Ph.D Thesis Synopsis Report

Multifunctionalization of Nanoscale Particles for Cancer Theranostics

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Cancer is a highly heterogeneous disease with inter-patient variations, which prevent conventional chemotherapy from being fully effective.[1-2] Herein, we explore the possibility of using multifunctional theranostic nanoparticles (MFTNPs) for simultaneous imaging, targeting and therapy of cancer cells *in vitro.* This could be useful for addressing challenges arising out of inter-patient variations and to further develop personalized therapeutic strategies thus reducing the vulnerability and increasing the chance of patient survival.[1-2] The main focus of the current thesis is the development of MFTNPs by unifying discrete "functional components" into a single nanosystem with the potential of simultaneously performing multiple task including multimodal imaging of the disease site, successful delivery and controlled release of the drug, providing with additional therapeutic module in the form of hyperthermia and magnetically targeted therapy *in vitro*.

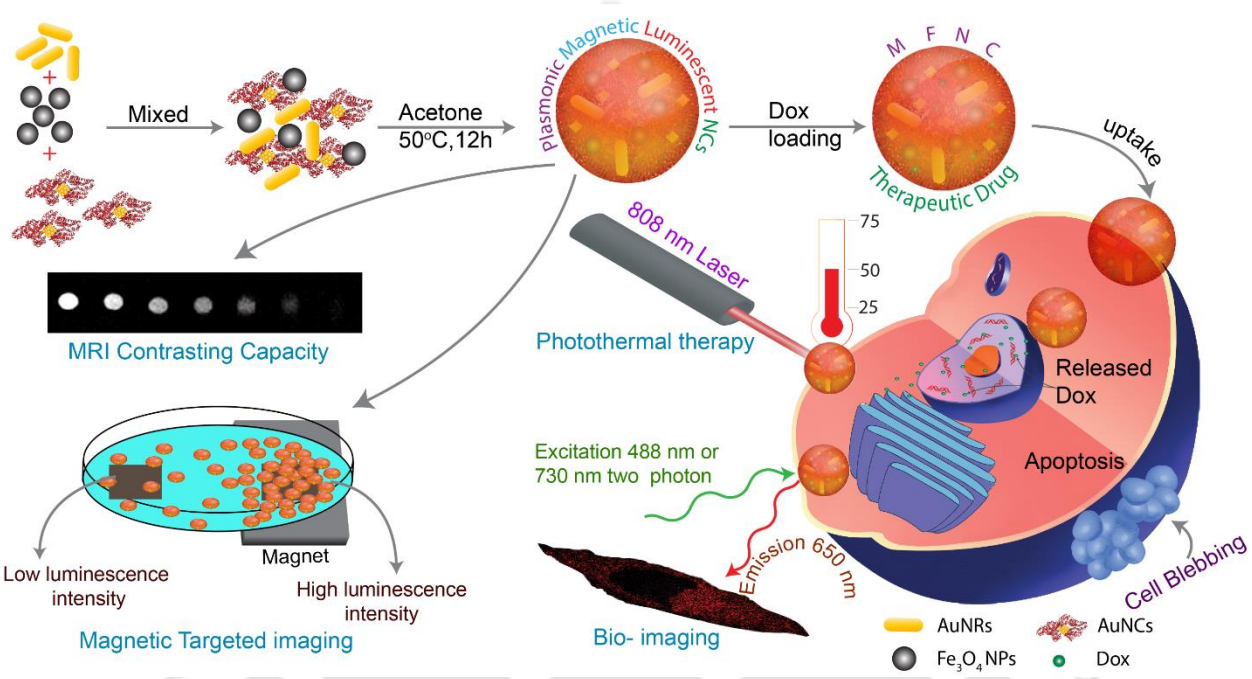
The thesis addresses two different approaches for the fabrication of the MFTNPs. The first strategy employs biocompatible and biodegradable protein (like bovine serum albumin and lysozyme) matrices for the unification of different functional nanomaterials for fabricating the MFTNPs. In this approach, we have developed an ideal plasmonic and magneto luminescent MFTNPs by integrating the three main functionalities of inorganic nanomaterials namely magnetic, plasmonic and luminescence and demonstrated successful application of these MFTNPs in bioimaging, magnetic targeting, plasmonic photothermal therapy and drug delivery. On the other hand, in the second strategy, we explore the newly developed method of "surface complexation" on nanomaterials for the fabrication of a single nanocrystal based MFTNPs capable of possibly single particle-level bioimaging and cancer therapy.

