2. Review of Literature

The utilization of fluorescent nanoclusters in the field of biomedicine is of paramount importance due to their exceptional biocompatibility, ultra-small size, and high quantum yield.[175] These properties in nobel metallic nanoclusters may render them a suitable alternative to the more toxic quantum dots, which possess high quantum yield, bright emission, multiple synthesis options, and photostability, making them practical for several applications.[176] The presence of unmetabolized nobel metallic nanoclusters, in turn, makes them unsuitable for biological applications, as they possesses long-term toxicity, due to damage of vital organs like liver, kidney, and heart. Given the inadequacies of unmetabolized nobel metal nanoclusters for long term use, novel class of luminescent materials such as alkaline earth metals are often investigated by various scientific groups for biological applications.[177] Before moving towards the synthesis of alkaline earth metal based biocompatible nanoclusters, a short description about development and properties of metallic quantum nanoclusters are discussed below:

2.1 Development of Metallic Quantum Nanoclusters

Metal nanoclusters bridge the gap between single atoms and metallic nanoparticles, as they consist of a small number of metal atoms ranging from just a few to around 100.[62] These clusters are typically smaller than 2nm, which is comparable in size to the Fermi wavelengths of electrons.[63] The incredibly small size of these nanoclusters, measuring only 2nm, 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 clusters make them a suitable candidate for various applications like catalysis, optoelectronics, sensing, and biomedicine field.[65] These metal nanoclusters have been devised by

various scientific communities over the past few decades. Among them, gold was the earliest to be discovered and continues to hold sway alongside other metallic nanoparticles such as copper, silver and calcium. Historically, colloidal gold served as the precursor for synthesizing ruby glass and as a coloring agent for ceramic pots as early as the 4th century BCE. For example, the "Lycurgus Cu" cup was colored with a colloidal gold solution that displayed distinctive colors contingent on the phenomenon of light reflectance and transmittance. When coated with a gold colloidal solution, this cup appeared green in reflected light and red in transmitted light.[178] Since the 7th century BCE, gold has also been employed as a vital ingredient in ayurvedic medicines as a "bhasm," with later research uncovering the existence of gold nanoparticles in bhasm samples.[179] In the 16th century (1676), a book was published by German chemist Johann Kunckels which included a pink solution of drinkable gold can cure several diseases[180] and further such colloidal gold solutions were utilized for medicinal purposes to remedy ailments such as arthritis, syphilis, and tumors.[181] During the 17^{th} century, a coagulation mixture of gold and tin oxide nanoparticles was adopted as a coloring agent known as the "Purple of Cassius", and a complete piece of writing about gold colloidal solution was mentioned by the Hans Heinrich Helcher. In the 18^{th} century, a doctor and philosopher enhanced the stability of gold colloidal solution by the use of boiled starch.[180] By 1818, the rationale for different color emissions in gold solutions was expounded. The yellow color resulted from particle aggregation, while the purple or pink color was attributable to the presence of fine particles.[180] In 1857, Faraday discovered the synthesis of the gold colloidal solution by reducing chloroauric acid ($HAuCl_4$) by using phosphorus in diethyl ether.[182] He generated thin films through dried colloidal gold solution and demonstrated the effect of mechanical compression on color changes within the films.[180] Faraday's discovered colloidal solution was optically

stable for up to 160 years and was kept in the Royal Institution Faraday's laboratory as a display. This Faraday work made possible a generation of a new era in colloid science. After that, a different reducing agent, formaldehyde, was used by another researcher, Richard Adolf Zsigmody, to synthesize a gold colloidal solution. By 1903, for observation of gold particles, an ultramicroscope was discovered by Richard Adolf Zsigmody and Henry Siedentopf.[183] The scientist Richard Adolf Zsigmody was awarded the Nobel Prize for his contribution to the field of colloidal solution for the interpretation of colloids[184]. By the year 1911, solid solution of colloidal gold was created in the medium of stannic oxide.[185] Subsequently, till the year 1918, The size of colloidal particles was determined using a ultramicroscope[183] and with the aid of X-Ray diffraction[186]. The popularity of these methods for size determination makes the use of such methods to date. Moreover, By the year 1940, under the stokes law of sedimentation, the ultracentrifugation technique was used to measure the size of colloidal solution.[178] By, 1933, Ernst August Friedrich Ruska and Max Knoll invented the TEM setup, and after World War II, the first commercialization of the setup was accorded. In line with that, after the synthesis of gold (Au) nanoparticles via reduction of chloroauric salt through citric acid, the first TEM image of gold nanoparticles was captured with a TEM setup[187]. Subsequently, a detailed investigation of nucleation and growth patterns during synthesis of gold nanoparticles was also accorded[188]. In addition to that, correspondingly, comprehensive methods of the synthesis processes for various metal quantum nanoclusters are also invented. In early 1960, an american chemist F.A. Cotton used the term ‘cluster’ for compounds containing metal-metal bond.[189] By 1965, triphenylphosphine ligands were used for the synthesis of various quantum clusters of gold (Au), silver (Ag), and copper (Cu). These synthesized gold (Au) quantum clusters contained five atoms of gold (Au_5)[190]. The same group synthesized gold quantum

clusters with 6 atoms (Au_6) using the different ligands, abbreviated as 1,2 Bis (diphenylphosphino) ethane. A separate exchange-based reaction, and infrared and conductivity analyses were performed to determine gold clusters in the solution precisely.[191] The synthesis of stabilized cluster $Au_{11}(PPh_3)_7(SCN)_3$ was disclosed, which was composed of a metal-metal bond containing 10 gold atoms bonded with one central gold atom.[192]. Subsequently, in 1970, many groups synthesized different metallic clusters composed of rhodium, ruthenium, niobium, osmium, manganese and copper.[193-200]. Thus, many researchers adopted different strategies based on the ligand augmentation technique to synthesize quantum mechanical clusters.[201, 202].

