1.1 Introduction

The field of bioimaging and cancer diagnosis, is witnessing a remarkable focus on a novel class of nanomaterials. These materials are gaining considerable interest towards developing sustainable and biocompatible nanomaterials to drive the advancement in the area of nanomedicine. The utilization of toxic nanomaterials in bioimaging and cancer diagnosis applications presents key challenges, including cellular and tissue responses, potential long-term toxicity, challenges in elimination and clearance, and the crucial requirement for sustained stability over extended time periods. Therefore the current goal is to utilize green synthesis route for the synthesis of biocompatible nanomaterials, with direct applicability in the area of bioimaging and photothermal therapy, offering potential solutions to overcome the aforementioned issues. Further, fundamental aspects of high temperature (100 ℃- 500 ℃) nucleation and growth, will be studied in detail to understand the mechanism of nucleation at subnanoscale. This information will allow us to estimate nucleation rate kinetics with the help of mathematical models. Enabling to fine tune the process parameters like temperature, heating rate to get crystal structure of desired size and morphology.

1.2 Brief Summary of Cancer Disease

Despite several advancement in the field of medical science and technology, cancer still remains a great threat, even with the availability of ample diagnostic and theranostic tools.[1] The major mortality and disability is observed at metastasis stage with higher recurrence phenomenon.[2] In 2018, there were 18.1 million reported cases of cancer, resulting in 9.6 million recorded deaths. According to the reports of Global Cancer Observatory (GCO), beyond the year 2030, the number of deaths by cancer disease will reach up to 30 million per year.[3] Such drastic increment in cancer patients will also

cause a huge economic and financial burden on the family of cancer patients. Thus, it is necessary to quest various efforts for prevention, diagnosis and treatment of cancer. Gene mutation is the main cause of cancer generation and subsequent proliferation. Normal cells get proliferated by several mutations, causing uncontrolled growth. These cancerous cells typically grow at a specific location inside the body and may reach up to different organs by the mechanism of Metastasis. [4]

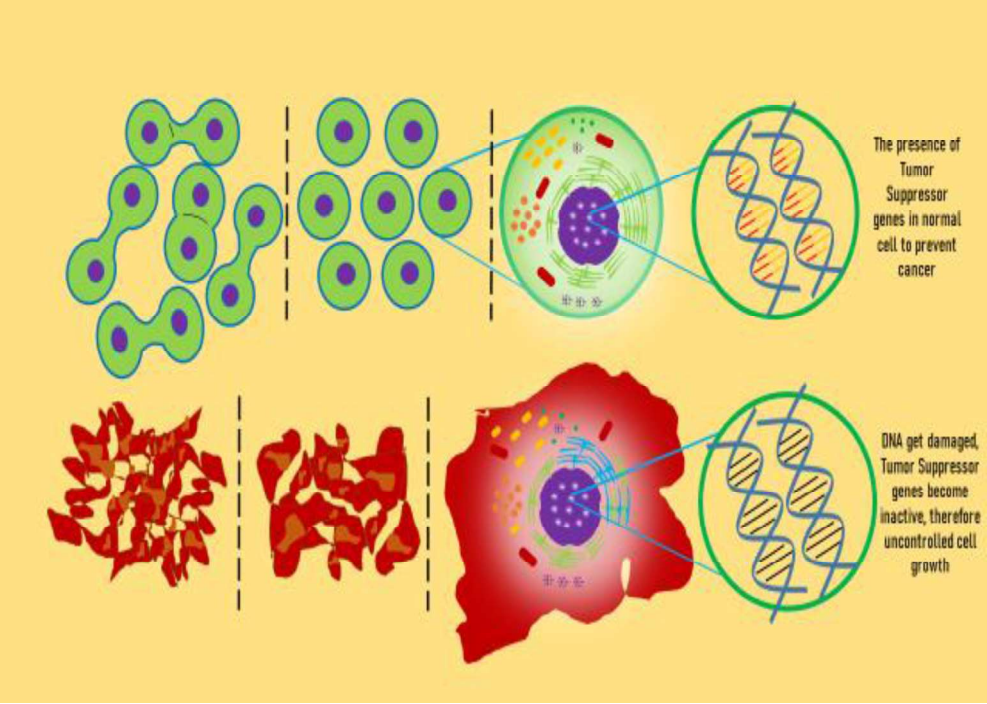

Figure 1.1 Distinction between Normal Cells and Cancerous Cells in terms of Controlled and Uncontrolled Growth.

The main mechanisms that contribute to additional toxicity in the human body during cancer treatment include nonspecific targeting[5], off-target effects[6], drug resistance[7], metabolism[8], and combined therapy [9]. In nonspecific targeting , the injected drug can target rapidly growing cancerous and healthy cells also. The treatment drug can suppress the production of blood cells in the bone marrow, white blood cells, and platelets, leading

to anemia, infection, and bleeding symptoms.[10] Whereas, Off- targeting results in interference with other biological pathways in addition to than its real target. For instance, Tyrosine kinase inhibitors (TKIs) destroys the specific signaling pathways for growth and survival of cancerous cells[11], but also target similar structured other kinases such as c-Kit, causing myelosuppression (reduced production of blood cells).[11, 12] Further its metabolization leads to to generation of toxic byproducts, which adhere extra load on the vital organs liver and kidney and impart toxicity.[13] The utilization of multiple drugs in drug resistance and combined therapy during cancer treatment can result in increased toxicity to healthy cells.[14] The aforementioned mechanisms are known to elicit toxicity in the human body. In addition to the aforementioned factors, there are specific barriers present at the tumour site that impede the successful diffusion of pharmaceutical agents. Enhance permeability and retention effect (EPR), diffusion resistance from tumor extracellular matrix (ECM), and tumor associated macrophages.[15] Therefore, for the purpose of treatment of cancer cells more amount of cancer drug is required at the tumor site to enhance efficacy for the treatment. The aforementioned challenges represent fundamental constraints that impede the optimal utilization of drugs in the context of cancer therapy.

