Performance Liquid Chromatography (HPLC) [71] or Mass Spectroscopy (MS) [72] based. A fluorimetric technique to measure the oxidative stress has been developed in this dissertation employing the native fluorescence of serum.

1.6. Molecular origin of magnetism in biomolecules

Pauling showed that the proteins are diamagnetic in nature due to induced ring currents in aromatic side chains of the aromatic amino acids (tryptophan, tyrosine and phenyl alanine) [73]. After that several studies were conducted, which reported the SMF induced orientations of retinal rod outer segment [74], Chloroplasts
[75,76], purple membranes [77], photosynthetic bacteria and algae [78,79] and nucleic acids [80,81]. The inherent diamagnetic anisotropy of aromatic amino acids within proteins [73] and bases within nucleic acids [80,81] were thought to be the main reason for observed magnetic orientations. Later on, the observation of magnetic orientation of polypeptides containing no aromatic amino acids [82-84] needed reevaluation of the molecular origin of observed diamagnetism in proteins. Lonsdale [85] and Worcester [86] showed that diamagnetic anisotropy can arise due to presence of planer resonance structures like ester groups and peptide bonds.

Lonsdale provided a theoretical formulation to measure the diamagnetic anisotropy of the ester groups. According to Lonsdale, the diamagnetic susceptibility of an ester group is

$$\Delta K = K(\text{parallel}) - K(\text{perpendicular}) = -8.8 \times 10^{-6} \text{ cm-gm-sec emu} \quad (1)$$

where $K(\text{parallel})$ and $K(\text{perpendicular})$ are the susceptibilities parallel and perpendicular to plane of ester group. Magnetic orientations effect can only be observed if energy difference between $K(\text{parallel})$ and $K(\text{perpendicular})$ become comparable with thermal energy (kT). Using this value Worcester showed that the diamagnetic anisotropy was higher for α-helix than β-sheets secondary structures [86]. The diamagnetic anisotropy of peptide bonds is tenfold lesser than aromatic amino acids [86] and the aromatic side chains are supposed to be normal to the peptide plane. Hence, the magnetic orientation of proteins is solely dependent on
the number and positions of aromatic side chains with respect to the helix axis. Regarding this, three cases may arise:

(a) Orientations of α-helix is parallel with field directions, which indicates the major contribution towards anisotropy comes from the peptide bond within helix like tubulin [87] and fibrin [88,89].

(b) Orientations of aromatic side chains is parallel with field direction implies major contribution aromatic side chains towards anisotropy.

(c) No orientations due to compensation.

Pauling also reported the existence of diamagnetism in peptides, but his calculation resulted in lower value of diamagnetic anisotropy than calculated by Lonsdale [73].

Apart from anisotropy calculation of peptides Worcester considered the parameters required for observation of magnetic orientation through overcoming the thermal noise. In this context he also gave a hint about formation of giant diamagnetic anisotropy in ordered biological structures [86].

1.7. Diamagnetic Levitations

Diamagnetic anisotropy of lipid bilayer [90] and secondary structures of proteins [86] make cells diamagnetic in nature. Now cells can be levitated using super conducting magnet [91,92] of very high field strength. When magnetization force is equal to the gravitational force then levitation occur through counteracting of gravitational force. The theory of diamagnetic levitation was reported by several groups [93,94]. Briefly according to Qian [95],

\[ f_B = m \chi_B B \cdot \frac{dB}{dz} \]  \hspace{1cm} (2)
where \( m \) is the mass of the material, \( \chi_\rho \) is magnetic susceptibility per unit mass, \( B \) is the flux density and \( f_B \) is the magnetization force or Kelvin force, a body force like gravity. Again according to Qian [95] in presence of field the total force exerted on a diamagnetic material is,

\[
f_{\text{total}} = f_g + f_B = -m \left( g - \chi_\rho (B \frac{dB}{dz}) \right)
\]

(3)

where \( f_g \) is the gravitational force. When \( f_g \) and \( f_B \) shows anti-parallel vectorial relations of same magnitude then \( f_{\text{total}} \) is zero. In this condition a diamagnetic material can be levitated.