Moreover, alkali, alkaline-earth metals, and nobel gas clusters were also reported by many scientific communities. The magic clusters of Xe Nobel gas were formed as the first scientific input related to the formation of such clusters. Other Nobel gas clusters like He, Ar and Kr were also synthesized in the same patterns with the specific magic number.[203, 204]By the year, 1967 synthesis of sodium clusters in the gas phase was reported to carry eight sodium atoms.[205] In another study, calcium-based triplets[206] were synthesized. On the contrary, alkaline earth metals cryptates were also synthesized[207].

Alkaline earth metals (Ca, Mg) fluorescent nanoparticles are also developed in response to the generation of astonishing biocompatibility when compared to nobel metal clusters. Calcium and Sulphur are biocompatible materials when engaged to the human body and are also a good source of H_2S within the human body[208]. Thereby, a biocompatible system i.e. calcium sulphide fluorescent nanoclusters (CaS) has been developed for various applications like drug delivery, sensing, bioimaging labeling and in photovoltaics field. The synthesis of fluorescent CaS was not well established, despite its remarkable importance and possible applications within the human body.[209] In the review of Smet,

et al.[210] CaS was proposed as a photoluminescence material for LED applications. CaS has been doped with many rare earth metals to avail phosphorescence and fluorescence properties for desired intact wavelength emitting light.[211]. By doping these rare earth metals toxicity as well as the cost of synthesis get enhances, and without doping the mechanism of fluorescence in native CaS nanomaterial becomes unclear.[209] Absorption and emission spectra of CaS nanostructures were not well studied, thereby to fill the research gap an experimental and theoretical approach was adopted to study the absorption characteristics of such nanomaterials.[209] When CaS nanomaterial is synthesized by the reaction of $Ca(CH_3CO_2)_2$ in DMSO solvent, an large and wide absorption band is detected in the wide range from ultraviolet (UV) and to the above 1000nm wavelength. Upon excitation with 405 nm, the maxima of emission comes at 500nm wavelength with a tail of wavelength above 600 nm. The generation of optical properties in the $(CaS)_n$ molecular clusters was directly dependent on the size of nanocluster. The tetramer and monomer forms of CaS both showed the existence of absorption spectra from the region of UV to the near infrared region. For n=5, the light absorption prevails and extends to the near infrared region.[209]In another approach, molecular zinc carbonate highly luminescent clusters were also synthesized.[212] Another investigation was based on the synthesis of biocompatible BSA capped magnesium fluorescent noncomplex[213], all with the intention of generating biocompatible nano systems. Prior to delving into the more research investigations on biocompatible nanoclusters based on alkaline earth metals, it is imperative to understand the fundamental properties of metallic fluorescent clusters. These properties are elaborated upon below.

2.2 Physical Properties for metallic fluorescent clusters

The physical property is an important entity which is used to characterize the ultra-small metal nanoclusters. A few physical properties related to metallic nanoclusters such as, optical absorption, photoluminescence, ultrafast dynamics and chirality are shown below:

2.2.1 Optical absorption

Gold nano spheres absorption wavelength was found to be at 520nm due to the effect of surface plasmon resonance and can be easily fit to maxwell equation. On reducing the particle size to ~ 2nm the absorption peak at 520disappears[214] due to a strong quantum confinement effect, which is apparent in the case of ultrasmall nanoparticles only. This faciltates its bahviour as molecular species, thereby resulting in the formation number of discreated energy states. These quantum states are influenced by the number of atoms present in the ultrasmall cluster. As the number of atoms increases, the energy gap between the states varies as a function of $E_f/N^{1/3}, where\ E_f$ is bulk metal fermi energy, N is the number of atoms present in metallic fluorescent nanocluster. However, this energy gap discretization and as well as quantum confinement was not known till the study of nucleation of crystal structure via cluster was taken up. The Prof. Jin's group in 2008 [215] for the first time crystallographic structure of $Au_{25}(SR)_{18}$, where he elaborated relation between optical property and crystal structure by performing density function theory calculation. In this study, three different absorption peaks were observed such as HOMO- orbitals (d-band) to LUMO inter-band transition, HOMO-LUMO intraband transition and mixed based transition. The study state spectra of ultrasmall nanoclusters and nanoparticles were also investigated. The surface plasmon resonance peak was not detected in metallic quantum cluster (Au_{25},Au_{55}) but on the other hand the same SPR peak was observed in nanoparticles(Au_{2406}). On the other hand, when the

simulation were performed according to Mie theory for Au_{25}, which resulted in a specific SPR peak, that summarizes that the Mie theory is not applicable for ultrasmall metallic cluster.[214]

