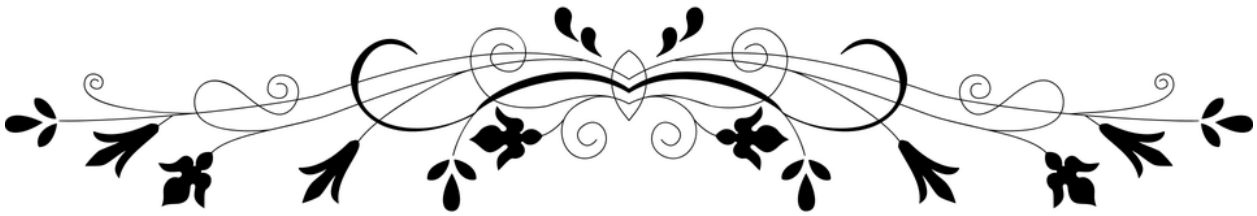

# *CHAPTER 1*

# *Introduction*

# CHAPTER 1

## 1.1 Introduction

New materials envisaging multifunctional traits need to be developed to tackle major problems in the area of nanomedicine employed in cancer theranostics. Among different reasons small diffusion lengths, formation of the corona over nanodrug, cytotoxicity, gradual resistivity to the nanomedicine as well as associated antibiotics, problem-related to secondary infections, non-informative image acquisitions from the growing tumors are some of the cited problems. The current thesis, therefore, is planned in view of synthesizing new nanomaterials using the green route and investigating them for obtaining properties such as biocompatibility, fluorescence, photons to heat conversion ability, magnetism, and electrochemical activity. Ultimately there would be the development of biocompatible nanomaterials that may demonstrate multiple functionalities in the diverse field of advanced cancer theranostics such as bioimaging, photothermal therapy by sidelining problems associated with conventional techniques. Further, evaluation of additional photo-physical, magnetic and electrochemical properties, facilitate development of electrochemical energy storage, bio-sensing, magnetic resonance imaging (MRI) technologies to probably complement with the theranostics process. Additionally the important physicochemical objectives related to electrochemical energy storage and magnetic devices also can be fulfilled.

## 1.2 Brief overview of Cancer Disease

Cancer disease is severe in humans and it causes ~10 million deaths per year worldwide [1]. Normal human cells become cancerous (Fig. 1.1) when they undergo a series of mutations, ultimately causing uncontrolled growth and division [2].

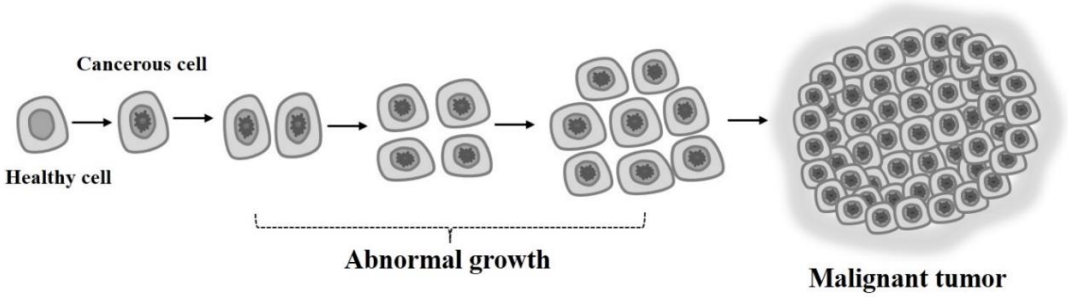


**Figure 1.1** The growth process of a tumor from a healthy cell.

The cancer cells grow inside the human body at specific location, but they metastasize to several body parts depending upon the conditions. The major anticancer drug's poor pharmacokinetics and non-specificity during cancer treatment lead to additional cytotoxicity inside the body. Further, effective distribution of chemical drugs in the tumor environment (Fig. 1.2) depends upon many factors, namely: enhanced permeability and retention (EPR), uptake by tumor-associated macrophages, effective distribution inside the tumor & surrounding micro-environment (TME), and diffusion resistance from the tumor extracellular matrix, etc.[3] Thus there are likelihood of reduction in the effective delivery of anticancerous drugs towards the location of diseased cells and high dosage of drug needed for maximum treatment efficacy. Similarly, the anticancerous drugs have also exhibited toxicity to highly blooming cell lines, e.g., hair follicles, stomach lining, and bone marrow, etc.

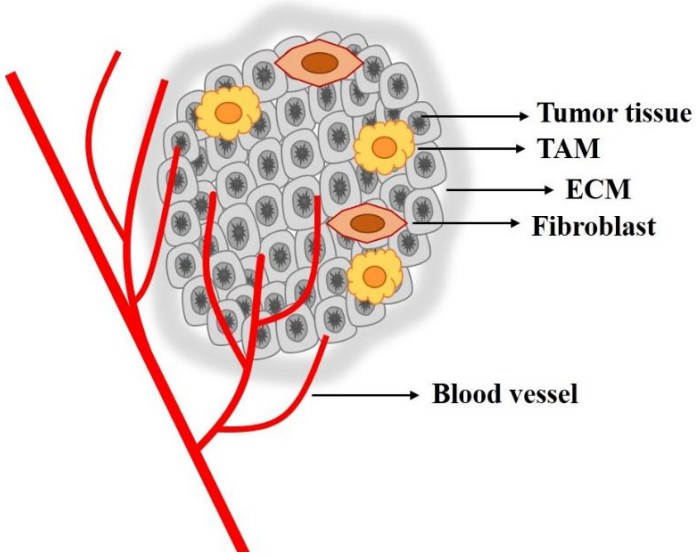


**Figure 1.2** Schematic showing tumor micro-environment.

Further in cancer theranostics, there is a higher priority for personalized medicines, those are based on the tuning of drug delivery dosage and treatment strategies adopted. This process aims at reducing undesirable side effects from the use of various kinds of medicines and treatment processes, ultimately yielding improved overall outcomes. Hence, over the last decade, there has been enhanced focus on optimization of cancer treatment strategies available to patients.

In order to tackle the above discussed constraints associated with effective cancer treatment, successful diagnostic regimens should be employed in prior. Diagnosis by "imaging" technique not only ascertain the exact physiology and phenotype of the tumor tissues inside the body, but also can guide corresponding treatment processes. Similarly during the supply of anti-cancerous drug to active tumor sites, for ascertaining drug's effective bio-distribution advanced imaging technique is very much inevitable. The term imaging is a virtual process yielding a two-dimensional picture of an entity that can be represented in pseudo colors. Supply of a sufficient amount of imaging contrasting agents

at the concerned region of interest could deliver the best possible results of the diseased state.

### 1.3 Common Bioimaging Techniques adopted for cancer diagnosis

Generally adopted imaging modalities in cancer diagnosis are computed tomography (CT) and positron emission tomography (PET) as well as single-photon emitted computed tomography (SPET), optical imaging, magnetic resonance imaging (MRI).

Computed tomography (CT) imaging relies on the difference in attenuated X-ray radiation at different angles resulting from the various biological components: fat, muscle, bone, water, etc. The anatomical study from the CT scan has good spatial resolution and three-dimensional detailing. However, it suffers from the drawback that it is a costlier imaging tool and involves highly ionizing radiation causing long-term toxicity.

Single-photon emission computed tomography (SPECT) and Positron emission tomography (PET) are radionuclide-based molecular diagnosis probes used for cellular imaging. In SPECT, radiative isotopes involving Tc-99m, I-131, and Ga-67 can circulate in blood after injection. Upon successful accumulation at the selected tissue regions, a 3D-gamma detector is employed to visualize emitted γ radiations. Similarly, in PET, the radionuclides involved In-111, Cu-64, and F-18, etc.

Unlike SPECT, the radionuclide involved in PET emits a positron which on subsequent interaction with electron produces two γ rays, those are detected by two oppositely mounted cameras in time bound manner.

Though both these techniques generate 3D images, the involvement of toxic and costlier radionuclides, low spatial resolution than other imaging techniques, and participation of cumbersome machinery limit their usability in cancer diagnosis.

Pertaining to above mentioned disadvantages associated with the conventional tumor imaging modalities such as PET, SPET and CT, state of the art optical and magnetic resonance imaging (MRI) could be the viable alternatives.