To address the aforementioned constraints and enhance the efficacy of cancer treatment, it is crucial to implement a diagnostic regimen as a preliminary step. Imaging techniques for diagnosis not only provide a precise understanding of the physiology and phenotype of tumor tissues within the body but also serve as a guiding tool throughout the cancer treatment process. Similarly, for effective bio distribution of specific drug at the tumor site use of imaging technique will provide a new insight for its efficacy. The term imaging corresponds to a two dimensional image of the prototype under observation. The

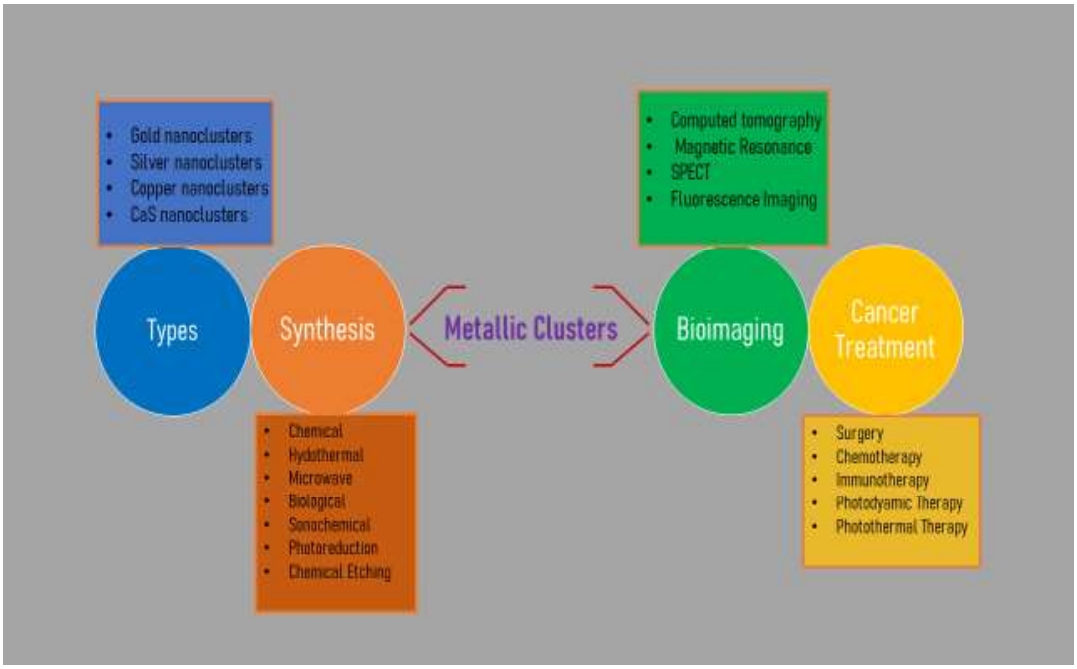

Figure 1.2 illustrates various synthesis methods for metallic clusters used in the fields of bioimaging and cancer treatment.

presence of imaging agents to the concerned region of interest can provide a detailed best possible pictured image, that can be utmost helpful during cancer treatment.

Principal Bioimaging Techniques for Cancer Treatment

The typical bioimaging technique for cancer diagnosis, tracking, and treatment purpose includes Computed tomography imaging, Magnetic resonance Imaging (MRI), and Single-Photon Emission Computed Tomography (SPECT), bioluminescence imaging and fluorescence imaging. The concise description of the above techniques are given below:

1.2.1 Computed tomography imaging

Utilizes a narrow beam of x-rays to image the patient's body and also around the body and generates computer generated 3Dcross-sectional images. These 3 D images are generated by assembling multiple cross-sectional images to access the

diseased region.[16] For improving the image signal to noise ratio liposomal-iodine is used as a contrasting agent. Good contrasting images are used to examine the benign or malignant tumours morphologically by visualizing the blood vessels.[17] The main disadvantage of CT imaging is its high cost, low sensitivity and repeated use of high dose harmful ionized X-ray radiation necessary for diagnosis and treatment purposes. Despite its eminent deep tissue penetration depth (40 cm), 3D imaging capability and existence of high spatial resolution its repeated use is unavoidable.[18]

1.2.2 **Magnetic Resonance Imaging** involves utilization of strong magnetic field used to polarize of hydrogen nuclei present in the fluorinated/heavy water molecule samples.[18] The polarized nuclei are subsequently detected with the means of radio waves signals and detected by the radio frequency coils (RF), employed to produce image for concerned region of interest.[19] These images are characterized by three distinct relaxation times, longitudinal relaxation time (T1), transverse relaxation time (T2), and T2 without rephasing (T*).[20]The imaging employs contrasting agents that exhibit either T1 relaxation or T2 relaxation time. For soft tissues of the same origin T1 and T2 relaxation differs. This differences in relaxations paved the way for successful imaging through MRI technique.[20] Currently, a multiparametric MRI is utilised in MR spectroscopic imaging (MRSI) combined with T2-weighted , diffusion-weighted images, and dynamic contrast-enhanced MRI.[21] Instead, Magnetic Resonance Imaging (MRI) provides the most accurate anatomical positioning of the cellular graft, with the advantage of f unionized radiation, 3D imaging capability, deep penetration (depth 50 cm). Despite of these advantages it fails to differentiate between mortal and l viable cells further it cannot distinguish between the behavior and proper functioning of

transplanted cells, and has very low acquisition time, low sensitivity, and generally produces false positive results.

1.2.3 Single-Photon Emission Computed Tomography (SPECT)

Technique involves the use of gamma rays emitting radioisotopes within the blood stream of the patient to be diagnosed.[22] SPECT has a very crucial role in the field of nuclear medicine imaging. The various radiopharmaceuticals used for the purpose of SPECT imaging are typically trialed at the level of clinical diagnosis for the treatment of the cancer, for example, cardiac ischemia is diagnosed by the use of radiopharmaceutical [^{99m}Tc]-sestamibi and other radiopharmaceuticals like [^{99m}Tc]-tetrofosmin or [^{99m}Tc]-labeled diphosphonates used for the diagnosis of bone metastasis in prostate and bone cancer.[23] The main advantage of SPECT imaging involves deep tissue penetration (50 cm), and 3D imaging capability but uses harmful ionizing radiations, and has long acquisition times with low spatial resolution.

1.2.4 Fluorescence Imaging

Technique was discovered several decades ago consisting of bioluminescence and luminescence.[18]

Bioluminescence imaging technique

Involves the emission of light by living organisms, which may/may not be genetically modified for the tracking purpose of cell localization. Thus bioluminescence imaging does not require excitation with external light of specific wavelength. In bioluminescence imaging, luciferin substrate is injected to the body of organism. Luciferase enzymes, is either naturally present in the body

or it is genetically modified that can interact with the luciferin substrate, causing emission of light.[24, 25] The specialized camera is typically used for detecting emitted light. Therefore, the main advantages of bioluminescence includes no use of external light source, which offers advantage of minimal light scattering, no use of ionizing radiation, high spatial resolution, accurate assessment of cell viability, and zero back ground noise, with the limitations of low penetration depth (2 cm) and difficulty in genetic manipulation.