2.2.2 Photoluminescence Property

Band flexibility for fluorescent metal nanoclusters from visible to NIR is a key factor for its use in biological field. In previous investigations, thiol based fluorescent metal nanoclusters were synthesized and their quantum yield was found less than 1%.[81, 216, 217] Later, many scientific communities adopted different methods to enhance the quantum yield for fluorescent metal nanoclusters. For example, changing capping agent, doping with metallic ions, changing solvent, and aggregation induced emission means were adopted to enhance the quantum yield.[218, 219] [220]Wang et al. synthesized fluorescent nanocluster with the aid of polar ligands. They used ligand exchange reaction on the $Au_{38}(SC2Ph)_{24}$ with many polar ligands, which resulted in to the enhancement of fluorescent intensity by replacing various polar ligands.[221]

Furthermore, another group studied variation in fluorescent intensity of gold clusters as function of charge transferring ability and hydrophobicity of the ligands.[222] In this investigation, they found that the gold clusters having hydrophobic ligands {$[Au_{25}(C_2H_4Ph)_{18}]^-$, $[Au_{25}(C_{12}H_{25})_{18}]^-$, $[Au_{25}(C_6H_{13})_{18}]^-$} resulted in intense less fluorescent clusters as compared with gold clusters with hydrophilic ligands $[Au_{25}(SG)_{18}]^-$. The sequence of fluorescent intensity in decreasing order with hydrophobic ligands were as follows: $[Au_{25}(C_2H_4Ph)_{18}]^-$> $[Au_{25}(SC_{12}H_{25})_{18}]^-$> $[Au_{25}(SC_6H_{13})_{18}]^-$, this pattern directly follows electron donating capacity of ligands $PhCH_2CH_2$>$C_{12}H_{25}$>C_6H_{13}. This group also investigated the efficacy of charge enhancement of fluorescent intensity as function of charge on the metal core More positive charge (-1, 0, +1, +2) resulted in

increase in the magnitude of fluorescent intensity.[222] In summary, this group found that the increase in the fluorescent intensity of metal clusters depends on electron donating capability of the ligand, presence of more positive charge in the core region or by using electron rich ligand groups. By the same group, it was observed that lesser the electronegativity more intense is the fluorescent intensity for ultrasmall Au_{36} metallic clusters. Whereas, when electronegativity of the ligands is increased in the sequence: CPT (cyclopentanethiol)<TBBT (4-tert-butylbenzenelthiol)<BBT (4-bromobenzenethiol)<FBT (4-fluoro-thiophenol), the fluorescent intensity of metallic clusters were found to decrease. Further, polymer with different structure were used as a capping agent to the metallic clusters and effect of polymer steric hindrance and electron donating capability on the magnitude of the fluorescent intensity was observed. To fulfil it, three different types of polymers were developed and used as a capping agent for the synthesis fluorescent metal clusters. These polymers were basically PTMP-PMMA, PTMP-PBMA, and PTMP-PtBMA. The polymer capped gold clusters quantum yield was in the order Au-PTMP-PtBMA (20.1%), Au-PTMP-PBMA (14.3%), Au-PTMP-PMMA (3.8%), which is directly propotional to electron donating capacity of polmers in the order PTMP-PtBMA> PTMP-PBMA> basically PTMP-PMMA. On the contrary, the presence of higher steric hindrance in PTMP-PtBMA matrix increase the quantum yield of Au-PTMP-PtBMA metallic clusters. [140]

Prof. Xie group[223] reported the phenomenon of aggregation induced emission in enhancing the fluorescence of Au metallic clusters by incorporation of Au-I thiolate complexes. This group synthesized the Au(0)@Au(I) complexes. Later, they used Ag(I) group as a bridge between Au(I) groups, which resulted in the formation of Au(I)/Ag(I) thiolate complex that resides on the surface of Au quantum clusters and thus produces an intense fluorescence.[223] On the other hand, the fluorescence of Au quantum clusters

can easily be synthesized by doping of Ag ions. Further, Au ions doping also found to increase the fluorescence in Ag quantum clusters. The mechanism behind the fluorescence after doping of metal ions is not yet known very clear. But it is supposed that, upon Au doping electronic dynamics and relaxation of doped clusters are disturbed substantially. PMMA/PMMA-PMAA capped Ag clusters are formed and solvent dependency of luminescence is checked thoroughly. When organic solvents were not added, hydrophobic domains are formed by alkyl chains, and hydrophilic groups like alkyl chains are loosely bound. Moreover, after the addition of organic solvents, hydrophilic groups like carboxylic acids better stabilize the Ag quantum clusters by collection around the surface of clusters. This phenomenon resulted in repression of non-radiative transition between quantum clusters and solvent, which enhances the luminance intensity substantially. [224]

2.2.3 Ultrafast dynamics of metal clusters

Ultrafast dynamics in metal clusters typically govern various mechanisms such as electron-phonon coupling, relaxation kinetics, and radiative emission. Techniques such as femtosecond time-resolved fluorescence and transition absorption differentiate metallic as well as molecular behavior. Au quantum clusters were found to have longer fluorescence lifetimes than Au nanoparticles, with power-independent electron-phonon coupling. The existence of ultrafast fluorescence in various sized metallic clusters typically originates from the metal core, while the sustained emission in the NIR region comes from surface states. Prof. Jin's group explained the relation between ultrafast dynamics and atomic structure, finding rapid decay (1.5 ps) in Au_{38} clusters and even faster decay (1 ps) in $Au_{38}T$ due to its unique core structure.[225] [226-230]