The present thesis is comprised of six chapters as described below.

Chapter 1 provides an introduction of the thesis and literature review regarding the applications nanomaterials in cancer theranostics. This chapter presents a brief idea about different theranostics applications of plasmonic, magnetic and luminescent nanomaterials. It also discusses the processes for developing multifunction nanocarriers, multifunctional theranostics nanomaterials and their advantages in cancer theranostics.

Chapter 2 describes the fabrication of plasmonic and magneto-luminescent multifunctional nanocarriers (MFNCs) by assembling gold nanorods, iron oxide nanoparticles, and gold nanoclusters within BSA nanoparticles (**Scheme 1**). The MFNCs showed self-tracking capability

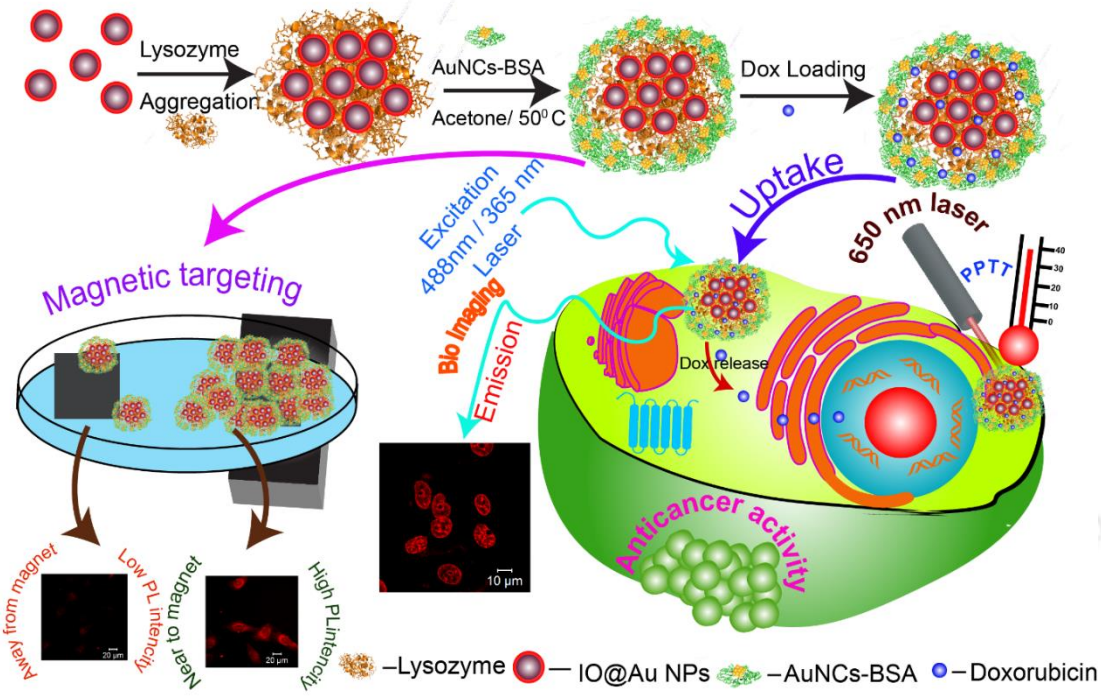
through single and two-photon imaging, and the potential for magnetic targeting *in vitro*. Appreciable T_2-relaxivity exhibited by the MFNCs indicated favorable conditions for magnetic resonance imaging. In addition to successful plasmonic-photothermal therapy of cancer cells (HeLa) *in vitro*, the MFNCs demonstrated efficient loading and delivery of doxorubicin to HeLa cells leading to significant cell death. These MFNCs with their multimodal imaging and therapeutic capabilities could be promising candidates for cancer theranostics.