Fluorescence imaging:

Fluorescence is a phenomenon in which fluorophore is excited by external incident light of lower wavelength, resulting in the emission of light of higher wavelength. The fluorescence cell labeling can occur either *in-vitro* (Outside the organism) or *in-vivo* (inside the organism) by using nanoclusters as fluorophores. Thus these labeled cells are excited by specific wavelength of light to detect emitted light signal of specific wavelength detected by an optical microscopy.[26] Despite this imaging technique offers low tissue penetration depth (1cm-2cm), but provides several advantages including high spatial resolution, high sensitivity, and utilizes wide range of fluorophores of specific spectral properties. Additionally, this imaging technique produces non-invasive visualization of cells within the body. It is typically cost-effective, uses non-toxic bioimaging agents with no use of harmful ionizing radiation.[27] [28]

Despite the fact that the aforementioned techniques such as CT- imaging, MRI, and SPECT produce 3D images of the disease of interest, they require the use of toxic and expensive radioactive, contrasting and MRI agents, during the diagnosis of cancer when compared to fluorescence imaging. The fluorescence imaging involves several more benefits over conventional CT, MRI, and SPECT imaging because it involves capturing

changes in various biological system as they occur in the real time. This enables tracking of tumor growth, drug response, and metastasis mechanisms in real time. CT, MRI, and SPECT imaging, on the other hand, require a lengthier acquisition time due to which rapid changes in tumor are difficult to capture in real time. [29-31] On the other hand fluorescence imaging utilizes non-ionizing radiation, thus preventing patients from its exposure during diagnostic and therapeutic procedures.[32, 33] Therefore, fluorescence imaging is a safe option for diagnosis and treatment processes for cases involving monitoring of tumor growth, repeated imaging requirements, pediatric patients, or pregnant patients due to the absence of harmful ionizing radiation and other factors, as discussed.

1.3 Main Techniques for Cancer Treatment

In addition to the aforementioned diagnostic processes, the treatment of cancer is a significant milestone to be addressed. Despite spanning several decades, the quest for an effective treatment of cancer has always been an important issue, among various scientific groups. The generally used therapeutic methods for cancer treatment includes surgery, radiotherapy, chemotherapy, immunotherapy, and photodynamic therapy.[34]

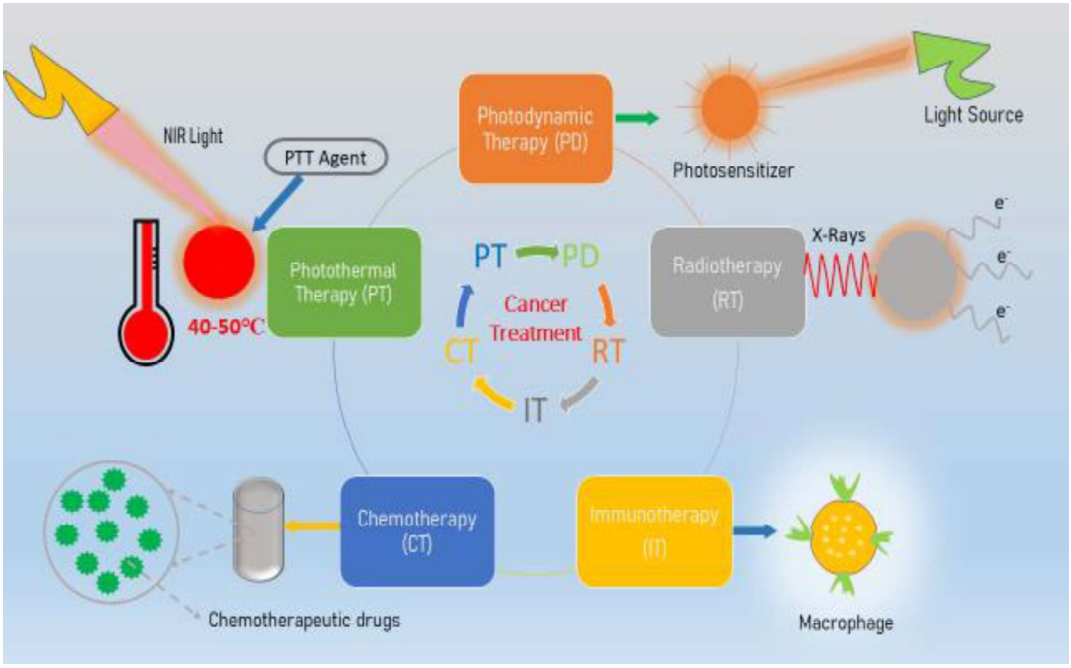

Figure 1.3: Illustration of Diverse Approaches Utilized in Cancer Treatment.

Surgery is the primary treatment option often employed for the removal of cancerous tumors from the body. The advanced surgical procedure for the removal of cancerous tumors involves the utilization of cryogenic procedures and advanced laser techniques. Typically, self-pressurizing spray guns utilizing liquid nitrogen are employed for treatment purposes. Cryogenic treatment is a therapeutic approach that involves the application of sub-zero temperatures, typically around -50℃, to the affected area of cancer. The treatment of malignant skin cancer requires a temperature of -50℃, whereas benign lesions necessitate a temperature range of -20℃ to -25℃.[35] The manifestation of inflammatory symptoms appears to be present subsequent to cellular damage induced by cryoablation. Simultaneously, lasers with high energy have been popularly used for the intention of cellular ablation. The aforementioned procedures are typically effective for tumors of a smaller size, however, they are associated with several drawbacks such as inflammation, pain, and vomiting. Additionally, complete tumor removal cannot be assured.[36]

Chemotherapy is a procedure of cancer treatment in which one or more powerful anticancer drugs are administered to neutralize and to slow down the growth of cancerous tumors. These drugs typically induces cell death in cancerous tissues by DNA damage, thereby interfering in the enzymatic process for DNA replication subsequently stopping the cell division and mitosis.[37]Additional therapies such as surgery, chemotherapy also helps in reducing the size of cancerous tumors.[38] The major disadvantages of chemotherapy includes, fatigue, vomiting, nausea, anemia, hair loss, weak immune system, gastrointestinal problems, and vital organs (heart, lungs, liver, kidney, and spleen) damage .[39] In contrast, other treatments such as radiation therapy utilize intense radiations consisting of electrically charged particles, such as x-rays and gamma rays, to induce cell death in cancer cells and reduce tumor size through mechanisms such as apoptosis, necrosis, mitotic catastrophe, autophagy, and senescence. [40]Such high intense charged particles destroys the genetic material (DNA) present in the proliferating cells, which leads to cell death. These therapies targets the tumor tissue with high accuracy although inflicts injures to the surrounding healthy tissues, during this process most of the tumor cells becomes resistant to the exposed radiation.[41] In another approach, the elimination of cancer cells by the immune system itself is a technique known as immunotherapy. Immune system is externally simulated by immune checkpoint inhibitors, monoclonal antibodies, cancer vaccines, and other adoptive cell transfer mechanisms.[42] This method has broad applicability and accuracy for treating a variety of tumor types. Specifically, in the case of "immunoinflammatory" tumors, immunotherapy is effective and the improved long-term survivalrates. This approach generally fails when the form of tumor is "immune exclusion type" or "immune suppression type. “Immunotherapy with immune checkpoint inhibitors results in the onset of autoimmune diseases, which can result in mortality, as well as high treatment costs and