2.2.4 Chirality Properties

Chirality is an important property in metal nanoclusters that distinguish them from their mirror images and which enables them for multimodal application, notably in enantiomer separation as well as chiral molecule recognition.[231, 232] The origin of this property typically arises from various factors such as specific arrangement of chiral legends on an achiral metal core, the presence of chirality in ligand carbon tails, and existence of chiral inner metallic cores. For example, in $Au_{20}SR_{16}$ clusters, although it is well known that the ligands and inner core may be achiral, despite this, the arrangement of surface protecting units imparts chirality. This ligand-based chirality is evident in clusters like $Au_{25}(SR)_{18}$. Achiral ligands like phenylethylthiolate yield achiral clusters, while chiral ligands like (S)-2-phenylpropane-1-thiol induce chirality, impacting the electronic structure.[233, 234]

The synthesis of nanoclusters utilizing noble metals such as Ag, Au, and Cu is a widely studied in the field of nanotechnology.[235] These metals are known for their biocompatibility and resistance to oxidation in atmospheric conditions, making them suitable candidate for the synthesis of fluorescent nanoclusters. For instance, gold clusters have been found to be biocompatible, leading to their application in nanomedicine and bio-imaging. However, long-term biocompatibility of these clusters remains a concern due to its retention in body after 28 days of injection of BSA-protected gold clusters into mice, where only 5% of the gold was found to be digested, and toxicity in mice was observed in the form of damage to vital organs such as the kidney and liver.[154] Therefore, the requirement for a biocompatible system has led to the development of biocompatible alkaline earth metal systems, as illustrated below.

2.3 Development of alkaline earth metal based nanoclusters

Despite their biocompatibility, nanoclusters made from alkaline earth metals like calcium and magnesium have received less attention in the area than clusters made from noble metals.[236] These elements play crucial roles in governing hemostasis due to their presence in phosphates within the cell nucleus and cytoplasm. Specifically, magnesium ions assist in the production of metal phosphates within the cell, which regulate the cell's location, while calcium aids in heart rate regulation, muscular contraction, bone health, and the regulation of the fluid balance of cells.[236] Furthermore, calcium plays a crucial role in regulating bio mineralization, osmotic pressure, and cell signaling mechanisms. Despite these numerous benefits, calcium is known for its highly reactive nature, which can render it unstable.[237]

Thus, many synthesis techniques were used to construct nanomaterials based on Ca and Mg elements. To the far that Ca is involved, the CaS phosphors triggered by rare earth elements have been employed in cathode ray tube phosphors since 1971 because they are said to exhibit superior fluorescence.[238] Due to their outstanding characteristics as a spectroscopic material, bare CaS and activated CaS are vastly used for application in a variety of research areas, including imaging, radian dosimetry, and alloyed semiconductors. It is crucially necessary to have the ultra-tiny size of biological material become smoothly suspended in the experimental solution when it comes to the investigation of biochemical as well as biological kinetics.[239] On the other hand, fluorescent semiconductor-based nanocrystals are mostly used as DNA, antibody, and cell detection sensors. Due to the existence of low toxicity, a significant Stokes shift, and a large sensitivity, inorganic materials were shown to have several benefits over organic materials, making them the ideal option to be employed for biological purposes.[240]

Europium-doped alkaline earth metal sulphides were among the sulphides shown above. These sulphides are regulated mostly by their preeminent ionic nature and they typically exist as FCC structure. A multiwavelength excitation of these nanomaterials results in strong fluorescence, and they usually exhibit a high quantum yield and are photostable. CaS phosphors with rare earth stimulators are manufactured by sulfurizing calcium salts and rare earth stimulators in carbon disulfide or hydrogen sulfide environments, however this process is costly and time-consuming.[241, 242]

Despite the good biocompatibility of doped luminescent alkaline earth metal sulphides bulk phosphors, they cannot be used for drug research, disease diagnostics, or other applications in the life sciences due existence of their large particle size.[236] The capacity to tailor the size and crystallinity of a manufactured nanomaterial directly affects its essential qualities. For instance, hydrolysis of CaS nanoparticle in the aqueous solution is the main obstacle for the synthesis of luminescent CaS nanoparticles in aqueous solution. Additionally, it is discovered that the fluorescence intensity of CaS nanoparticles in water is quite low. Consequently, this circumstance prompted the development of Europium-doped fluorescent CaS nanoparticles in ethanol in order to increase quantum yield and retard hydrolysis in water.[243] To control the size, hydrolysis and crystallinity of CaS nanoparticles during the synthesis, wet chemical method was employed, that created luminescent triethanolamine-capped calcium sulfide nanoparticles [243]

After conducting an analysis of diverse biocompatible molecular clusters containing calcium, including calcium carbonate ($CaCO_3$), a group of researchers synthesized amorphous $CaCO_3$ clusters (1.4 nm) protected by 10,12-pentacosadiynoic acid, consisting of seven $CaCO_3$ molecules. In the same way, ultra-small $CaCO_3$ clusters (4.9 nm) were made using the solvothermal method in the solvent ethanol. Also, computer simulations

showed that $CaCO_3$ clusters are made up of units of $CaCO_3$ ions that switch places. Also, the ion-pair technique showed that the $CaCO_3$ prenucleation clusters could be stabilized.[244-246]