### 1.4 Multimodal imaging involving use of Optical and MR Imaging

The optical imaging is based on the photons emitted from a chemo/bioluminescent fluorescent probe with the advantage of being highly sensitive, involvement of minimal non-ionization radiation, deep tissue penetration facility, and use of the tunable wavelength spectrum. It is practically achievable and inexpensive for its real-time usability in cancer diagnosis. Optical imaging e.g. fluorescence imaging, is broadly used in the histologic investigation of cells, and has gained clinical attention for its intraoperative potential. Clinical studies have also established that the optical imaging-guided tissue resection mediate improved removal of cancerous tissue and thus there is reduction in the local recurrence. It may suffer slight noise due to scattering and interference from biological auto-fluorescence, but efficaciously utilized by slight up-gradations. Overall, optical imaging is indeed a novel approach in cancer diagnosis. It is safer, non-invasive, less complex, generate highly resolute images, and cost effective than other described techniques. Moreover difficulties associated with optical imaging can be successfully addressed by combination of two or more imaging techniques, in terms of multimodal imaging. This has become a key way to overcome the limitation posed by fluorescence imaging. Multimodal imaging is very much essential in accurate cancer diagnosis and delineation of diseased lacerations. Since each imaging contrast has a

distinct chemical composition, size, solubility, and pharmacokinetic, it is problematic to mix all together in a single dose for achieving spatiotemporal consistency [4]. However, the use of a single probe having the capability to exhibit multiple imaging facilities is highly favorable and forms the basis of multimodal imaging contrast. The multimodal imaging technique can reduce the corresponding toxicity and complex pharmacokinetic involved during the preclinical research and have more control in further translational research.

Magnetic resonance imaging (MRI) is one such widespread diagnostic technique employed mainly for getting high-resolution anatomical images of abnormal tissues e.g. tumor, with outstanding contrast and resolution. MRI revealed the physiochemical state of tumor tissue and neurological data based on the time difference between longitudinal (T1) and transverse (T2) relaxation. More importantly, its non-invasive nature and involvement of non-ionizing radiation don't interfere with the physiology of surrounding healthy tissues. The best practical realization can be achieved using a lanthanide-based MRI contrast agent [5]. The MRI method can be employed synergistically with luminescence-based imaging practice for efficient cancer diagnosis by following the concept of multimodal imaging.

### 1.5 Brief description regarding effective treatment of cancerous cell

Apart from diagnosis, cancer treatment strategies have been modified over time through the evolution of various therapeutic methods such as surgery, chemotherapy, radiation, immune and photodynamic therapy.

The primary methodology adopted for cancer treatment is surgery, in which the tumor is surgically removed from the body. The advanced technique adopted for effective surgical resection of tumor tissues is cryosurgery and lasers. In cryosurgery, extreme cold

conditions created by liquid nitrogen destroy tumor tissues. At the same time, highly energetic lasers are the cause of abnormal tissue obliteration in laser surgery. Moreover, surgical procedure is mainly helpful for treating tumor tissues grown peripherally or premalignant tumors. Furthermore, surgery is primarily an invasive technique, which creates additional side effects like inflammation, pain, etc., and doesn't guarantee tumor recurrence [6].

Similarly, Chemotherapy is another treatment, involving use of chemicals or drugs, ultimately causing methodical cancer cell death. The primary mechanism of action of chemotherapy drugs against cancer cell lines includes DNA damage, replacing standard building blocks of DNA/RNA, altering enzymatic activity for DNA replication, inhibition of cell mitosis and division, etc.[7] However, several disadvantages associated with chemotherapeutic treatment may be immediate or create chronic toxicity. Direct toxicity is reflected on skin & hair tissues, bone marrow, blood, the gastrointestinal tract, and the kidneys. The various neurotoxic effect may be observed, such as somnolence, paresthesia, paralysis, ataxia, spasms, and coma. The chronic side- effects include resistivity to drug application, and infertility, etc.[8]

Another rapidly adopted method "radiation therapy" uses high dosages of ionizing radiation to kill cancer cells or destroy tumor tissues. The electrically charged particles/ions involved, provide energetic effects to cancer cells causing their death or mediating genetic changes/damage to arrest their proliferation. The therapy is synergistically used with surgery for practical tumor shrinkage. The therapeutic modalities can be controlled externally by generating high-energy ionizing particles, and the internal mechanism involves radioactive sources present inside catheters for direct delivery into the tumor sites [9]. However, radiation therapy may cause various side

effects via disruption of multiplicative cell renewal system and vascular tissue damage, fibrosis, ultimately affecting the cell re-growth process. Also, in pediatric patients, it may cause secondary cancers due to mutations [10].

Contrary to chemo and radiation therapy, a modern non-invasive technique, namely immunotherapy, encompasses exploiting of the body's immune system as a selective therapeutic tool against cancer. Rapidly developing cancer cells metastasize to different body parts via immunosuppression mechanisms that enable them to be unrecognized by the body's immune system. Thus, immunotherapy aims to passively or actively distinguish these cells or abnormalities, destroy them, and diminish their recurrence. Identifying and eliminating cancer cells is an intricate pathway coordinated by innate (e.g., monocytes, macrophages, dendritic cells) and adaptive immune (e.g., T cells and B cells) components. But immunotherapy is subjected to several limitations, such as damaging immune homeostasis catalyzed by an immune reaction, which is not suitable for healthy cells. Hence adverse immune side effects, namely inflammation, colitis, and pruritus, could be observed [11].

Modern Photodynamic therapy (PDT) is one of the most efficient methods discovered till now for treating cancerous tissues by synergistically combining a photosensitizer and appropriate light. PDT mainly comprises of three components "NIR light", "photosensitizing molecule", and "oxygen ($O_2$)". When NIR light-triggered a photosensitizing agent, it transfers the absorbed energy to the adjoining oxygen environment, yielding singlet oxygen ($^1O_2$) activity. Destruction of tumor tissues is carried out by the cytotoxic effect of generated singlet oxygen species, as this reactive oxygen species (ROS) can oxidize nearby biomolecules. Surplus oxygen supply is a criterion for successful PDT for cancer cells. However, due to tumor tissue's rapid growth

and metastasis, the oxygen concentration rapidly depletes in the tumor microenvironment. Thus the efficacy of the PDT is significantly hampered under such hypoxic conditions [12].

### 1.6 Importance of Photothermal Therapy in Cancer Treatment

To overcome the above-discussed difficulties allied with various therapeutic techniques used in cancer treatment a noble, cost-effective, and practically achievable treatment modality has been evolved called "Photothermal therapy (PTT)" [3, 13]. Photothermal therapy utilizes the near-infrared radiation (NIR) for an exciting photothermal agent to convert light into thermal energy. This thermal energy is responsible for the localized killing of cancer cells [14]. It can also act effectively in the necrotic and hypoxic regions of tumor tissue unlike PDT, without compromising the photothermal destruction efficacy [12]. Additionally, the localized heating tendency is fruitful for properly treating diseased states without affecting the physiology of surrounding healthy tissues.

Temperature is one of the critical parameters influencing the dynamics of biological systems ranging from a single cell to sophisticated organs [15, 16]. Any temperature increment above average human body temperature (~37 °C) is harmful, leading to severe organ damage [17]. However, temperature increment when proceeds through controlled manner could positively impact patients undergoing cancer treatment [18]. As tissue/Cells provide a transparent window for NIR radiation [19], the photon absorption process become easier for the photothermal agent present deep inside the tumor [20]. The resulting thermal energy causes the killing of cancer cells via protein denaturation, cell membrane rupture, simulated body fluid evaporation, etc. [21]. Photothermal therapy causes programmed cell death rather than cell necrosis, where the inflammatory response of the biological system may hamper therapeutic efficacy [22]. However, some

researchers reported that both apoptosis and necrosis are associated with the cancer cell death process caused by PTT [23]. Apoptosis is an active mechanism that triggers cell death without activating an immune or inflammatory response. At the same time, necrosis is a passive mechanism causing cell death due to inflammation resulting from cell membrane damage and the release of impaired molecular patterns [24]. The beauty of PTT is that it can be synergistically used along with other conventional therapies for successful antitumor response [25].

The effectiveness of photothermal treatment for tumor ablation depends on two primary factors, e.g., duration of therapy and subsequent temperature increment [26]. The likely changes occurring during the destruction process of cancer cells by photo-generated heat can be segregated into different temperature zone (Fig. 1.3), and subsequent mechanisms are represented in figure 3 [27].