low treatment efficacy.[43] In another more efficient approach than as described above, called photodynamic therapy. Photodynamic therapy is an efficient technique encompassing Photosensitizing molecule, NIR light, and oxygen (O_2) molecule. When photosensitizing molecule absorbs NIR light, and transfer to oxygen molecule to produce singlet oxygen ($^1O_2$) species, which is highly reactive and can oxidize adjacent molecules, leading to restriction in tumor growth. When the cancerous tumor reaches to final stage of metastasis, the depletion in oxygen concentration happens very rapidly, and efficacy of PDT get hampered. [44]

1.4 Photothermal therapy's significance in cancer treatment

Photothermal therapy (PTT) has been proposed as a cost-effective approach to address the limitations associated with conventional cancer treatment modalities, as discussed earlier. By utilizing near-infrared (NIR) light, this therapy has demonstrated promising outcomes in effectively eradicating cancer cells. The near-infrared (NIR) light is transformed into thermal energy through the photothermal agent, which facilitates the destruction of cancer cells.[45] Various scientific groups have employed different photothermal agents with varying photo-to-heat conversion efficiencies. These include Au nanoshells (15%)[46], Gold nanorods(21.3%)[47], Pt-PEG nanoworms(38.9%)[48], Pt nanoparticles(22.98-30.88%)[49], Pt cubes(32.3%) [50] and Cys-CuS NPs(38%)[47]. Consequently, these nanomaterials possess all the requisite attributes for utilization in photothermal systems. Despite Au nanorod and Au nanoshell provide a broad absorption spectrum for their utilization across multiple wavelengths, both Au nanorods and Au nanoshells pose significant challenges. Also it was noticed that with the prolonged therapy, the absorption peak shifted due to change in the aspect ratio of Au-nanorods. This resulted in the decreased treatemtn efficacy. Besides, its synthesis is complex, expensive, and often associated with high toxicity.[51-53] Additionally, their size typically exceeds the renal

clearance threshold limit of 10 nm.[54] Furthermore, despite the green synthesis, it was observed that the presence of platinum nanoparticles at significantly low concentrations, ranging from 12.5 to 200μg/ml, resulted in a substantial reduction of relative cell viability. [55] Moreover, the significant toxicity of CuS nanoparticles synthesised through biological means was observed in zebrafish, even at lower concentrations greater than 80 μg/mL.[56] Hence, there is an urgent need to develop a novel photothermal agent that surpasses the reported photothermal efficiencies of commonly used nanoparticles, as mentioned above, while also possessing a size less than the renal clearance threshold limit (10nm). Furthermore, such photothermal agent should exhibit exceptional biocompatibility to ensure their safe utilization for biomedical applications. Furthermore, the main advantages of photothermal therapy over various aforementioned discussed treatments are as follows: Photothermal therapy uses nanoparticles that can be designed to specifically target localized tumor site. Due to such localized treatments the minimal damage to healthy tissue occurs with good precision, leading to reduced side effects in comparison to those associated with radiation and chemotherapy. NIR photothermal therapy is invasive in nature and penetrate to a deep tissue without any complications that typically arise from radiation and chemotherapy.[57] NIR photothermal therapy provides rapid response to the cancer treatment. Upon activation by near-infrared (NIR) light, nanoparticles generate localized heat, resulting in hyperthermia. Elevated temperatures within the human body, exceeding the standard physiological temperature of 37°C, have the potential to cause a programmed cell death and may result in damage to critical bodily organs. Many scientific groups identified the reason of cell death by PTT are apoptosis and necrosis.[58] The beauty of PTT lies in the fact that it can be combined with other therapies for successful treatment of cancerous tumors.[59] For the recurrent cancerous tumors, and at metastasis cancer stage surgery often fails but due to non-

invasive nature PTT can be used multiple times at the site of cancer without any significant complications.[60] In short, this technique has many significant advantages over conventional cancer treatments, including simplicity of procedure, fewer complications, a quicker recovery, and a shorter hospital stay.[61] Consequently, PTT is a safe option for the diagnosis and treatment of malignant tumors due to its numerous advantages over conventional treatment methods, as discussed previously.

1.5 The Significance of Fluorescent nanoclusters and Metal nanoparticles in Optical Imaging and Photothermal Therapy

Metal nanoclusters bridge the gap between single atoms and nanoparticles, as they consist of a small number of atoms ranging from just a few to around 100.[62] These nanoclusters are typically smaller than 2nm, which is comparable in size to the Fermi wavelengths of electrons.[63] The incredibly small size of these nanoclusters, grants them exceptional chemical and physical characteristics like fluorescence, discrete redox behavior, quantized charging ability, chirality nature, and molecular magnetism phenomenon.[64] Such unique properties of ultra-small nanoclusters make them a suitable candidate for various applications like catalysis, optoelectronics, bioimaging, sensing, and biomedicine field.[65] Fluorescent metallic nanoclusters (Au, Ag, and Cu) are most suitable candidate that is used by various scientific groups over the decades for the purpose of optical fluorescence imaging because of their large stokes shift, high photostability, emission wavelength tunability, and good biocompatibility when compared to organic fluorescent dyes and quantum dots.[66] Noble metal nanoparticles like Au, Ag and Cu are the focus of interest among various scientific groups, due to their strong absorption and scattering of light with tunability in excitation and emission according to their size. The quality of converting NIR light to heat is due to existence of resonant oscillation of free electrons, also abbreviated as localized surface plasmon

resonance (LSPR). Due to the phenomenon of Plasmon resonance, noble metal nanoparticles can either radiate light (Mie scattering), for imaging purpose, or rapidly converted to thermal energy. Owing to their, strong NIR absorption, precise control on heating, high photothermal efficiency, biocompatibility, and multifunctionality, these nanoparticles are suitable for biomedical applications.[67] Another class of nanomaterials are used for photothermal therapy are semiconductor materials, such as metal oxides and chalcogenides, due to their low cost, low toxicity, and one pot facile synthesis. These nanoparticles and semiconductors typically have strong extinction coefficient in NIR region with tunable absorption wavelengths, when excited with NIR light compared to organic photothermal materials.[68] Before starting the application part of metal quantum nanoclusters in optical bioimaging and metal nanoparticles in photothermal therapy, it is important to know their synthesis processes. The various versatile synthesis processes for metallic quantum nanoclusters are given below:

1.6 Synthesis methods required for fluorescent metal nanoclusters

The synthesis parameters like pH, temperature, and concentration are of utmost importance to regulate the morphology, size, capping agent, stirring speed, and optoelectronic properties of such clusters.[69-71].It is known that optoelectronic properties of nanoclusters degrades with the increase in concentration of capping agents. Many such capping agents are used to synthesize metallic nanoclusters like thiolates, polymers, peptides, protein templates, amino acids, etc.[70, 72-75] Nanoclusters synthesis can be carried out either using Top-down method or Bottom-up approach. The top-down method consists of a chemical etching technique, while the Bottom-up approach is based on the use of reducing agents based on chemical and green approaches. Despite the

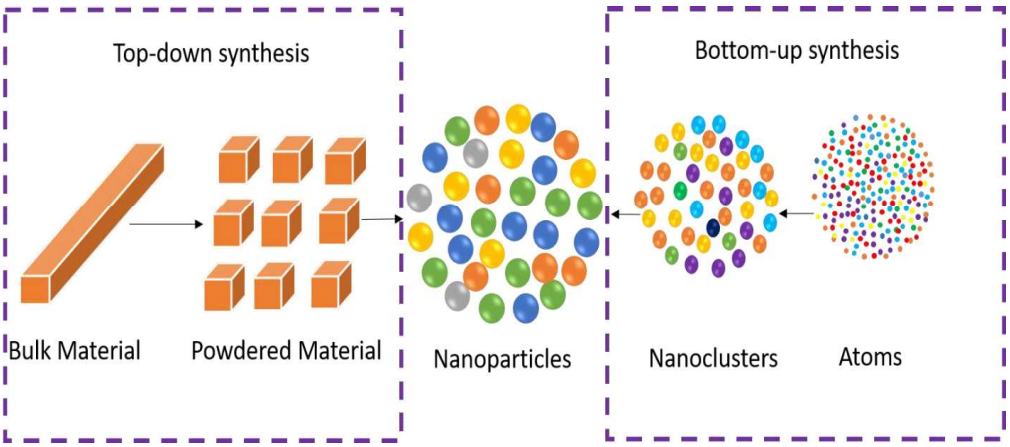

Figure 1.4 illustrates the differentiation between bulk material, powdered material nanoparticles, nanoclusters, and atoms, highlighting two distinct processes known as Top-down synthesis and Bottom-up synthesis.

above, the other methods like the hydrothermal approach and microwave synthesis are also included in the Bottom-up approach. The detailed processes pertaining to metallic cluster synthesis are given **below:**

1.6.1 Chemical based synthesis routes

Various types of chemicals reducing agents were used for synthesis purpose of metallic nanoclusters. Sodium borohydride ($NaBH_4$), hydrazine, alcohols, and citrate ions are used as reducing agents and utilized in the synthesis of nanomaterials.[76] For instance, Ni_{39} and Ni_{41} nanoclusters were obtained in methanol and THF solvent by the use of reducing agent $NaBH_4$.[77] At, the initial stage thiol containing ligands like alkyl thiols are basically used for the quantization of fluorescent Au nanoclusters through the thiolate bond formation. Due to poor solubility of alkyl thiol in water, glutathione is basically used to make water soluble nanoclusters in place of alkyl thiols[78]. The tripeptide glutathione composed of l-glutamic acid, glycine and l-cysteine makes the synthesized Au nanoclusters water soluble.[79-82] By reducing $CuSO_4$ with hydrazine hydrate for 19

hours at 95°C, stable copper fluorescent nanoclusters with a quantum yield of 3.8% in ethanol were also produced.[83]

In addition to thiol, number of polymer based capping agents like polyethylene glycol, polyethyleneimine, and poly-amidoamine (PAMAM) dendrimers have been also used for capping of highly fluorescent metal nanoclusters. The OH terminated PAMAM dendrimers were typically used for synthesis of highly fluorescent Au[84] and Ag[85] nanoclusters, for the first time. Later, PAMAM capped Au_8 nanodots were prepared by the same Dickson group with a high quantum yield of 42%.[84] The same protocol was adopted for the synthesis of fluorescent Au nanoclusters with tunable emission from visible to NIR[86]. In the same synthesized nanoclusters the origin of luminescence might be due to PAMAM dendrimers without existence of Au clusters, that complicated the integrity of nanoclusters formation[87]. To solve the problem, updated synthesis procedure was adopted to monitor the fluorescence of Au nanoclusters in absence of PAMAM dendrimers[88]. The blue emitting PAMAM dendrimers capped Pt nanoclusters were also synthesized with 18% quantum yield[89]. The BSA capped red luminescent Au_{25} fluorescent nano clusters were synthesized in water as solvent.[90] The synthesis of PEI conjugated blue luminescent Ag nanoclusters has been reported in the area of sensing of metal ions, pH, temperature, halide ions, and organic molecules[91-94]. In spite of that, thiolated PEG and other block copolymers capped Au quantum nanoclusters were synthesized.[95, 96].

On the contrary, various proteins, peptides and amino acids were also employed to prepare water soluble metal nanoclusters to use it as biocompatible material, and to maintain proper nucleation and growth of such quantum nanoclusters.[97-104] Bovine serum albumin (BSA) is globular protein was first time used for the synthesis process for red luminescent nanoclusters[90]. High reaction pH (>10) favors the reduction of gold

ions capped by BSA protein. Subsequently, various protein templates such as lysozyme, trypsin, pepsin, urease, horseradish peroxidase (HRP), insulin, DNase I, and ribonuclease A were typically used for synthesis of fluorescent metallic clusters[71-73, 105-118]. As mentioned earlier, pH plays a crucial role towards the formation of different size nanoclusters. For an example, different sized (Au_5, Au_8), Au_{13} and Au_{15} of gold (Au) quantum clusters with respect to different emitting colors of blue, green and red were obtained upon changing the pH from 9 to 1 and finally 12, respectively[71]. On the other hand, HRP capped gold clusters showed peroxidase activity and insulin protected clusters were found to maintain blood glucose level[114, 118].