Hence, use of high field strength (>several kilo Gauss) is a situation similar with simulated microgravity. While the role of gravitational field in maintenance of lives is unclear, there are several reports on effects of high field strength in cellular level. For example, orientations of erythrocytes using field strength of 4 Tesla [96]. Morphological changes of smooth muscle cells using 5 Tesla filed strength, where the targeted cellular components are intracellular microtubules and microfilaments [97]. Extensive studies were also performed using oosteocytes to observe the effects of diamagnetic levitation on morphology, cytoskeleton and focal adhesion protein expression level [95].

Apart from diamagnetic levitation induced effects there are several reports on cellular level achieved through use of moderate field strength.

### 1.8. Effects of Magnetic field of moderate field strength on Cells
The study on magnetic field effects in biology can be classified in two groups, the first involving effect of oscillatory or pulsed fields [98-106] and the second describing the static magnetic field SMF [107-114] effects. The clinical manifestation of time varying fields is known to significantly differ from the SMF effect [115,116]. The SMF effect poses a challenge, as the classical electromagnetic theory is insufficient to explain the effect. On the other hand, one is unsure of how quantum effects can intervene with the biological information transfer explicitly.

Time varying field $\delta B/\delta t = -\text{curl}(E)$ may affect the Columbic or non-covalent interactions (both being primarily of electric origin) at the macromolecular level [116]. The presence of a fluctuating field would induce thermal effects by local dissipation with internal entropy production, $T\left(\frac{d\delta S}{dT}\right) = -<\mu>\cdot \frac{\delta B}{\delta T}$, with $<\mu>$ being the average magnetic moment. In case of SMF ($\delta B/\delta t =0$) such dissipation would disappear. The SMF effect cannot be explained in simple energy term. One may consider the Zeeman splitting of spin states. Energy between split states are typically (for protons under 1T SMF) of the order of $1.76 \times 10^{-7}$ ev, this being several orders of magnitude lower than the thermal energy (0.04ev) at 300K [117]. Thermal energy would therefore mask any population distribution among the spin states whose degeneracy is removed by the SMF. Alternate mechanisms like radical pair (RP) is proposed explaining the SMF effect [118]. It is thus not surprising that the recent attention on SMF effects is owing to this not so well understood correlation between the short lived spins states [119] and the long term biological effect. One is however compelled to introduce the concept of long term coherence, an idea
introduced by Frohlich and his coworkers [120-122] several decades ago. The basic dilemma one faces is that how the quantum information is transferred to information transfer at the cell biological or gene expression level.

1.9. Modulation of fluorescence emission by SMF

Degeneracy of electronic levels can be lost in presence of SMF in optically excitable systems [123]. The enhancement of fluorescence emission by SMF is already reported. In solid state (tetracene crystals) the observed enhancement in fluorescence is due to triplet-triplet fusion and formation of excited singlet through triplet state annihilation [124]. Next, in liquid state (solution of Chlorophyll-a) enhancement of delayed fluorescence (luminiscence) by SMF was observed [125]. According to the authors in Radical-Pair (RP) systems, where electron donor-acceptor is present, the fluorescence yield increases due to Zeeman interaction between singlet-triplet states and hyperfine coupling between electronic and nuclear spins [126,127]. SMF induced enhanced fluorescence was also used in Confocal imaging platform to increase the image contrast.

On the other hand, self-assembly or aggregation of fluorophores generally decreases the fluorescence yield due to charge transfer from excited states [128]. Recently, some polymeric derivatives have been designed, where fluorescence yield increases upon aggregation [129]. The synthesized derivatives are derived from phenylsilole groups [130].

To observe SMF induced fluorescence enhancement, the presence of SMF during fluorescence measurement is must. In this dissertation it has been shown
that pre-exposure of magnetic field can also result in enhanced fluorescence emission of pi-ring containing fluorophores through directional self assembly. Now SMF induced differential assembly may be due to inherent diamagnetic susceptibility of biomolecules.