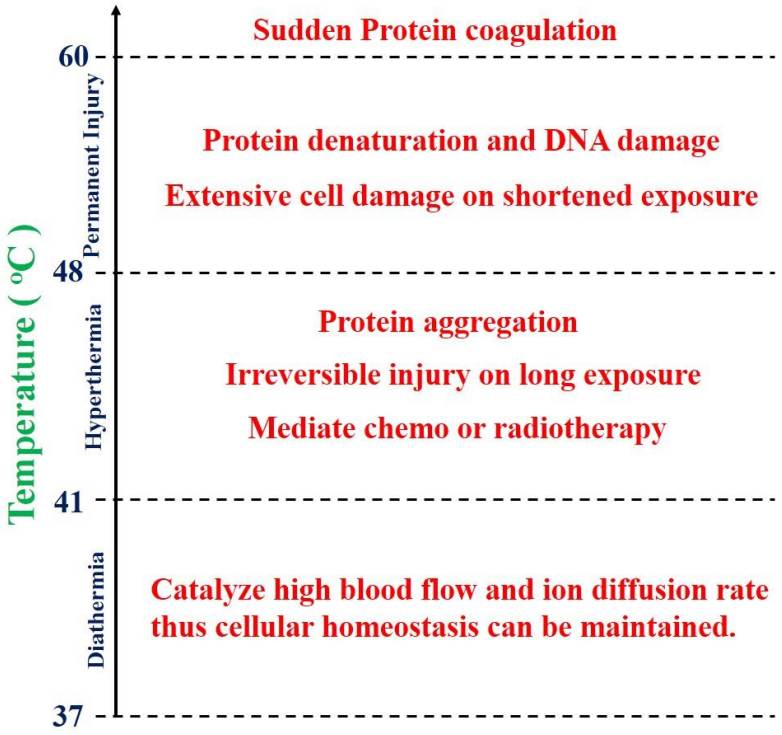


**Figure 1.3** Different zones of temperature are responsible for causing various physiological changes during tumor treatment by photothermal therapy.

The different zones of temperature raised during photothermal therapy cause cellular damage to variable extents. These are classified into mainly three types;

**(i)** **Irreversible Injury**: Tumor temperature rises above 48 °C within a minute, and drastic cell death is achieved due to coagulative necrosis. This process of cell death cannot be reversed; hence injury occurring to tumors is termed irreversible. But this process can be non-selective, thus hampering the physiology of nearby healthy cells [28-30].

**(ii)** **Hyperthermia Treatment:** The treatment of tumors carried out within the clinically significant temperature range (41–48 °C). Within this temperature range, denaturation of cellular protein leads to aggregation-induced destruction of cells.

**(iii)** Also, thermo resistive protein secretion can be minimized by prolonged exposure of tumors to this temperature range, ultimately causing cell death by oxidative damage or necrosis [31, 32].

**(iv)** **Diathermia treatment:** Passive cancer treatment process, carried out at a temperature below 41–48 °C for a prolonged duration. This ultimately enhances the efficacy of cancer drugs on tumors by assisting adequate blood flow, oxygen supply, pH balance, and ion diffusion across the cellular membrane of resilient tumor cells [33, 34].

Hyperthermic zone or hyperthermia range of PTT is popular among the scientific community due to practically achievable (Fig. 1.4) temperature range with a minimalistic negative impact on healthy cells as mentioned earlier [35].

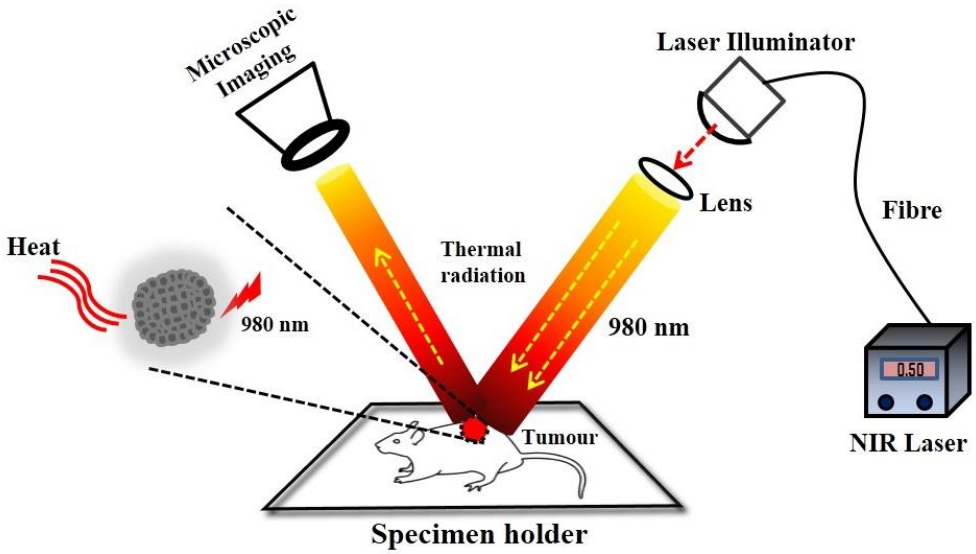


**Figure 1.4** Schematic showing *in Vitro* hyperthermic activity in a mouse model for treatment of tumor.

## 1.7 Requirement of Multifunctional Nanomaterial (MFN)

Simultaneous achievement of above discussed advance optical and MR imaging capabilities along with noble photothermal functions can be successfully realized via development of multifunctional nanomaterial (MFN). These nanomaterials are important flag bearers of the current emerging research trend "Nanobiotechnology," and a single nanomaterial can accomplish multiple objectives in cancer theranostics through innovative functionalization. There exists an optimized interplay between nanostructure & its physicochemical characteristics, attached ligands, diagnostic realm, and therapeutic agent in a unified platform.

For various tissue depths or treatment stages, multimodal imaging facilities (e.g. fluorescence & MRI) offered by multifunctional nanomaterials offer better images. Thus precise disease diagnosis and corresponding image-guided therapy can be successfully accomplished. Apart from this, embedded plasmonic characteristics or energy transitions involved in multifunctional nanomaterial is suitable enough for developing a noble

photothermal agent to be employed in the thermal ablation of cancer cells. Furthermore, as highlighted earlier, the exceptional characteristics of multifunctional nanomaterials and their size effect has unbolted exciting pathways for analytical chemistry or energy-related applications. These characteristics may span from optical to electrochemical activity. The optical property has its self-diversification in cancer diagnosis and electrochemical activity may lead to the development of potential biosensors for the detection of biomarkers closely related with cancer metastasis. Unlike invasive, expensive, and high false positivity associated with the standard histopathological techniques used in early cancer diagnosis, electrochemical biosensor delivers rapid and reliable detection mechanism for tumor angiogenesis and metastasis [36]. It comprises various cancer biomarkers such as altered genomic sequence, circulating DNA, microRNA, antigens, cytokines, exosomes, floating tumor cells [37], and electrochemical signal transduction process, namely cyclic voltammetry square-wave voltammetry, electrochemical impedance spectroscopy, chronoamperometry, etc. [38]. Therefore the multifunctional nanomaterial with advanced electrochemical and photo-physical activity could find greater applicability in the theranostics field. Overall, these electrochemical properties also could lead to the fabrication of a miniature electrochemical device/battery with improved charge storing and delivery capability. Additionally, the enhanced magnetic activity of multifunctional nanostructure can be suitable enough for fabrication of magnetic storage devices or magnetic energy-related applications besides cancer diagnosis via development of MRI contrasting agent.

There are a number of multifunctional nanomaterials have been synthesized for theranostics applications, built on inorganic and organic-based precursors. These precursors mainly include proteins, polymers, lipids, noble and rare-earth-based metals, etc. [39]. The most expedient luminescent imaging technique and photothermal therapy

though efficaciously realized using organic molecules, but the concept of photo bleaching is major a shortcoming [5]. On the contrary, inorganic materials pertaining to advanced optoelectronic, magnetic, and plasmonic characteristics are suitable enough for development of multifunctional nanomaterials [40-42]. Specifically, inorganic multifunctional nanomaterials based on noble metals (Au/Ag/Cu) draw wide attention due to their large optical fields of absorption, and scattering. In noble metal based nanostructures, there is an advancement in the optical and photothermal properties resulting from the resonant oscillation of surface free electrons under electromagnetic radiation. A hefty electric field is induced near their surface due to coherent oscillation of conduction band electrons resonating with externally applied electromagnetic light (~NIR). Thus surface plasmon resonance (SPR) band is observed as a result of this generated electric field. This localized surface plasmon resonance can have dual purpose: radiation via "Mie" scattering and or fast non-radiative relaxation into heat energy; thus, both imaging and therapeutic requirement can be fulfilled. Previously plasmon resonance nature of gold and silver metal-based nanomaterials was harnessed in applications like disease diagnosis, biophysics, and clinical therapy. However, due to low cost, abundant availability, improved physicochemical and plasmonic characterstics "copper" is suitable material of choice for the synthesis of multifunctional nanomaterial. Besides, copper has the highest electrical conductivity ($6.5 \times 107\ Sm^{-1}$) as compared to gold ($4.9 \times 107\ Sm^{-1}$) and silver ($6.6 \times 107\ Sm^{-1}$) [43]. Further, Copper metal also has a thermal conductivity value (401 W/m.K) and melting point (1083 $^{o}$C) comparable or high with respect to silver metal, having thermal conductivity and melting temperature 429 W/m.K and 1064 $^{o}$C, respectively. Copper also facilitates easy transport of conduction electrons, which is very helpful in plasmonic or nano-photonic applications, thus minimizing joule heating, and accelerates smooth propagation of surface plasmon polariton (SPP) waves [44]. Lastly,

the copper-based nanomaterials are reported to have excellent magnetic and electrochemical properties also.