1.6.2 Hydrothermal method synthesis

The hydrothermal method employs uniform high pressure heating, which is green in approach, affordable, and scalable route for typical synthesis of metal oxide nanomaterials. Nanoparticles size and morphology are dependent on the time and temperature patterns in the hydrothermal processes.[119] Ag fluorescent clusters were first synthesized by this method by the aid of poly(methacrylic acid sodium salt) (PMAA)[120]. In spite of that, hydrothermally route was adopted for synthesis of carbon dots, used in detection and imaging applications [121, 122]. Hydrothermal techniques was also adopted for fabrication of metal sulphide nanomaterials. When compared to the sol-gel and ceramic based high temperature annealing synthesis routes, the crystalline materials can easily be synthesized at lower temperatures by hydrothermal route. On the other hand, non-toxic high boiling point solvents are usually utilized in the hydrothermal process, indicating its environmentally friendly nature.[119] A number of scientific group synthesized lithium iron phosphate nanoparticles by the use of continuous flow hydrothermal routes.[123] Hydroxyapatite nanotubes with nanorods, and metal organic

framework (MOF) synthesis is also executed through continuous flow hydrothermal method.[119]

1.6.3 Microwave based synthesis

In comparison to conventional synthetic routes, microwave assisted synthesis is an interesting and unique approach for nanomaterial synthesis. This approach results in production of uniform sized nanomaterials in short time in comparison to conventional approach.[124] It becomes possible only due to its rapid and uniform heating as compared to conventional heating processes. Due to which nucleation and growth pattern during can easily be altered to produce multi and uniform sized nanomaterials.[124, 125] Microwave assisted synthesis was performed to prepare the lysozyme protected nanoclusters for antimicrobial applications[126]. The microwave approach was also adopted to synthesize dihydrolipoic acid BSA protein protected Au clusters, which resulted in fivefold increase in quantum yield and reduced reaction time[127, 128]. Trisodium citrate was used as a capping and reducing agent for the synthesis of Ag nanoparticles.[124] On the other hand, the synthesis of gold clusters from gold nanoparticles by topdown approach can also be prepared by the microwave assisted synthesis[129].

1.6.4 Biomineralization mediated Synthesis

Biomineralization is a process that involves utilization of biological entities, such as proteins, peptides, dendrimers, polymers or cells, to direct the nucleation and growth of metal clusters with desired fluorescence properties. Highly fluorescent Pt clusters were synthesized by biomineralization technique.[130] BSA protein was used to biomineralize the NIR emitting silver clusters. [131] Biomineralization is employed as a solution to the issue of biocompatibility, as it effectively addresses this concern. Therefore, for the

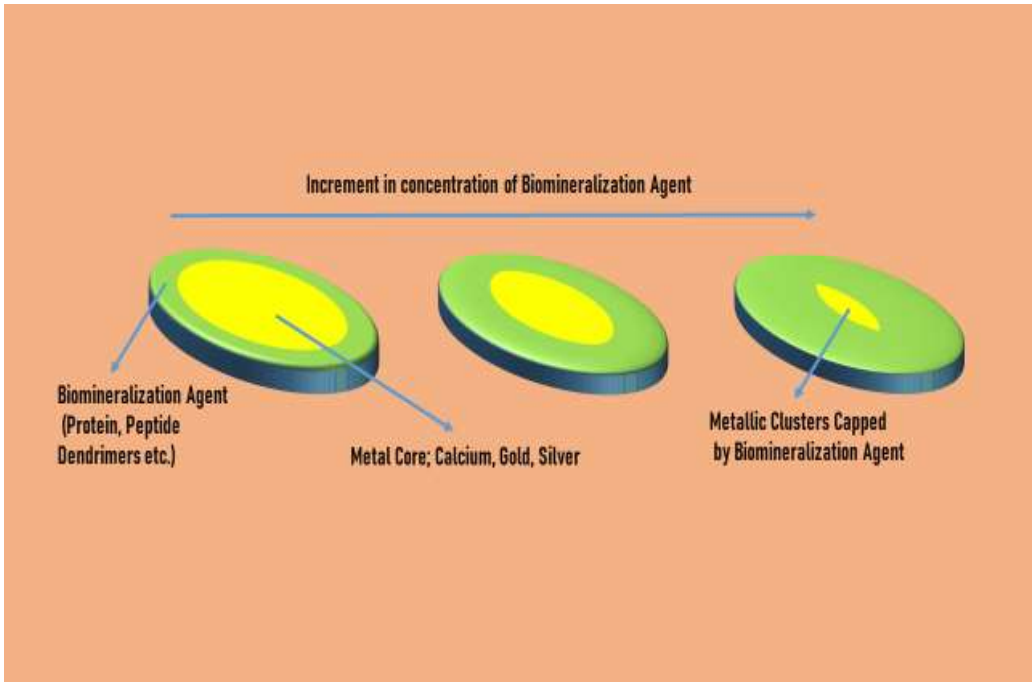

Figure 1.5 Increasing the biomineralization agent concentration confines the metal clusters tightly.

biomedical use the synthesis of clusters and nanoparticles are biomineralization centric .[132] In summary, biomineralization-mediated synthesis offers an environmentally friendly approach that minimizes the generation of toxic wastes and promotes sustainable synthesis. The synthesized nanoclusters by this technique are highly suitable for biomedical applications due to excellent biocompatibility.[133] Additionally, this synthesis method provides excellent control over the size and shape of the clusters, directly influencing their properties.[134] These advantages have increased our interest in utilizing biomineralization as a sustainable, biocompatible, cost-effective, and energy efficient method for nanocluster synthesis.

1.6.5 Sonochemical assisted synthesis

The sonochemical mediated synthesis utlizes initiation and continuation of chemical reactions by the impact of powerful ultrasound waves (0.02-10 MHz).[135] Due to the effect of ultrasound radiation an acoustic cavitation is generated in the liquid being irradiated. Subsequently, the radiation of ultrasound is able to trigger various reactions like reduction, oxidations, hydrolysis, and decomposition.[136] The fluorescent silver nanoclusters are synthesized by sonochemical method[137, 138]. During the process of ultrasonication the highly reactive species are generated such as $HO_2^{\cdot}$, $H^{\cdot}$and $OH^{\cdot}$ by the action of localized hot spot temperature (5000K) and high pressure (100 bars)[138].

1.6.6 Photoreduction method

The main principle behind the photo reduction is the formation of metal clusters via irradiation of light. This method does not require any reducing agent and it is non-toxic, green and less time consuming synthesis as compared to the conventional methods[139]. Functionalization of Poly (methacrylic acid) via pentaerythritol tetrakis 3-mercaptopropionate was used as a capping agent for the synthesis of water soluble copper, silver and gold clusters[139]. Subsequently, different polymers including poly(methyl methacrylate), poly(n-butyl methacrylate and poly(tert-butyl methacrylate) were used for synthesis of gold fluorescent clusters using the same approach as mentioned earlier[140]. As this method does not require presence of any reducing agent, thus it is considered to be non-toxic, clean and quick synthesis approach.