Numerous researchers attempted to render $CaCO_3$ material luminous. To do this, $Gd^{3+}$and Eu^{3+} are doped into the $CaCO_3$ matrix, resulting in fluorescent $CaCO_3$ crystals. $CaCO_3$ Gd^{3+} becomes luminous with a lifespan of 6.19 ms owing to the $^6P_{7/2}$-$^8S_{7/2}$ transition induced by doping of Gd^{3+}. [247]When Mn^{2+} is utilized as a dopant, $CaCO_3$:Mn^{2+} crystals have a very long lifespan (50 ms), indicating that the 3d-3d transition is successful.[248] By doping Calcite crystals with Mn^{2+} and Ce^{3+}, blue fluorescence emission is detected; the 5d to 4f transition is effective in this instance, notably for Ce^{3+}.[249] The dopants , Gd^{3+}, Eu^{3+}, Ce^{3+}, and Mn^{2+} exhibited the longest fluorescence lifetimes on average (ms). Due to existence of the larger size of these crystals, they were often used as phosphors. [250] Despite these findings, sustainable fluorescent matrix composites of calcium carbonate with carbon dots have been created.[251] In addition, sulfonated fluorescent dyes were integrated into the calcite crystal to provide a stable and biocompatible framework for comprehending the path of additional material obstructing in the calcite crystal during dye fusion. Using this data, a stable, biocompatible, white glaring light-emitting was created.[252] On the other hand, $MgCl_2$ was used to create BSA-capped fluorescent magnesium clusters. The ultra-small magnesium clusters produced exhibited fluorescence in the blue and green bands, with a blue quantum yield of 0.17. A549 cells were bio-imaged using thiolate-capped Mg-S clusters. It was shown that when BSA is eliminated from the synthesis, large magnesium nanoparticles are synthesized and Mg(0) clusters are not created. Thus, BSA protein was employed to reduce the size of Mg nanoparticles from big to ultra-small. Therefore, the inclusion of BSA protein is required for the creation of ultra-small nanoclusters. The produced MgS nanoclusters have an

exceptional quantum yield, minimal toxicity, and restricted color emission. The aforementioned MgS cluster synthesizing method is based on bio-mineralization. This biominerlization procedure is regarded as a very fascinating and biocompatible synthesis route for ultra-small clusters.[213, 253]

Thus, red-emitting fluorescing gold clusters were previously produced using the bio mineralization approach.[90] As the BSA protein includes 17 disulphide bonds and cystein residues, these bonds and residues aid in the capping agent during ultrasmall cluster production. The usage of green synthesis is based on the assumption that it is inexpensive and environmentally beneficial compared to chemical synthesis.[254, 255]

During the formal synthesis of $CaCO_3$ nanoparticles, granulated stem peel was commonly used. The presence of phenolic reducing agent in extract of stem peel granulates facilitated in the synthesis of $CaCO_3$ nanoparticles.[255] Similar to this, M. oleifera flower extract was previously used in the fabrication of ultra-small gold nanoparticles measuring 3-5 nm in size. The leaf extract of M. oleifera is a promising tool for reducing metal ions to metallic nanoparticles due to the presence of a substantial quantity of ascorbic acid.[256, 257] Additionally, M. oleifera has antihypertensive, anti-inflammatory, and antitumor effects.[258] M. oleifera flower extract is thus utilized as a reducing agent in the fabrication of ultra-small gold nanoparticles.[258] All researches as elaborated above are discussing development and utilization of platform pertaining to calcium based luminescent biocompatible nanoclusters. In addition to above, to understand the thermodynamics and kinetics dependent nucleation kinetics of ultra-small clusters that produces crystals of desired shape and size at low temperature (<100℃), various methods are proposed by several groups, which are mentioned below.

2.4 Nucleation Rate and Interfacial Energy Analysis of $CaCO_3$ nanomaterials on various substrates and additional approaches to measure nucleation rate of other nanosystems

Various engineered technologies were developed for computation of nucleation rate and interfacial energies for ultra-small clusters in a low temperature range (0-100°C), to understand their formation.[174] The above methodology relied on the calculation of nucleation rate based on the Classical Nucleation Theory (CNT). As, it is well known, according to CNT, nucleation begins at a particular supersaturation value or by the influence of high temperature on solid state amorphous particles.[259] Homogeneous nucleation comprises the fundamental processes of growth, crystallization, aggregation, and phase change.[260] During the nucleation process, the interfacial surface is of utmost importance, which aids several interfacial-based synthesis procedures, such as CO_2 sequestration, biomineralization, battery operations, and scaling control procedures.[261-264]