### 1.8 Copper oxide/hydroxide based biomineral for MFN synthesis

Due to their biological origin, copper metal-based semiconductor nanomaterials (especially oxide/hydroxide-based systems) are termed as copper-based biominerals. On a laboratory scale, these biominerals are investigated to have unique physical properties, fluorescent nature, plasmon resonance, and electrochemical as well magnetic activity [45, 46]. These copper biominerals could suitably exhibit multiple functionalities in the field of theranostics, bio-analytics, and energy. Additionally, the quantum confinement effect improves the photo-physical property in copper oxide or hydroxide-based semiconducting nanomaterials [47]. Generally, fluorescence in semiconductors nanomaterial is an intrinsic phenomenon induced by impingement of electromagnetic radiation of suitable energy. Basically, it's a stoke process (Fig. 1.5) and generates light of higher wavelength when excited by light of lower wavelength, as explained by Jablonski [48].

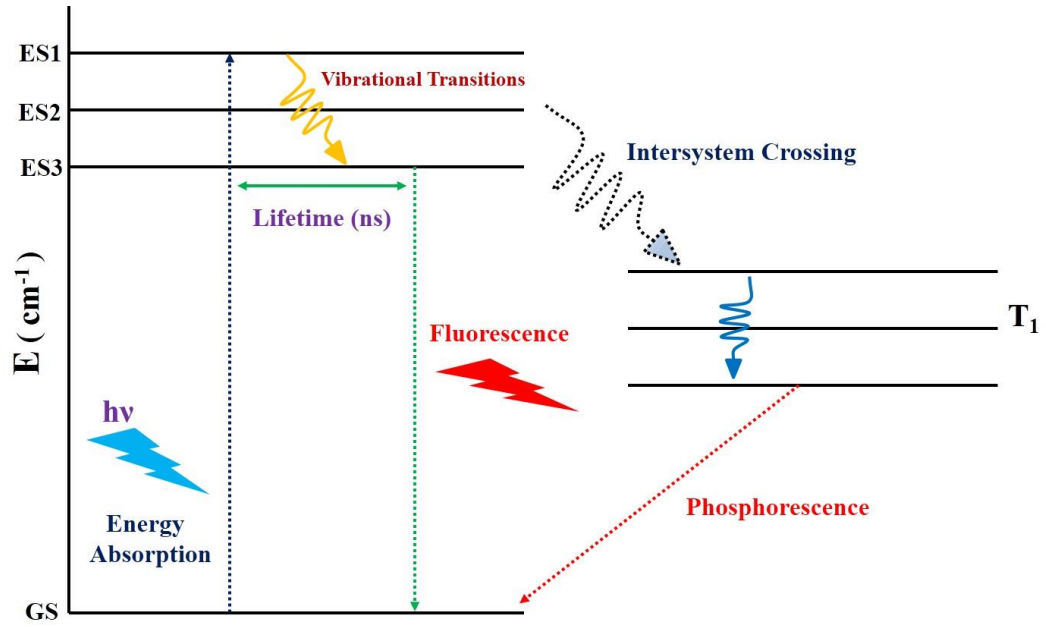

**Figure 1.5** Jablonski Energy Diagram explaining the fluorescence Mechanism. ###ES: excited state, ##GS: ground state, #T: Triplet state.

In the field of *in Vivo* cellular imaging like cancer diagnosis, the use of fluorescent semiconducting nanomaterials as an imaging probe is an exciting and technologically advanced phenomenon. It not only facilitates efficient bioimaging of targeted sites during disease diagnosis but also detects the biological and chemical species that are not intrinsically fluorescent.

Moreover, while developing copper-based semiconducting nanomaterial for end applications like “theranostics” and “bio-assay” deep tissue penetrability, minimal environmental interference, improved signal to noise ratio, high image resolution are some factors that need to be taken care of. All such factors can be efficiently addressed by developing rare earth doped multifunctional UCPs, those not only have deep tissue penetrability, excellent image resolution, high signal-to-noise ratio but also have high heat generation capability. In fact, during different bio-analytic/sensing processes, their unique detection mechanism and signal processing mechanism is robust and effective.

## 1.9 Upconverting phosphors (UCPs) exhibiting Multifunctionality

Upconversion is the non-linear optical absorption process. The sequential uptake of two or more photons with low energy, generate luminescence in the broad wavelength region (UV to NIR) [49]. This process may not be thermodynamically feasible, but achievable from the quantum mechanical point of view [50]. Unlike fluorescence and phosphorescence, Upconversion is the anti-stoke process where emission wavelength is smaller than the exciting photon wavelength [51]. Concurrent multiphoton absorption mechanism proceeding through virtual energy levels in case of fluorescent dyes and quantum dots, but Upconversion banks on the sequential uptake of low energy photons

through the ladder-like energy structure of doped lanthanide ions [52]. Most UCPs are irradiated through less expensive continuous-wave diode laser [53]. Moreover, NIR laser (transparent for biological window) excitation capability, high contrast optical signal strength and high heat generation properties (Fig. 1.6) lead to better prospective in biomedical engineering when there is use of UCPs [54-56].

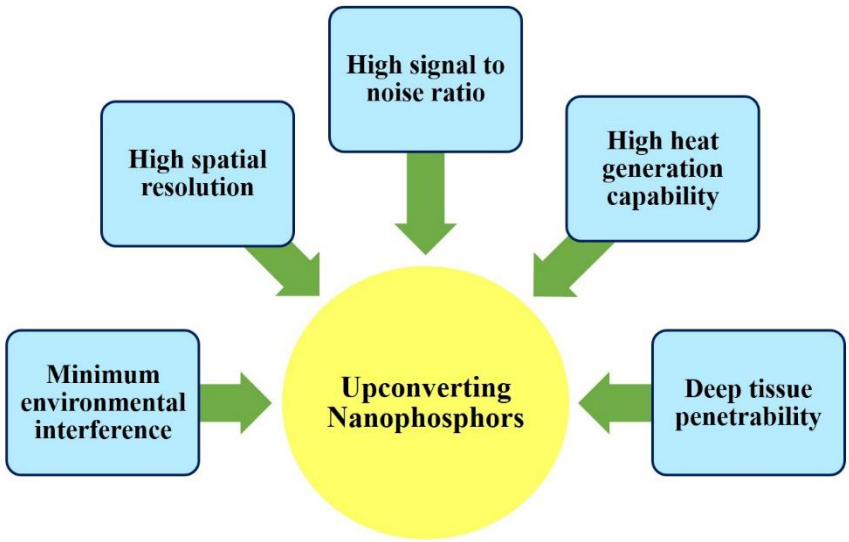


**Figure 1.6** Interesting characters of Upconverting phosphors (UCPs).

Upconversion mechanism proceeds through intermediate energy levels of host lattice, those act as short-lived reservoirs for pumped photons generated via dopant ions before relaxing to ground state for generation of luminescence [57, 58].

Trivalent lanthanide ion doping imparts unique photo-physical property to Upconverting nanomaterials by $4f^n5s^25p^6$ electronic arrangements with exponent 'n' varies within 0-14. Depending upon variation in 'n' value, there are 14 possible energy levels and emission results in UV, Visible, and NIR regions. More importantly, the f–f electronic transitions of doped lanthanide ions in the host lattice generate a set of sharp atomic emission peaks. Sharp emissions not only reduce the probability of spectral overlapping but also facilitate the recovery of screened signals. Additionally, the parity forbidden

transitions among $4f^n$ electronic states to give rise to long-duration luminescence lifetime ('ms' scale), which is extremely useful for time-resolved fluorescent imaging, biomolecule sensing, and multiplexing. The collective emission from dopants in a single Upconverting crystal does not cause photo blinking, most important for single-molecule imaging experiments those can be observed for long-time and good for deep tissue imaging [59]. It also carries out several *in Vivo* photochemical reactions using absorbed NIR radiation and frequency converting ability. Further, due to the shielding of 4f orbital by outer filled 5s & 5p orbitals, the Upconversion emission is also unaffected by changes in the surrounding environment and also there is minimization of oxidative induced photo bleaching.