Scheme 1: Schematic depiction of preparing MFNCs, their capacity for *in vitro* MRI contrasting and magnetic targeting, Two-Photon imaging, plasmonic photothermal therapy, and inducing cell death in cancer cells (HeLa), following successful loading and delivery of anticancer drug doxorubicin (Dox).

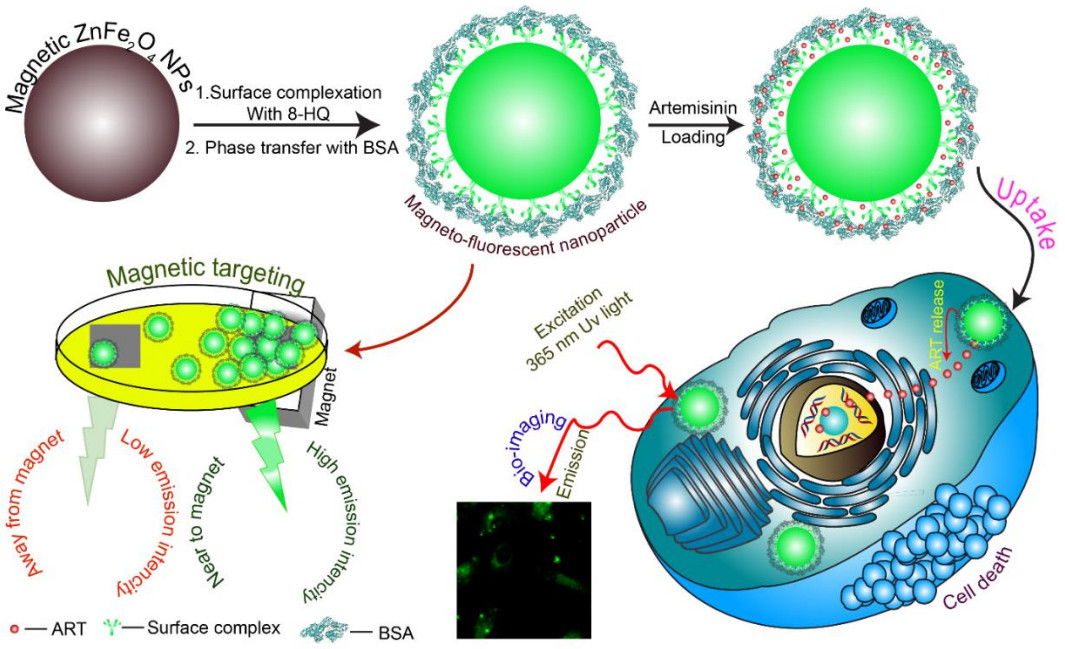
Chapter 3 discusses the fabrication of a plasmonic-magneto-luminescent multifunctional nanocarrier (PML-MF nanocarrier) by lysozyme-mediated agglomeration of gold-coated iron-oxide nanoparticles (IO@AuNPs) and subsequent coating of these agglomerates with BSA-stabilized gold nanoclusters (BSA-AuNCs, **Scheme 2**). Agglomeration-mediated red-shifting of the plasmonic absorbance peak of IO@AuNPs within PML-MFNCs towards NIR-biological window helped in plasmonic photothermal therapy (PPTT) by PML-MFNCs. PML-MFNCs demonstrated excellent in vitro bioimaging and magnetic targeting capabilities due to strong

photoluminance and superparamagnetism of the constituent AuNCs and IO@AuNPs, respectively. PML-MF nanocarriers showed successful loading and delivery of doxorubicin to cancer cells with significant killing efficiency that could be synergistically improved by combining with PPTT.