While calculating nucleation rate, a firm grasp of the two barriers (kinetic and thermodynamic) is necessary. The nucleation rate is always a product of $J_o = A_\alpha exp(E_a/RT)$ -(Kinetic barrier) and $exp(\Delta G/RT)$ (Thermodynamic barrier), Here E_a is the activation energy of nucleation, ΔG is the Gibbs free energy barrier, and T is the varying temperature.[174] Interestingly, proper information on the calculation of J_o is seldom available when calculating the nucleation rate, and the majority of previous research have used J_o as a constant factor.[174, 265-267] For homogeneous nucleation, a few experiments were conducted at room temperature to compute J_o as (D)/(5d), where D is the monomers diffusion coefficient d is the monomer diameter.[268] These calculations were performed in order to determine the homogeneity of the nucleation process. In

addition, further research carried out at room temperature was employed for the calculation of J_o; this was accomplished with the use of an atomic force microscope (AFM). For instance, the value of J_o for the nucleation of silica on NH_3/COO^- as well as carboxyl mixed composite platforms is as high as $10^{14.8\pm1.4}$ nuclei m^{-2} min^{-1}, and $10^{13.5\pm0.7}$ nuclei m^{-2} min^{-1}respectively.[174, 269] However, the aforementioned methods were used to calculate nucleation rate at room temperature. In spite of above proposed methods for computation of kinetic barriers for nucleation rate, estimation of nucleation rate for ultra-small gas molecules (<10 nm) to gas hydrate systems was presented by several scientific groups at low temperature (100-200K) and high pressure for understanding condensation pattern.[270] As an illustration, SAXS (Small Angle X-ray Scattering) methodology was adopted to understand the nucleation rate kinetics of heavy hydrocarbon gases and alcohols with temperature and specific supersaturations experimentally[271, 272]. On the other hand, various other groups used FLUENT and programmed the condensation process of light hydrocarbons on the basis of nucleation rate variations.[273] In the supersonic nozzle, the mechanism of condensation of methane gas was investigated, when CO_2 gas was used as a carrier gas.[274] These aforementioned analysis of condensation mechanism were directly dependent on the inlet temperature, inlet pressure and content of carrier gas. However, it is generally recommended to incorporate existing nucleation models to generate a deep insight condensation mechanisms.[270] In another approach, Molecular dynamics simulations (MDS) were used for understanding the nucleation rate mechanisms. The main theory behind the MD simulations relied on interactions and physical properties of the nucleating objects.[275] This MD simulations generated various methods for computation of nucleation rate for various condensing systems. For example, stillinger criterion was developed to determine clusters nucleation rate theoretically for condensing systems with the aid of MD simulations.[276] Further, it

was assumed that clusters usually exists in liquid systems with higher local density when compared gas densities. Ultimately the definition of liquid cluster should pass two criteria; one is validating the stillinger criterion and other is presence of at least five adjacent particles. Thus, different ways for computation of nucleation rate is being proposed by several scientific groups based on clusters identification.[270]. For example, MD simulations were used to compute condensation rate of nucleation of Lennard-Jones fluid (Supersaturation:6.8), when temperature drops below the three-phase point with super saturation of water vapor to be at 14.6 at 350K.[270] The Mean first passage times technique (MFPT) was acquainted by Wedekin et al. to compute nucleation rate with the aid of MD simulations.[277] The survival probability (SP) approach, direct observation technique, and the other cluster based methods were utilized by Julin et al. via single simulation approach.[278] Chkonia et al. adopted a comparison methodology for different techniques for nucleation rate calculations by choosing condensation of Argon gas.[279] All the aforementioned techniques which was developed by various scientific groups for calculation of nucleation rate of nanoclusters and ultra-small gas molecules for low temperature range (<100°C and 100-200K). But at high temperature (>100°C), the existence of large complexity and limitations in microscopic functions and the inclusion of bulk components in the computation of the nucleation rate are the primary causes of the ignorance of kinetic factors involved in the nucleation rate calculation for ultra-small solid nanoclusters.[280] Consequently, the further investigation is required for the calculation of J_0 for nanoclusters through a precise calculation of A (pre-exponential kinetic factor) and E (nucleation activation energy) at high temperature (>100°C).[281] These kinetic factors can possibly be computed using the most precise iso-conversional techniques through the data extracted through non-isothermal TGA machine.[282] [281]In addition, previously, the activation energy of dehydration has been calculated

during the phase shift of amorphous calcium carbonate clusters to crystalline calcite at 315℃.[283] The nucleation rate of ultra-small osmium clusters was calculated to be 78.8 and 176.5pm/min when the temperature is raised from 20 to 100℃, respectively.[284] Despite the aforementioned findings, no other practical technique exists to calculate the nucleation rate of solid state ultra-small clusters over 100℃.[284] In addition, some studies have only calculated the activation energy of nucleation for In-Se crystals at high temperature (550℃) using iso-conversional techniques after TGA.[283] To compute the activation energy of nucleation and subsequent analysis of kinetics through the thermogravimetric analysis (TGA) at high temperature accurately, several groups adopted various model-free approaches, as discussed below.