Structurally during Upconversion process, the trivalent lanthanide ions are doped in the various nanostructured matrix namely fluorides, chlorides, iodides, bromides, oxides, silicates, borates, phosphates, etc. [60]

**1.10 Constituents of Upconverting phosphors**

Most of the Upconversion materials consist of host matrix, doped with rare-earth ions those are constituents of the third group of the periodic table, i.e., Sc, Y, La and 14 elements of the lanthanide family Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu. These 15 chemical elements with atomic number 57-71 (La- Lu) are collectively called lanthanide (Ln) series elements/rare earth elements [61]. They are also sometimes coined as f-block elements due sequential adding of '14' 4f electrons to the base electronic configuration of lanthanum ([Xe] $4f^{n:0-14}5s^25p^6$) [51]. All lanthanide elements exist in trivalent cationic ions; their chemistry largely depends upon the ionic radius and unchanged outer electronic configuration. Based on this they display similar type of chemical properties. In rare-earth elements also the electrons in the 4f shell are shielded by outer 5s and 5p orbitals, thus spatial distribution of energy levels remains unaffected

by the change in host matrix [58]. At the same time, the energy levels of sublevels are ascertained through the presence of nearby rare-earth ions by stark effect. Upon irradiation, lanthanide ions can be excited via three types of transitions: 4f-4f, 4f-5d, and a less common charge transfer process. Generally, Upconversion luminescence occurs via 4f-4f transitions.

The distinctive ability of lanthanide ions to emit luminescence in the broad wavelength region, e.g., ultraviolet, visible, and NIR region, is helpful in various applications: theranostics to optoelectronics [39].

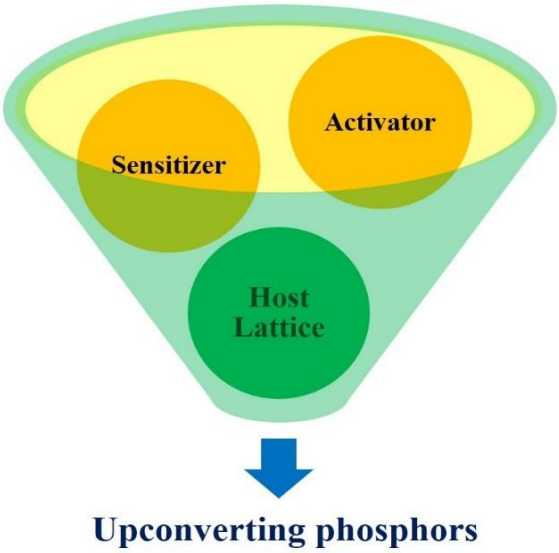


**Figure 1.7** Schematic showing constituents of Upconverting phosphors.

Upconversion system is based on the guest-host arrangement, where guest rare-earth ions are doped into host solid crystal. Generally, Upconverting system (Fig. 1.7) consists of three main components: Host lattice, sensitizer, and activator [62, 63]. One doped rare-earth ion serves as a sensitizer (e.g., $Yb^{3+}$), while the other serves as an activator (e.g., $Er^{3+}$, $Tm^{3+}$, $Ho^{3+}$). Sensitizer acts as a NIR radiation-absorbing center and transfers its energy to the activator ion through various mechanisms. Therefore activator serves as an energy emission center while the sensitizer performs the energy donation activity.

### 1.10.1 Sensitizer

The sensitizer can effectively absorb infrared photons and traverse from the ground to the metastable excited state. The sensitizer ion in an excited state transfers its energy to a neighboring activator ion by an energy transfer mechanism that may be radiative or non-radiative. Energy transfer through the multiphoton process is possible from the sensitizer because activator ions are situated at high lying energy levels as compared to absorbed IR photons. Generally, ytterbium and neodymium elements are used as a sensitizer in the Upconverting system. However, the $Yb^{3+}$ ion doping (~20-40 mole %) acts as a sensitizer in most Upconverting particle synthesis due to the high absorption cross-section at exciting NIR wavelength [64].

### 1.10.2 Activator

Activator ions act as the center of anti-stokes luminescence. They relax to the ground state preceded by absorbing energy from sensitizer ions through the energy transfer process. If there is no gradient in the energy levels of different activator ions, there will be unwanted non-radiative relaxations. The most commonly used activator ions are $Er^{3+}$, $Tm^{3+}$, and $Ho^{3+}$, which have discrete energy levels and metastable electronic states [65]. The range of applicability of Upconversion luminescence depends upon the nature of the activator ion.

### 1.10.3 Host Lattice

Apart from sensitizer and activator, the crystallinity of the host lattice and its interaction with the sensitizer and activator ions has a strong influence on the Upconversion luminescence. The host material is chosen to have a low lattice mismatch between dopant ions, close proximity between the dopant ions, and minimum phonon relaxation resulting from lattice vibrations [58]. For an ideal host matrix, phonon energy should be as low as possible. It is important to wisely select the composition of the host matrix to maximize

radiation and minimize non-radiative losses. Halide compounds, such as iodides, chlorides, and bromides pertaining to low phonon energy, could have been used in Upconverting nanomaterial synthesis [60]. However, due to their hygroscopic nature, they are not widely adopted. Though Oxides are chemically more stable, their high phonon energy could be an issue. Nowadays, semiconductor nanocrystals are also proposed to be viable alternatives, but this technique can be overruled due to the very different ionic radius between host and doping element. In this context fluorides with very little phonon energy and improved chemical stability can be used as a feasible host material during the synthesis of UCPs. Furthermore, this inorganic host lattice is found to be suitable for the synthesis of UCPs by doping of trivalent ions with similar chemical properties and comparable ionic radius. Host lattice based on cationic ions such as $Na^{+}$, $Ca^{2+}$, $Sr^{2+,}$ and $Ba^{2+}$ with ionic radii close to radii of the dopant ions, reduce lattice stresses and corresponding crystal defects, indeed a good choice for the synthesis of UCPs. Thus cation ($Na^{+}$, $Ca^{2+}$, $Y^{3+}$, $Gd^{3+}$) based fluoride materials are the best choice for host matrix in Upconversion experiments. Since the optical properties of UCPs depend upon the phase and crystallinity, the best host lattice is the hexagonal $NaYF_4$ (β-$NaYF_4$). The Upconversion efficiency of the β-$NaYF_4$:$Yb^{3+}$/$Ln^{3+}$ is roughly ten times higher than its cubic counterpart. Similarly, less expensive, improved crystallinity, low phonon vibrations are some excellent properties associated with the $NaYF_4$ host [66].

### 1.11 Mechanism of Upconversion Process

The entire Upconversion mechanism can be categorized into various types, namely: Excited-state absorption (ESA), Energy transfer Upconversion (ETU), Co-operative sensitization (CS), Photon avalanche (PA), and Triplet-triplet annihilation (TTA).

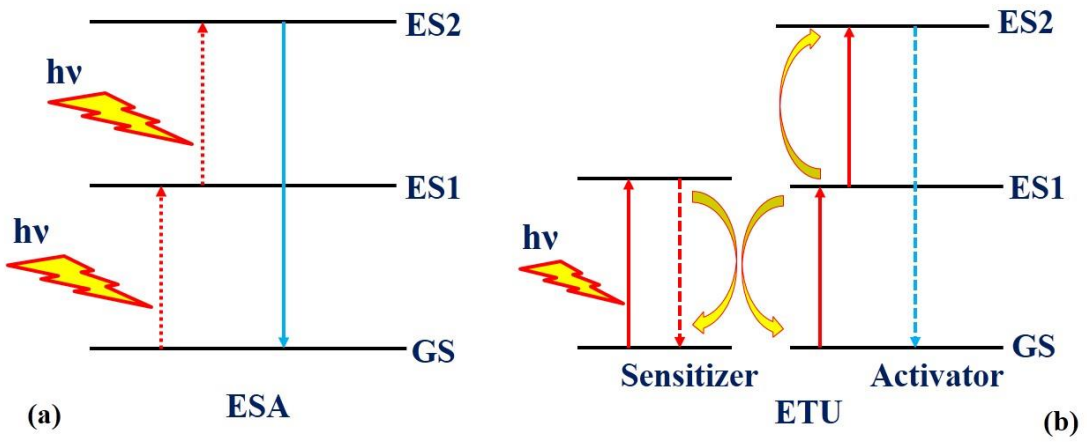


**Figure 1.8** Two popular Upconversion mechanisms are (a) Excited-state absorption (b) Energy transfer Upconversion. ### ES1: first excited state, ## ES2: second excited state, #GS: ground state, *hν: energy of the photon.