1.10. ‘Spins’: the mediator for bulk scale quantum effects

In 1935 Einstein [131] designed a thought experiment for complete description of the quantum mechanics. At the same time Schrodinger [132] coined a term ‘entanglement’, and mathematically showed that quantum entanglement arises from interaction between two particles through evolution of Schrödinger equation. In EPR paradox [131] entanglement was termed as ‘spooky action at distance’. Afterwards, experimental evidences in favor of the existence of quantum entanglement have been established by several scientists [133-136]. All the experiments and theoretical calculations indicated the existence of spins with any classical counterpart like mass and charge. Spin is the inherent property of relativistic quantum mechanics [137]. Hestenes [138] showed that the spin is responsible for the quantum effects of the Dirac electron and the imaginary number ‘i’, in the Dirac equation solely originates due to electronic spin. Hence spin is the candidate for quantum entanglement between two systems. Apart from technological advancement of information transfer and communication (like quantum dense coding [139], quantum teleportation, communication [140-142] quantum physicists got interests in existence of quantum coherence or entanglement in biological systems. The efficiency of information transfer in
photosynthetic energy transfer and geomagnetic sensing are much higher than manmade systems. These two systems show long lived quantum coherence between different spin populations at noisy physiological temperature [143-148].

This new biology often termed as quantum biology has emerged with promises of unraveling some novel unnoted features of biological systems. They may also lead to design of new methodology to track information transfer in biological systems. It is now believed that the reported quantum coherence in biological information transfer requires well organized special proteins (cryptochromes in magnetoception, light harvesting complex in photosynthetic energy transfer). Apart from achieving a little higher efficiency, the functional role of quantum mechanics over classical within living systems is a recent topic of interest [145,148] as the used technique to probe quantum coherence within the above mentioned systems is laser (coherent) based like 2D electronic spectroscopy [149,150], a four laser based system, whereas living system uses incoherent light sources. In all those reports the main concern is the life time (decoherence time) of the coherent state. There is no discussion on downstream translation of those short lived coherent state (functionality of quantum coherence) within living systems. Hence, to answer the open question regarding the functionality of quantum phenomenon more and more ‘simple’ experimental evidences are needed. In this context, the role of quantum tunneling (of electron, hydrogen and phonon) in enzyme catalysis [16,151,152] and odor sensing [153,154] are cited as experimental evidences of such quantum phenomenon. Precise functioning of living systems in thermal fluctuations cannot
be accounted classically unless nonclassical information transfer is assumed. According to a recent report the quantum coherent state exists through harnessing the thermal energy [155]. Living systems in course of evolution might have created structural niche that permits quantum coherence in presence of thermal noise. How such quantum coherent spin states play a role in cellular information transfer, remain again an interesting question. The quantum coherence may be restated as, whether non-local information transfer is limited to some specialized protein like cryptochrome or is applicable to a wider biological context.

1.11. Outline of the thesis

The present thesis is organized as follows:

Chapter II contains the synthesis, used methodologies and physical characterizations of different iron oxide NPs. Chapter III contains synthesis of a bifunctional NPs, that is plasmonic gold coated magnetic iron oxide NPs and systemic theoretical formulations to explain the observed enhanced magnetism in such synthesized nanocomposite. In addition, the theory behind observed magnetism in citrate coated diamagnetic Au NPs is also provided.

The magnetic moment of magnetic nanoparticles is measured using an optical technique based on quantum measurement problem. The principle, methodology and applications of the developed technique are provided in Chapter IV.

Chapter V starts with development of a synchronous fluorescence spectroscopy based technique for measuring body oxidative status and serum
ferritin. In next part of the Chapter V, magnetic property of horse spleen ferritin is studied and the differential magnetic environment of human serum is addressed by use of iron oxide NPs and SMF.

Chapter VI contains SMF induced enhanced fluorescence emission of pi-ring containing fluorophores and optical memory, resulted from SMF induced directional self-assembly. The SMF induced self-assembly of proteins (albumin, ferritin and fibrinogen) and their relaxation behavior is also discussed in terms of altered autocorrelation pattern in next part of the Chapter VI.

Observation of SMF induced altered assembly pattern in imaging platform is discussed in Chapter VII. The instant magnetic response of live cells in terms of altered sub-cellular streaming and retention of the said effects after withdrawal of the field are also discussed.

Finally the summary of the work performed is presented in Chapter 8.

1.12. References

Chapter-I

Chapter-II

Synthesis and Characterization of Iron Oxide Nanoparticles and Their Functional Aspects