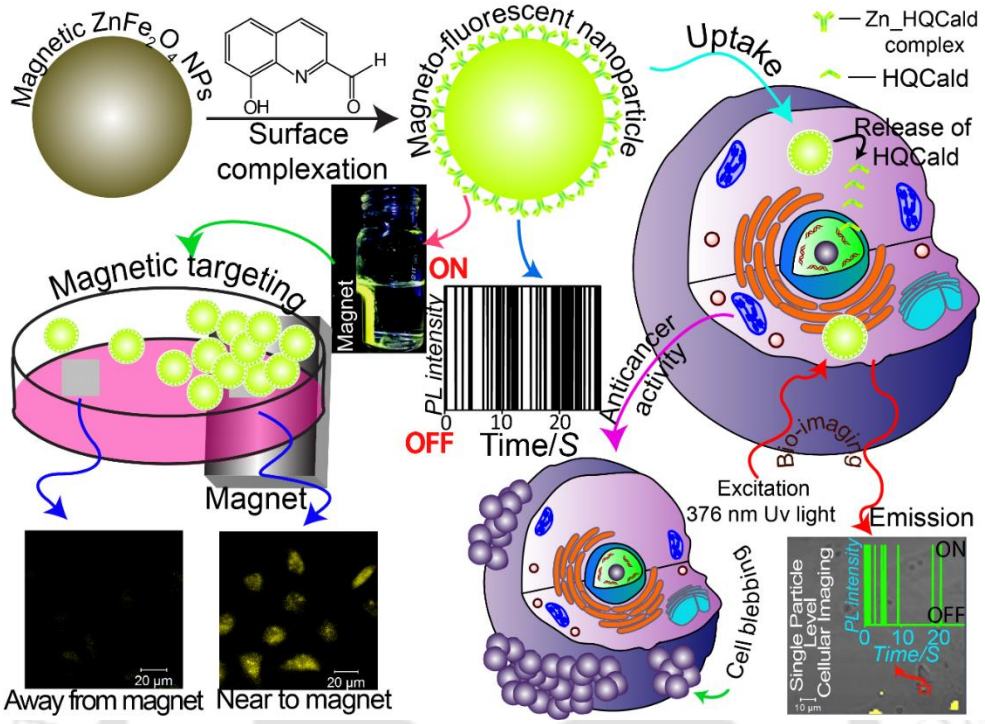
Scheme 2: Schematic representation of the fabrication of PML-MF nanocarrier and its capacity for plasmonic photothermal therapy, drug delivery, bioimaging and *in vitro* magnetic targeting.

Chapter 4 demonstrates the fabrication of a novel class of magnetofluorescent theranostic nanoparticles (MFTNPs) based on 'surface-complexation' of zinc ferrite ($ZnFe_2O_4$) NPs with 8-hydroxyquinoline (HQ, **Scheme 3)**. The potential of HQ surface complexed $ZnFe_2O_4$ NPs (HQ-ZFNP) in fluorescence-based bioimaging of different cancer cells was successfully demonstrated. The superparamagnetic behavior of the HQ-ZFNP was exploited effectively in magnetic targeting *in vitro*. Finally, a well-known hydrophobic anti-malarial and prospective anti-cancer drug artemisinin was efficiently loaded into the MFTNPs. Artemisinin loaded MFTNPs were observed to induce superior anti-proliferative response, as compared to free drug, in cancer cells in a synergistic mechanism with combination index of 0.1 or less.



Scheme 3: Schemetic representation of fabricating HQ-ZFNP-based magneto-fluorescent theranostic nanoparticles, and their use in bioimaging and magnetic targeting *in vitro*. Also depicted is the loading of artemisinin (ART) into BSA-coated HQ-ZFNPs with subsequent delivery of the drug resulting in killing of cancer cells.