ESA (Fig. 1.8a) is the most straightforward Upconversion mechanism in which dopant ion promoted to first excited state ES1 by absorbing a photon, also denominated as ground-state absorption. On absorption of energy from another pump photon, this ion excited to a higher energy level ES2. Subsequent release to the ground state results in luminescence [58]. One of the most cited Upconversion mechanisms is ETU (Fig. 1.8b). It involves energy transfer happening between two neighboring dopant ions, namely sensitizer, and activator, ultimately producing luminescence. Sensitizer ion first absorbs a photon and rises to the first excited state ES1. Then sensitizer relaxes back to the ground state by transferring its energy to the activator, which is then promoted to the intermediate excited state. Activator ion on absorbing another photon jumped to a higher excited state, and ultimately results in luminescence on ground-state relaxation [67]. Co-operative sensitization or co-operative Upconversion (CUC) also involves two types of luminescent centers, e.g., sensitizer and activator. However, unlike two ion systems involved in ETU, CUC is a three ion system, and the probability of energy transfer is low (Fig. 1.9).

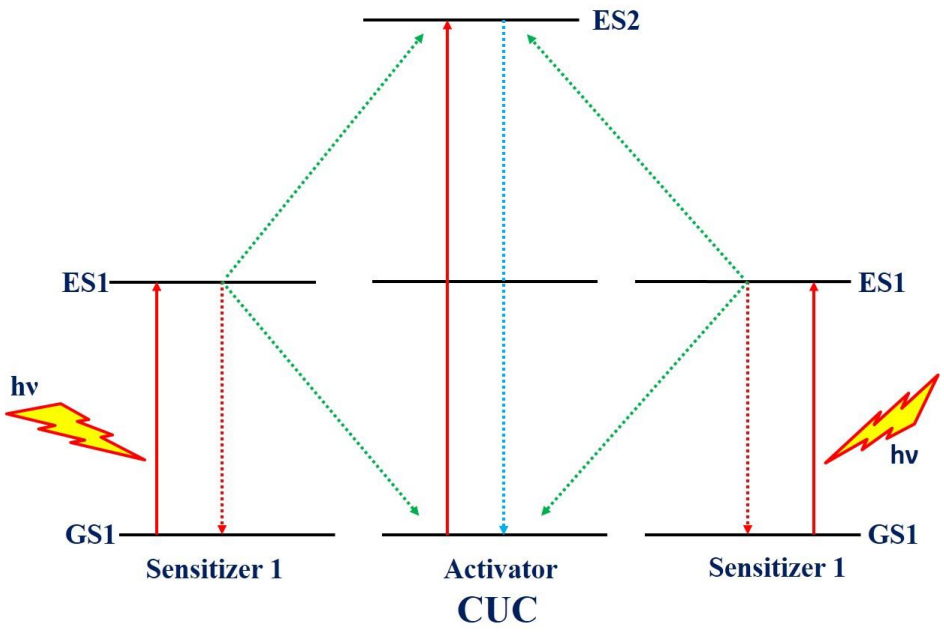


**Figure 1.9** The Co-operative mechanism involved in the Upconversion process.

The energy levels of activator ion populated with simultaneous energy transfer from two neighboring sensitizer ions. The activator ion relaxes immediately to produce luminescence due to short-lived intermediate energy states compared to sensitizer ions [68]. Photon avalanche (PA) is a unique mechanism that is less popular due to high photon pumping power requirements. The entire PA process is based on the cross-relaxation mechanism between two closely spaced ions in a material (Fig. 1.10).

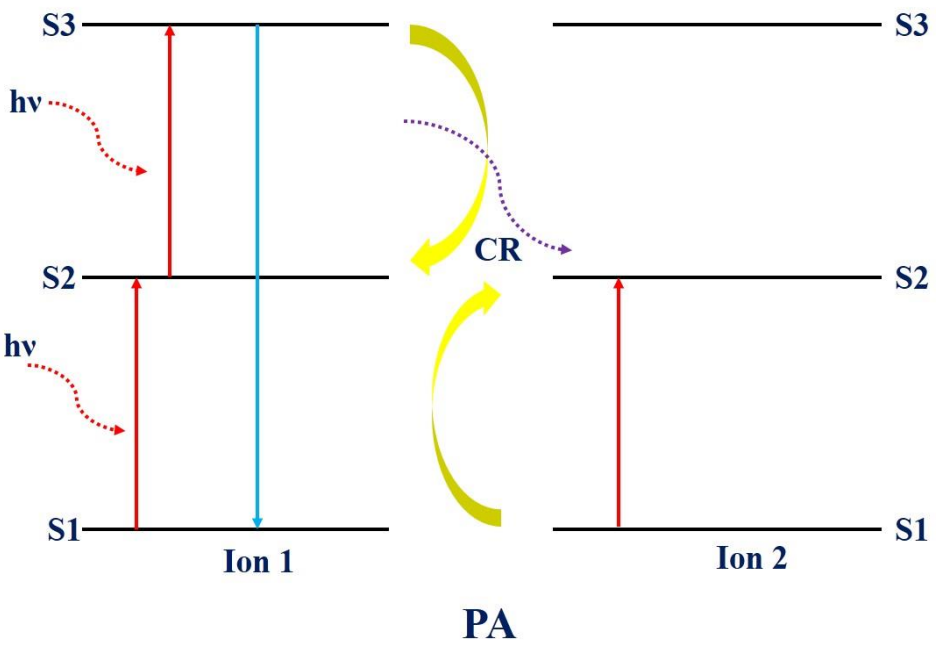


**Figure 1.10** Photon avalanche mechanism associated with Upconversion process.

The ion '1' present in the ground state S1 is promoted to state S2 and subsequently to state S3 by the energy transfer Upconversion process. When the ion relaxes back to the ground state, during that process it transfers its energy to another ion present in the ground state, which is again promoted to state S2. These two ions in excited state S2 populate two more ions in state S2 through excited state absorption and cross-relaxation energy transfer process, thus there is a production of an avalanche of ions in this energy level. Then Upconversion proceeds through direct excited state absorption from state S2 to state S3 and is followed by ground state relaxation [69].

Energy migration Upconversion (EMU) or triplet-triplet annihilation (TTA) is a novel and latest discovered Upconversion phenomenon occurring in core-shell type Upconverting nanostructures (Fig. 1.11).

This type of mechanism involves luminescent centers called sensitizers, accumulators, migrators, and activators. Sensitizer/accumulator ion is confined to the core layer of

UCPs which is connected to activator ion present in the shell via different migrator ions or vice-versa. Sensitizer undergoes excitation by GSA and subsequently transfers its energy to the accumulator. Upon excited to higher energy levels, there is energy transfer to activator ion via hopping mechanism catalyzed by migrator ions. The trapped energy from the migrator ion undergoes ground state relaxation from the activator's highest energy level, resulting in luminescence [70].

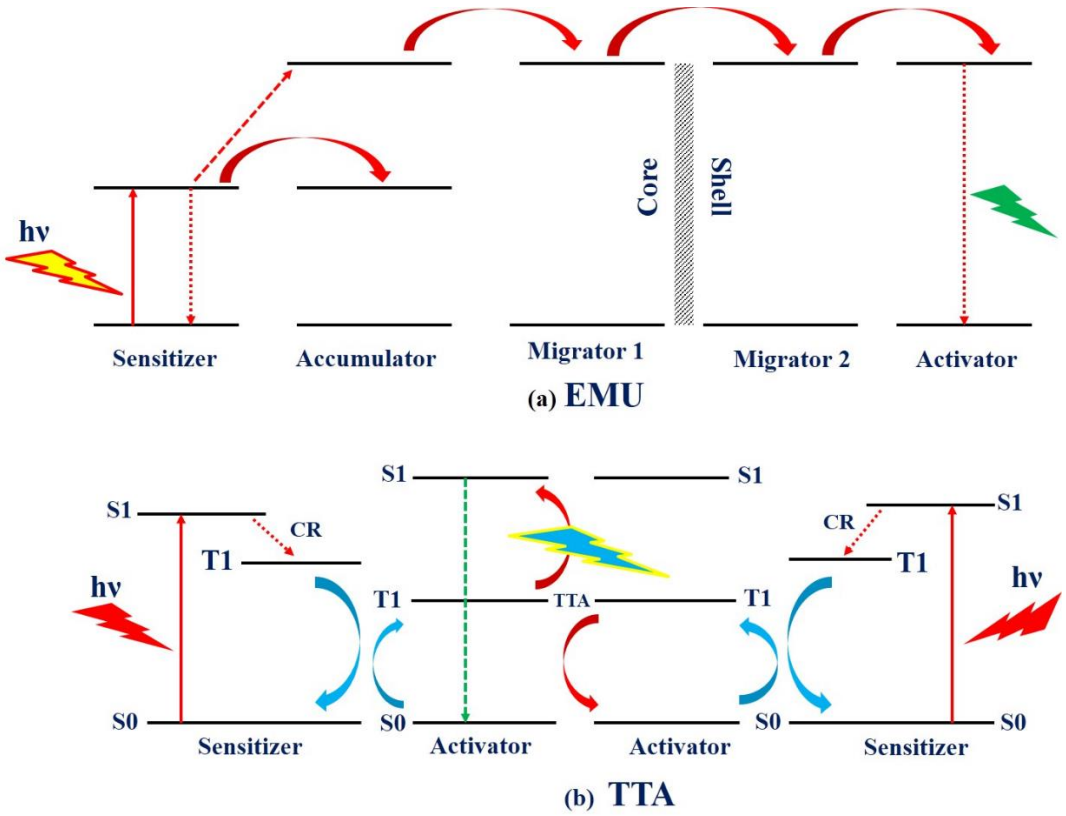


**Figure 1.11** Upconversion luminescence mechanism mediated by energy migration and triplet-triplet annihilation.

Overall it can be stated that Copper-based semiconducting and rare earth doped Upconverting nanomaterials endowed with both optical activity, and NIR excitation capability facilitate a minimally invasive, deeper penetrable imaging and photothermal agent for the successful cancer theranostics purpose [71, 72]. Besides, photostability,

yield predictable results, and unquenched luminescence by binding with cellular proteins are additional advantages in cancer theranostics field [73].