1.6.7 Chemical etching-based synthesis

In this approach, metal nanoparticles are utilized for synthesis of highly fluorescent nanoclusters by using organic ligands, like mostly thiols. Au clusters with core mass of 14

kDa were heated with dodecane thiol solution for the completion of etching process so here used as a precursor for the formation of Au fluorescent clusters of 6kDa[141]. Consequently, various ligands such as polymers, precursor, and simple molecules were used for the synthesis of fluorescent clusters by etching of metal nanoparticles. As an example, different ligands such as 11-mercaptoundecanoic acid, GSH, dopamine, tris(2-carboxyethyl)phosphine (TCEP), biomolecules, hyperbranched PEI, ethylenediamine, Au precursor ($HAuCl_4$) etc. were used for the etching based synthesis. In spite of that, many more synthesis were reported as a first time approach for the synthesis of thiolate Au1 complexes from GSH capped nanoparticle by performing hydroxyl radical based etching[142].This approach of synthesis was accorded as a relevant method for the extraction of gold from the scrap electronic materials. A different method was adopted by opting heterophase ligand exchange based etching process for conversion of gold nanoparticles to organic soluble fluorescent gold clusters.[143] On the contrary, as the research moved to forward direction, different approaches were created by many scientific communities. For example, fluorescent Ag nanoclusters were synthesized by a novel etching process of cyclic reduction via oxidative atmosphere.[144] Furthermore, aqueous organic phase was introduced for interfacial etching reaction for the synthesis of gold and silver nanoclusters[145, 146]. On the contrary, electrostatically mediated phase transfer etching was accorded for the formation of Ag/Au/Cu/Pt clusters[147, 148]. In addition, DHLA molecule was utilized as an etching agent media for the synthesis of water-soluble silver clusters[149]. Later, Platinum (Pt) nanoparticles were used for the formation of yellow emitting platinum clusters by performing glutathione based etching process[150].

In short, there are various methods available for synthesis of metal fluorescent clusters. Every method has its specific pros and cons that can be used for synthesis purpose as per

the required flexibility. As discussed earlier, hydrothermal method produced uniform size and shape of nanoparticles required due to the high pressure and uniform heating operation. But such method is very difficult to monitor and consumes ample amount of time for the synthesis process. Despite this, reaction monitoring facility is available in sonochemical and photoreduction processes. Sonochemical hydrothermal, photoreduction, and microwave assisted synthesis methods, on the other hand, are environmentally benign and non-toxic. Despite this, their installations are extremely expensive, consume a great deal of energy compared to conventional methods, and are difficult to construct. Despite non-toxicity of above discussed synthesis methods, for the application purpose in the field of biomedicine, the problem of eminent biocompatibility (near to 100%) is always seen as a great concern. One possible solution to the problem of biocompatibility as discussed earlier is process of biomineralization. Concerns about biocompatibility are reduced when proteins like BSA, HSA, and lysozymes are used. These proteins are naturally occurring, already present in the body, and useful for internal applications and helps in nucleation and growth of metallic clusters in controlled manner. Therefore, biomineralization is the safe, primary and important method for producing clusters and nanoparticles for applications in the field of biomedicine.[151]

The above-mentioned methods were employed to produce fluorescent noble metal clusters (Au, Ag, and Cu) as bioimaging agents. However, in order to enhance biocompatibility, the biomineralization technique stands out as a best technique among all synthesis methods for optical fluorescence bioimaging applications. Biomineralization effectively resolves the issue of low biocompatibility that is commonly associated with these metal clusters, making them more compatible for use in the field of biology.[151]To move forward in the direction biocompatible nanomaterials, various noble metal fluorescent clusters were synthesized by biomineralization technique.[90, 104, 152, 153]

Thus, gold fluorescent nanoclusters are supposed to be highly biocompatible, leading their extensive use in the area of bioimaging and nanomedicine. Such biocompatibility, therefore, is only limited for short-term usage. Furthermore, it was demonstrated while injecting BSA protected gold clusters within the mice, vital organs such as liver and kidney got damaged. During the biodistribution study of gold clusters inside the body of mice, BSA capped Au nanoclusters were found to get accumulated in liver and spleen, and the GSH capped Au nanoclusters were having low concentration in all vital organs. After 24 h of administration of GSH capped gold clusters, only 36% extracted out through the passage of urine, but in case of BSA protected gold clusters only 1% extracted out through the passage of urine. After observation period of 24 days, only 5% Au present in BSA capped clusters is renal cleared, and while for GSH capped clusters 94% Au was cleared through renal passage. Upon analysis of blood serum after 24 hours, the white blood cell (WBC) count was substantially increased in the mouse model for both types of injected clusters. On further demonstration, they found that the CREA of the GSH and BSA capped Au nanoclusters increased significantly, and the kidney function was significantly hampered. After 28 days of BSA-protected gold nanocluster administration to mice, infections and injury to vital organs such as the liver and kidney were observed, and only 5% of the gold present in the gold clusters could be metabolised.[154] To resolve above issue, there is an urgent need to develop and explore new biocompatible materials that are naturally found in the body and their decomposition products are not harmful.

1.7 Problem Statement defined

Developing biocompatible nanomaterials, naturally occurring in the body with non-toxic decomposition products, is crucial. Among these, calcium-based nanomaterials like calcium carbonate stand out for their eminent biocompatibility, due to vital roles of

calcium in physiological processes such as cellular signaling, muscle function, and bone health, making them a prefect material for various biomedical applications. Their compatibility with biological systems ensures safety and efficacy, emphasizing their significance in various medical fields, is a typical reason for selecting it in the current study.[155-157] These compounds even in excess amounts, they are efficiently eliminated from the body through urine and feces.[158] Among these, calcium carbonate nanomaterials have garnered significant attention due to their high biocompatibility and widespread utilization in the field of biomedicine.[159] Consequently, our objective is centered around the development of fluorescent calcium carbonate molecular clusters specifically for applications in fluorescence bioimaging. We have selected fluorescence imaging as the preferred technique for bioimaging of MG-63 cells using our biocompatible fluorescent $CaCO_3$ prenucleation clusters. This decision is based on the several advantages offered by fluorescence imaging compared to traditional CT, MRI, and SPECT imaging methods. Fluorescence imaging enables us to observe and analyze dynamic changes in diverse biological systems in real time. Upon using fluorescence imaging technique, we can accurately monitor tumor development, track the drugs response, and can easily investigate the mechanisms of metastasis as they occur in real time. In contrast, CT, MRI, and SPECT imaging typically require longer acquisition times, making it challenging to capture rapid changes occurring within tumors in real time. By employing fluorescence imaging technique, we typically add on the ability to observe dynamic biological processes as they happen in real time, providing valuable insights for various biomedical and clinical based applications. Hence, fluorescence imaging technique is considered as a best choice for diagnosing and treatment purposes that involve tracking tumor growth, repeated imaging requirements, and can be safely used in pediatric and pregnant patients. The notable astonishing advantage of

fluorescence imaging technique involves in its radiation-free nature, ensures a non-invasive and safe procedure for patients. Therefore, this technique can easily eliminates associated harm of using ionizing radiation and mitigates associated risks. Additionally, fluorescence imaging technique includes wide flexibility and adaptability, ensuring repeated imaging sessions without any adverse effect. This is especially valuable while tracking the tumor growth or monitoring treatment efficacy over time.