2.5 Use of Model free methods for computation of activation energy of nucleation

Over numerous centuries, inorganic materials' kinetic studies have been a major focus during the phase transition from solid to liquid and gaseous products.[285] In order to estimate the activation energy needs when researching the thermal degrading behaviors of several inorganic materials, many mathematical models have been constructed to date.[286] Through thermogravimetric TGA analysis, one may easily determine this activation energy of dissociation. The TGA machine provides mass loss data versus temperature and time in an experimental technique, which may be utilized to calculate apparent activation energy using the iso-conversional method. During the processing of the TGA scan, it is possible to make adjustments to the sample weight, heating environment, and heating rate with relative ease. Furthermore many model-free approaches were used by researchers in the scientific community in order to determine the apparent activation energy of degradation. Vyazovkin, Friedman, and Vyazovkin AIC are the model-free approaches that are being discussed here.[281, 287, 288] For instance, the calculated value of apparent activation energy using the Friedman technique is

thought to include less systematic error, but experimental noise causes such procedures to fluctuate somewhat, leading to the occurrence of instability.[289] Regarding the Vyazovkin approach at a given conversion point, the activation energy values were averaged from 0 to different conversion points. It produces flattened noise in the final converged activation energy values.[290] To eliminate the aforementioned flaws, the Vyazovin AIC approach was proposed, which included accurate activation energy estimates in the small region of $\Delta\alpha$.[291] In the Vyazovkin AIC technique, taking the derivative of the minimization function results in a complicated equation. The minimization of the performed function is time-consuming when determining apparent activation energy using the V. AIC approach. Thus, the iterative isoconversional methodology is adopted by several researchers, which has traditionally been regarded as a faster method of calculation than other isoconversional techniques. As a result, iterative integral iso-conversional approach is considered mostly for quick calculation of activation energy, while maintaining the accuracy in the small region of computation interval of conversion.[291] Moreover, compared to other nanomaterials, calcium is regarded as an excellent nanomaterial due to its exceptional surface area, presence of high reactivity, non-toxic nature, and existence of higher interfacial energy. Therefore, $CaCO_3$ is thought to be a flexible material with numerous potential medicinal uses, including in cancer treatment, medication delivery, protein adsorption, energy storage, and other fields.[288, 292] The conversion kinetics of $CaCO_3$ materials are being intensively studied by several different research institutes. Apparent activation energy is being computed to be in the range of 145 to 200 kJ/mol while evaluating kinetics of the material, and this distortion in diffusion for one and two dimensions makes this range a possibility.[293] Thus, several thermal kinetic investigations have been conducted for large $CaCO_3$ materials[294, 295], to understand their nucleation kinetics.

2.6 Development of various methods for synthesis of tin nanocrystals

Moreover, research into the synthesis of biocompatible nanomaterials is getting elevated day by day, with novel substances being constantly identified and evaluated for their potential utility in the healthcare sector. One particularly promising area of enquiry is that of calcium-based systems, which have the potential to serve as superstructure materials and thus open up new vistas for applications such as oncological treatments, also elaborated in aforementioned investigations. These materials represent a nascent yet promising field of study and have the potential to significantly impact the healthcare industry. When compared to other nano-materials except alkaline earth metals (Calcium and Magnesium) with potential biomedical applications, tin nanoparticles (Sn) have very little investigation in health care sector. Tin (Sn) possesses the remarkable property than can easily be altered at nano scale to show distinct characterstics.[296, 297] The structure of Sn metal provides insight into its basic characteristics. Tin element is basically found in two phases: β-Sn (tetragonal structure) and α-Sn (diamond cubic structure). When the room temperature falls below the marking of 13°C, β-Sn (metallic phase) automatically converts to α-Sn (zero band gap semimetal) material, resulting into increase in volume.[298, 299] The two forms of tin abbreviated as semi-metallic (gray tin) and metallic states (white tin) possesses different optical properties. These properties are again tuned upon changing of size of tin element to the nanoscale level [297]. Furthermore, ample amount of theoretical work has been demonstrated for existence of ultra-small tin nanocrystals, revealing their unparalleled scientific importance.[298, 300-302] This caused to facilitate synthesis of large sized beta tin (β-Sn) nanocrystals (>10 nm) [303-306] or use of charged particles and bulk materials to provide stability to β-Sn nanoparticles. [307] [308]

The synthesis of alpha tin (α-Sn) involves substantial difficulties and challenges (enumerate those challenges). As bulk α-Sn is thermodynamic unstable entity at ambient temperatures[309], a substrate such as InSb(100) is always used as a crystallization seed with matching lattice constant, for the stability and growth of bulk α-Sn on substrate. In another approach molecular beam epitaxy technique is adopted for growth of α-Sn in followed by 400°C annealing of thin films over an InSb(111) matrix.[310]. Similar type of research has been performed on the matrix of silicon wafers (1,0 0) with coatings of SiOx (70 nm)[173], InSb and CdTe and substrates[309, 311], and amorphous silicon based substrates.[312] In the latest research, SnGe carrying alloyed beta Sn, were heated at 300-350℃ on an silicon substrates with an oxide layer and, which resulted to the growth and stability of α-Sn with β-Sn, as detected by XRD analysis[313] to synthesize bulk α-Sn. Furthermore, in the nanoscale realm, α-Sn nanocrystals as well as α/β Sn nanocrystals were synthesized in ethanol solvent through the process of micro-plasma synthesis. These formed nanocrystals of tin showed tuning of band gap from 0 to 1.25 eV, which provided metastability to these crystals, since at the nanoscale realm Bohr exciton radius (12.5 nm) for α-Sn stability is larger than the size of ultra-small nanocrystals.[297, 301] Furthermore, the above method of synthesis by microplasma techniques presents a several benefits for tuning the size of nanoparticles , however, it requires an sophisticated as well as high gas temperatures (1426℃-1726℃) during the synthesis of nanoparticle[314]. Henceforth, the fabrication of water based ambient temperature synthesis of α-Sn nanocrystals appears to contain ample difficulties.