These inorganic semiconducting multifunctional nanomaterials (Fig. 1.12) also have their own set of exclusivity in energy-related applications, e.g., electrochemical energy [74, 75]. Further lanthanide-doped Upconverting phosphors are known to exhibit excellent T1 relaxation mechanism very much required for the development of MRI contrasting agent.

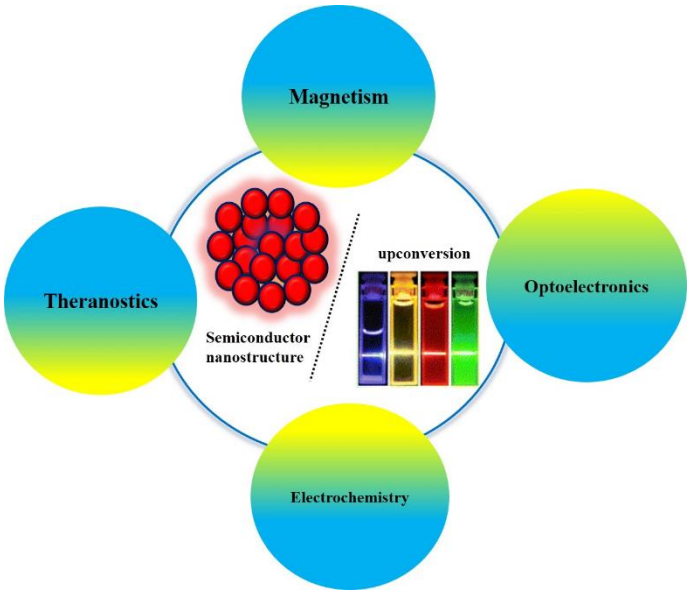


**Figure 1.12** Schematic representation of multifunctional attributes of semiconducting and Upconverting nanomaterials.

### 1.12 Effectiveness of MFN depending on Size, Shape & Functionality

Further the entire dynamics of the efficient multifunctional theranostics system depends upon the morphological (size or shape) and surface chemistry-related factors associated with developed nanomaterials. The size of the nanomaterial governs the surface-to-volume ratio and effective bio-distribution of nanomaterial within tumor tissue for targeted application. Since tumor vasculature dimension varies between 5-200 nm, thus intermediate size spanning from 10 to 100 nm is optimum enough for improved

biomedical application of nanomaterials due to adequate circulation time achieved. Generally, smaller nanomaterials (< 10 nm) are cleared through renal filtration while large particles (> 100 nm) are eliminated via "Kupffer" cells present in the liver and spleen. However, it is observed that upon intravenous injection, the nanomaerials get coated with serum protein due to circulation in the blood vessels [76], ultimately increasing their hydrodynamic radius. Thus, the nanomaterial's successful renal clearance may be hampered even after targeted use. Further absence of an effective reticuloendothelial system and in-significant uptake by non-targeted tissues might create additional problems [77]. This unsuccessful renal expulsion created additional toxicity in the body caused by the accumulation of theranostics nanomaterials. Therefore before theranostics use, tuning the size of synthesized multifunctional nanomaterial is very much crucial. The 40-50 nm and 10-100 nm sizes are reported to yield the best possible results during *in Vitro* and *in Vivo* applications respectively. Smaller nanomaterials overcome the cellular obstacle by some mechanism that is not specified, while larger ones sail through pinocytosis, phagocytosis, or other specific cell transport mechanism. Furthermore, for solid tumor applications, the optimum nanomaterial dimensions are reported to be 70-200 nm those exhibits the best bio-distribution [78].

Nanoclusters or quantum dots are proposed morphology regimens that need to be explored due to their fascinating photo-physical and biological activities, established previously [79-81]. Quantum dots have a size ranging from 1-12 nm and are semiconducting in nature [79, 82]. Synthesized from group II-VI elements in the periodic table, quantum dots are important optical probes whose fluorescent properties modulated according to their size & composition [83]. The fluorescence property of quantum dots is attributed to the recombination of electron-hole pairs (exciton) generated when absorbed sufficient amount of energy [84]. However, during several optoelectronic

applications, the surface defects associated with the quantum dots act as electron traps those delay the electron-hole recombination process, ultimately reducing the quantum yield and resulting in photo bleaching [85]. Additionally, quantum dots are also subjected to aggregation and size enhancement during cellular applications, thereby their renal clearance is reduced [86, 87].

On the other hand, nanoclusters are ultra-small sized structures ranging from 1-3 nm and consist of few to hundred atoms [88, 89]. Some researchers also reported the estimated size of nanoclusters to be within 1-10 nm [90, 91]. Further, for the multitude of end application purposes, fluorescent nanoclusters have been synthesized using templates such as nucleotides, polymers, proteins, peptides, biomolecules, small chain ligands, dendrimers, etc. (Fig. 1.13) [92, 93].

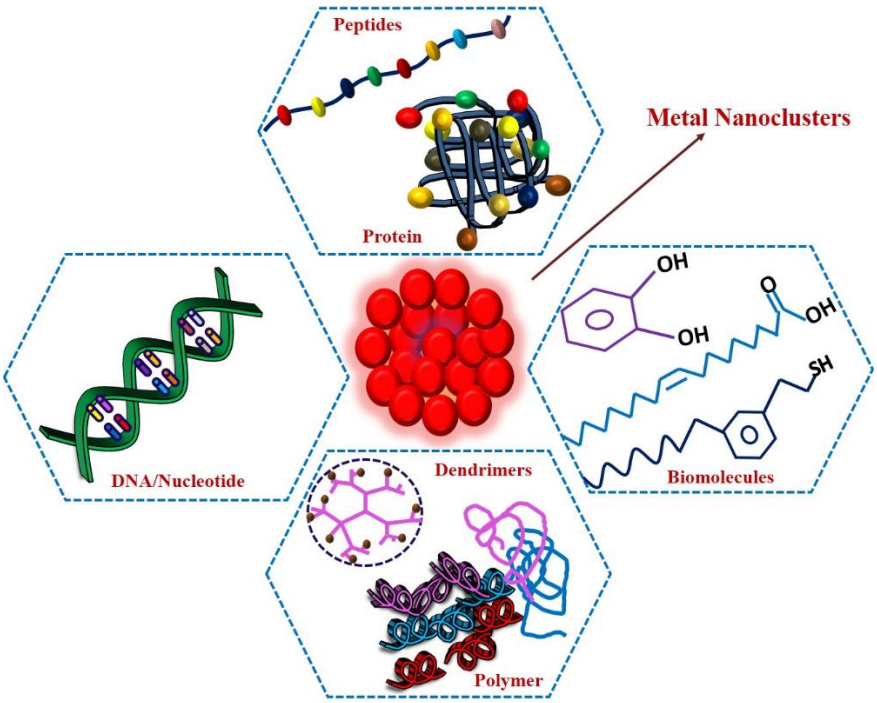


**Figure 1.13** Schematic representation of fluorescent metal nanoclusters protected by Nucleotides, Proteins/peptides, biomolecules, polymers/dendrimers, etc.