Furthermore, it is crucial to acknowledge that gold nanorods and gold nanoshells, despite their excellent near-infrared (NIR) absorption properties as photothermal agents, typically exceed 10 nm in size. This larger size poses potential long-term toxicity risks in the body since they are not naturally occurring components and are not efficiently cleared through renal processes. Additionally, other nanoparticles, such as platinum, have been shown to reduce cell viability. To address these concerns, the development of a new biocompatible nanomaterial with a diameter less than 10 nm becomes paramount. This new nanomaterial should not only exhibit superior photo-to-heat conversion efficiency compared to conventional nanomaterials but also mitigate the limitations associated with larger-sized particles. By focusing on the synthesis of such a biocompatible nanomaterial, we can ensure their safe integration within the body, overcome toxicity risks, and enhance overall therapeutic efficacy., On the same lines, Group IV elements (C, Si, Ge, and Sn) exhibit an eminent biocompatibility as similar to alkaline earth metals such as Calcium and Magnesium.[160] For instance, carbon based materials such as graphene and carbon nanotubes, have shown promise in biological applications such as drug delivery, tissue engineering, and biosensing due to their excellent biocompatibility.[161, 162] In medical procedures like hemodialysis and hemoperfusion, activated carbon is utilized to eliminate toxins from the body.[163] Silicon-based materials are frequently employed in construction of various drug delivery medical devices, including silicon-based implants

and prosthetics, owing to their biocompatibility and eminent mechanical strengths.[164]. Germanium is not extensively used in the body, but it is typically used as constrasting agent in the body, for computed tomography (CT) imaging scans and also in photoacoustic Imaging[165, 166]. Many studies used germanium nanoparticles for the purpose of targeted drug delivery and theranostic applications.[167] Tin nanomaterials (Sn), such as tin oxide nanoparticles, have been investigated for their antimicrobial properties[168] and potential use as an implant material due to their excellent biocompatibility.[169] Due to its ability to bond to other metals and produce stable compounds, tin has also been incorporated into dental materials such as amalgams.[170] Tin (Sn) is commonly found in two distinct forms, namely alpha tin (α-Sn) and beta tin (β-Sn). Alpha tin demonstrates superior NIR-II absorption in comparison to beta tin. The biomedical potential of bulk α-Sn has not been thoroughly investigated despite its significant NIR-II absorbance, primarily due to its considerable size (reaching up to 200 nm) [171] and the possibility of alloying with toxic metals, such as lithium, during the synthesis process [172]. A number of synthesis routes pertaining to α-Sn have been investigated on large substrates (>100 nm)[173]. Therefore, due to large size of nanoparticles on large substrates, their biological use have been mostly problematic. It is important to note that, NIR absorptivity and biocompatibility of the alpha-Sn instigated us to further investigate us to explore its applicability in biological systems. As a result, the difficulties mentioned earlier encouraged us to synthesize ultra-small (<10nm) and substrate free alpha tin nanocrystals, with the aim of utilizing them for photothermal purposes in the domain of cancer therapy. This innovative breakthrough marks the initial application of tin nanocrystals within the field of biological sciences. Therefore, we have selected photothermal therapy as the primary technique for treating malignant cells using our innovative biocompatible nanomaterial known as tin. The utilization of nanoparticles

in photothermal therapy enables the precise targeting of tumor sites that are localized. As a result of targeted therapies, there is minimal harm to healthy tissue with high precision, resulting in decreased adverse effects in contrast to those linked with radiation and chemotherapy. The non-invasive nature of NIR photothermal therapy enables it to penetrate deep tissues without the complications commonly associated with radiation and chemotherapy. Therefore, PTT is a safe option for the diagnosis and treatment of malignant tumors due to its numerous advantages over conventional treatment methods, as discussed previously. Therefore, the current thesis has actively taken up Ca and Sn based nanomaterials to study its use in biological system and evaluate its future prospects in the area of nanomedicine.

Thematic Connection: Diagnostic and Therapeutic Synergy, Size Compatibility and Biomedical Efficacy

1. **Diagnostic and Therapeutic Synergy:**

- Bioimaging by using biocompatible ultra-small $CaCO_3$ nanoclusters provides clinicians with precise visualization of physiological and anatomical structures at cellular and molecular levels, in real time. This detailed imaging aids in the detection and precise diagnosis of diseases by identfying the size, location, and specific features of tumors or affected tissues. Subsequently, the information obtained from imaging can guide the application of photothermal therapy using tin nanocrystals, enabling targeting of treatment specific to each patients requirements and sunsequent disease characteristics.

2. **Size Compatibility:**

- Both $CaCO_3$ nanoclusters and tin nanocrystals lie below the 10 nm kidney filtration limit, ensuring safe clearance from the body and do not induced long term toxicity.

3. **Optimized Circulation Time:**

- Their ultra-small size facilitates extended circulation in the bloodstream, enabling both sustained imaging as well as therapeutic effects, in real time with minimal long term toxicity or any adverse considerable impact.

4. **Enhanced Biomedical Efficacy:**

- As both entities are found to be highly biocompatible, thus their efficacy in biomedical applications advances them among the class of precision medicine and focuses on patient-centered care.

Moreover, in order to investigate the thermodynamics and kinetic behaviors of ultra-small clusters at high temperatures (>100°C), we have developed a novel approach utilizing thermogravimetric analysis (TGA) to evaluate the variation of nucleation rate and interfacial energy for tiny clusters of $CaCO_3$. While various tailored technologies have been employed to calculate nucleation rate and interfacial energies for ultra-small clusters in the low temperature range (0-100°C)[174], we have introduced TGA as a pioneering method for computing these parameters at high temperatures (>100°C) for the first time. By understanding the kinetics of nucleation rate through proposed mathematical models, researchers can precisely adjust process parameters such as temperature and heating rate to achieve the desired crystal structure with specific size and morphology. In summary, TGA can now be used as a valuable tool for generating nucleation kinetics for any type of ultra-small clusters at high temperatures.