2.7 Problem Statement

1. As discussed in the literature review above, long term toxicity of Au, Ag, and Cu quantum clusters and quantum dots instigated the synthesis of biocompatible fluorescent nanomaterials. Thus our synthesis approach is inclined towards the development of biocompatible multi-color fluorescent alkaline earth metal based $CaCO_3$ pre-nucleation clusters. To synthesize such ultra-small clusters a facile, sustainable and low cost bio mineralization technique is adopted by choosing leaf extract as a reducing agent and BSA protein as a capping agent.
2. The literature survey mentioned earlier only addressed the computation of nucleation rates for ultra-small osmium clusters within a low-temperature range (20°C to 100°C). However, no technology was available to compute these rates at higher temperatures (>100°C). To solve these problems we used thermogravimetric (TGA) technology to compute nucleation rate and interfacial energy of such ultra-small clusters at high temperature and respective conversions.
3. In another approach we created a novel synthesis method, through which we can fabricate alpha tin nanocrystals (~4.16nm) at normal ambient temperature. In previous discussed literature survey, alpha tin nanocrystal synthesis involves the utilization of bulky substrates line InSb, CdTe etc (>100nm) and high temperature (>100°C) operations. To address this challenge we devised a new technique based on reduction approach that allows synthesis of ultra-small alpha tin nanocrystals coexisting with beta phase at normal room temperature, and these alpha tin nanocrystals were utilized as a photothermal agent for the cancer treatment.

2.8 Aim and objectives of present work

The major objectives of our research is as follows-

- **Objective 1: Synthesis of biocompatible, BSA capped fluorescent $CaCO_3$ pre-nucleation nanoclusters for cell imaging applications.**
- Synthesis of ultra-small fluorescent BSA capped $CaCO_3$ pre-nucleation clusters.
- Characterization of ultrasmall clusters through TEM, XPS, HR-XRD, FTIR, Fluorescence lifetime, MALDI-MS, analyses.
- Confocal imaging of ultrasmall clusters in MG-63 cells.
- Fluorescence microscopic imaging of ultra-small clusters in MG-63 cells.
- CTCF analysis of florescent ultra-small clusters.
- Shelf life, pH stability, and photostability determination of ultra-small clusters.
- Quantum Yield determination of ultra-small clusters.
- Biocompatibility test of ultra-small clusters through MTT ASSAY.

- **Objective 2: A New Technique for Calculating Kinetic and Thermodynamic Barriers for Nucleation Rates and Interfacial Energy of $CaCO_3$ Prenucleation Nanoclusters at High Temperature Using TGA Models and In-Situ Crystallization.**

- Current research discusses computation of J_0 (kinetic energy barrier) at high temperature and respective conversion for ultra-small BSA capped $CaCO_3$ prenucleation clusters. Characterization of clusters is accompanied by XRD, FTIR, TGA, HR-TEM.
- A_α (pre-exponential kinetic factor) for ultra-small clusters was determined by adopting the differential function $f(\alpha)$ of the random nucleation process.

- E_α (Activation energy of nucleation) is computed by adopting most accurate isoconversional method, abbreviated as iterative isoconversional method.
- Thermodynamic parameters (ΔG, ΔH, and ΔS) are computed for ultra-small clusters at high temperature and respective conversions.
- Nucleation rate of ultra-small clusters at high temperature and respective conversions is computed by adopting classical nucleation theory equation.
- Interfacial energy of ultra-small clusters are also computed at high temperature and conversion.
- Mathematical models are also proposed for computation of nucleation rate and interfacial energy of ultra-small clusters at high temperature and respective conversions.
- Experimental validation is also performed to assess the existence of nucleation at high temperature and respective conversions.

- **Objective 3: A facile synthesis of Nano diamond tin nanocrystals at room temperature with their beta forms for in-vitro photothermal cancer research, *in-vivo* toxicology, and integrated Fourier Transform modeling.**

- A cutting-edge reduction technique has been developed to synthesize ultra-small α-Sn nanocrystals with β-Sn in an aqueous solvent at ambient temperature.
- Characterization of tin nanocrystals is accompanied by XRD, HR-TEM, Spectrophotometer, and SAED Patterns.
- The photothermal efficiency of ultra-small tin nanocrystals is calculated to be 42.4% upon shining (0.5 W), 980 nm NIR-light using CW-laser.

- These nanocrystals are utilized for *in-vitro* cancer cell treatment by incorporation of 980 nm laser, and subsequent Pre and Post photothermal MTT Assay is performed.
- Concentration and laser dependent photothermal property is also demonstrated upon excitation of 980 nm laser.
- Direct band gap is also calculated through Tauc plot.
- Using FFT-weighted bright field imaging technique, a sophisticated mathematical model has been proposed that foretells the behavior of cancerous cells pre and post photothermal operation.
- Biocompatibility assessment is also performed by injecting tin nanocrystals to Wistar Rats via tail vein. Histopathological and enzyme based analyses revealed the safety of tin nanocrystals inside the body of Rats.