Nanoclusters develop a missing connection between a single atom and plasmonic nanomaterials and demonstrate the discrete electronic configuration along with molecular behavior [94, 95]. Semiconducting nanoclusters exhibit distinct photo-physical, catalytic, electronic, and magnetic properties [96-99]. The nanoclusters display luminescence which is of rare occurrence, and the underlying principles are still under investigation. However, it is understood that luminescence can arise due to: (i) core (intrinsic confinement) and (ii) surface effect (core metal-ligand interaction) [100, 101]. However, due to their molecule-like electronic states, nanoclusters, when invaded by light of suitable energy, result in photoluminescence (Fig. 1.14). [93, 102]

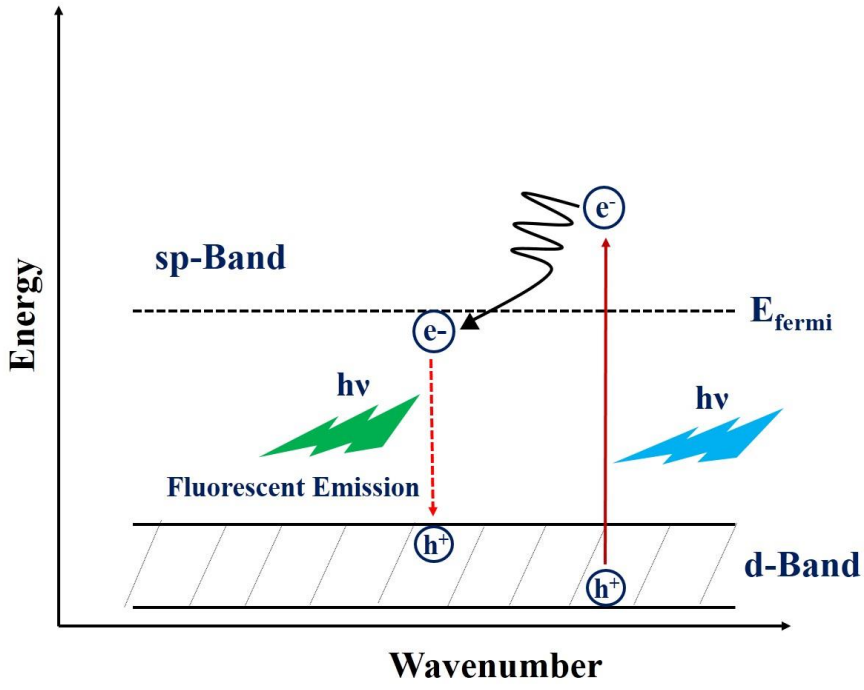


**Figure 1.14** Fluorescence mechanism in transition metal nanoclusters.

Moreover, ultra-small sized semiconductor nanoclusters are atomically expressed and metastable species normally encountered in synthesizing various nanocrystals [103, 104]. Their atomic and electronic configuration provides a reasonable understanding of

nanocrystal formation dynamics, thus unraveling the underlying synthesis mechanism also [105].

Semiconductor nanoclusters have a unique electronic configuration [103], which results in optical absorption and subsequent band-edge emission [106, 107]. These properties are also influenced by quantum confinement effect [108].

Copper is the environmentally active material of noble metal group, having low cost and is biotechnologically significant, therefore archived special role in nanocluster synthesis [109-111]. Generally, (i) complex synthesis procedure (ii) existence of strong interband transition energy, lying close to resonant Plasmon energy (iii) enhanced spectroscopy applicability, and lastly (iv) relatively more biocompatibility [112, 113] are some standout abilities associated with copper-based nanoclusters. Despite being interesting semiconductor [45, 114], not much effort has been devoid towards development of luminescent nanoclusters out of divalent copper-based systems or copper-based biominerals.

In addition to size, morphology also plays a pivotal role in regulating the effective interaction of multifunctional nanomaterial with tumor tissue and its distribution within. Thus requiring modulation of the shape of the multifunctional nanomaterial in cancer theranostics. Shape controls the hydrodynamic forces required and trajectory traversed during the circulation inside the body. Thus nanomaterial's adequate circulation and bio-distribution can be controlled. Nanomaterials with a more symmetrical structure like a sphere, cubes, etc., may experience uniform distribution of forces over them. Therefore remain positioned at the center of the blood vessel during circulation, ultimately effectively delivered at the diseased sites. On the other hand, more asymmetrical structures may experience uneven drag and torque during their motion, affecting their

tendency for accumulation at diseased state [5]. Interestingly spherical particles are reported to be experiencing shorter circulation time. Lastly, a nanomaterial's shape that can trigger maximum coverage over cell membranes is observed to be best for easy cellular uptake. Thus asymmetrical shapes with better aspect ratios are suitable enough for smooth internalization, and balance should be maintained during shape modulation for practical efficacy.

Therefore above discussed inorganic nanomaterial's successful cancer theranostics use can be successfully achieved via the development of very small-sized spherical structure besides nanocluster morphology. Spherical morphology as earlier mentioned could help successful delivery at tumor region and corresponding renal clearances [5]. Similarly, multiple functionalities of rare earth doped UCPs in cancer theranostics and magnetism can be achieved by controlling their morphology and crystallinity without compromising advanced photo-physical and luminesce attributes. Hence there is a greater need for the synthesis of Upconverting phosphors with diverse morphology and crystalline property.

In addition to size & morphology, the surface functionality of developed multifunctional nanomaterial is responsible for regulating overall nano-functional characteristics and imparting diverse theranostics as well electro-analytical facility. Modification in the synthesis strategies, reduction in size, and surface-to-volume ratio optimization result in improved surface functionality. The surface modification of nanomaterials by small molecules, ligands, particles, polymers, etc., could accomplish varied tasks. These techniques not only control the shape, size, and solubility but also resulted improved the physicochemical, optoelectronic, magnetic, and other spectroscopic properties [75]. The surface modification with various bio-molecules or polymers may bind with tumor-specific receptors [40]. Specifically, surface-functionalized ligands (e.g., protein, peptides, biomolecules, antibodies ) hold a solid capability to bind with overexpressed

receptor moieties on tumor surface [5]. Hence, the multifunctional nanomaterials are efficiently delivered to the diseased regions by these surface anchored active ligands [115]. Lastly all such discussions regarding inorganic multifunctional nanomaterial's shape, size, and surface functionality are related to their reduced toxicity and effective bio-distribution for successful application in cancer theranostics.

### 1.13 Problem Statement Defined

Normally the majority of developed inorganic multifunctional nanomaterials, have gained varied functionality via external surface modification mechanisms. The external surface functionalization strategies mainly include (i) electrostatic adsorption, (ii) chemisorption and (iii) affinity-based association process [116]. In electrostatic adsorption, positively charged protein or antibodies adhered to the nanomaterial surface those kept intact with the help of anionic citrate or carboxylate moieties. Similarly, in chemisorption, multifunctional nanomaterials become functional with thiolate-containing ligands through a bi-dentate covalent linker. The most advanced technique is the "affinity-based conjugation", in which surface attached ligands of multifunctional nanomaterial have enhanced empathy for proteins and oligonucleotides present in the diseased state. The above-discussed external mechanisms are very much complex, labor some, and cost-intensive. Also, adopted strategies are sensitive to the external environment, and adhered biomolecules are prone to alteration in their basic skeletal structure and character.

There is extensive use of chemical reagents during the synthesis of semiconducting copper-based biominerals [117, 118]. The reagents mainly include hydrazine hydrate, ethylene glycol, sodium borohydride, etc. These chemical reagents are not only toxic but corrosive as well. Similalrly, the strategies involving the use of templates such as protein,

biomolecules, and polymeric substances during the synthesis of fluorescent nanoclusters, add to the cost factor. Further trivalent lanthanide ion-doped Upconverting phosphors are earlier reported to be synthesized using high boiling solvents namely oleic acid, oleylamine, octadecylamine, oleamide, trioctylphosphine, trioctylphosphine oxide, Sodium citrate and low boiling solvents namely ethylenediaminetetraacetic acid (EDTA), diethylenetriamine pentaacetate (DTPA), egtazic acid (EGTA) and tetraethylene pentamine (TEPA), etc. [119]. These used solvents as probable nucleating agent are not environmentally friendly and expensive also. Synthesized Upconverting phosphors further undergo surface modification and functionalization by sophisticated techniques such as ligand exchange, ligand oxidation, ligand removal, silanization, layer-by-layer arrangement, and polymer coating for enhanced Upconversion efficiency, easy dispersibility in biological buffers, and smooth bio-conjugation with targeted biological entity.

All such discussed templates, nucleating agents, and surface-active materials besides synthesis techniques and surface functionalization mechanisms limit the use of inorganic nanomaterials in diverse biological and physicochemical applications from a toxicity, cost, and complexity point of view. Hence for imparting multiple functionalities to copper-based semiconducting biomineral and rare earth doped Upconverting phosphors a facile, less expensive, environmentally benign synthesis strategy is very much necessary. Pivotally the synthesis of small size & morphology tunable nanomaterials with advanced surface functionalities is indispensable for multifunctional nanomaterial research.