Chapter 5 discusses fabrication of novel magnetofluorescent nanoparticles by complexation of zinc ions present on the surface of zinc ferrite nanoparticle with 8-hydroxy-2-quinolinecarboxaldehyde (HQCald, **Scheme 4)**. The as prepared HQCald-complexed $ZnFe_2O_4$ NPs showed good quantum yield (3.62%), high photostability, considerable excited state lifetime (5.31 ns) and high saturation magnetization (12.7 emu g^{-1}). These magnetofluorescent nanoparticles demonstrated bioimaging capability both at the ensemble and single particle levels, and *in vitro* magnetic targeting. Moreover, the pronounced anti-proliferative efficacy of these nanoparticles against cancer cells, with appropriate targeting strategies, can lead to potential cancer theranostics.



Scheme 4. Schematic representation of fabricating HQCald-surface complexed $ZnFe_2O_4$ NPs and their application in ensemble and single particle level cellular imaging, magnetic targeting and *in vitro* anticancer activity.

Chapter 6 contains conclusion and future prospects.

In summary, the present thesis reports the fabrication of four different multifunctional theranostics nanomaterials (MFTNPs) for potential cancer theranostics. This has been achieved by: (i) by assembling gold nanorods, iron oxide nanoparticles, and gold nanoclusters within BSA nanoparticles and subsequently loading doxorubicin in it, (ii) preparing lysozyme mediated nanoscale aggregates of gold coated ironoxide core-shell nanoparticles followed by coating them with luminescent BSA stabilized gold nanoclusters and loading chemotherapeutic drug doxorubicin in it, (iii) by developing green emitting complexes on the surface of zinc ferrite nanoparticles with HQ and subsequently loading artemisinin on it, and (iv) fabricating yellow emitting complexes on the surface of zinc ferrite nanoparticles with derivative of HQ 8-hydroxy-2-quinolinecarboxaldehyde. All the MFTNPs developed here has been demonstrated for

fluorescent bioimaging, *in vitro* magnetic targeting and anticancer efficacy. Moreover, some of them have also been demonstrated for photothermal therapy, combination therapy, two-photon or single particle based bioimaging and magnetic resonance imaging. Taken together, all the MFTNPs showed significant cancer theranostics effect in *in vitro* model. However, future studies should be carried out in animal models in order to employ them clinical treatments. Additionally, the unification strategies used here to fabricate MFTNPs namely use of protein matrix and surface complexation are versatile in nature and hence should be further explored for the development of newer MFTNPs with novel theranostics potential.

References:

1. Bao, G.; Mitragotri, S.; Tong, S., Multifunctional nanoparticles for drug delivery and molecular imaging. *Annu. Rev. Biomed. Eng.* **2013,** *15*, 253-282.
2. Bigall, N. C.; Parak, W. J.; Dorfs, D., Fluorescent, magnetic and plasmonic—Hybrid multifunctional colloidal nano objects. *Nano Today* **2012,** *7* (4), 282-296.

List of Publications:

1. **Pan, U. N**.; Khandelia, R.; Sanpui, P.; Das, S.; Paul, A.; Chattopadhyay, A. Protein-Based Multifunctional Nanocarriers for Imaging, Photothermal Therapy, and Anticancer Drug Delivery *ACS Appl. Mater. Interfaces*. **2017**, *9*, 19495– 19501. DOI: 10.1021/acsami.6b06099
2. **Pan, U. N.;** Sanpui, P.; Paul, A.; Chattopadhyay, A. Synergistic Anticancer Potential of Artemisinin When Loaded with 8-Hydroxyquinoline-Surface Complexed-Zinc Ferrite Magnetofluorescent Nanoparticles and Albumin Composite. *ACS Appl. Bio Mater.* ***2018,*** *1,* 1229−1235. DOI: 10.1021/acsabm.8b00358
3. **Pan, U. N.;** Sanpui, P.; Paul, A.; Chattopadhyay, A. Surface-Complexed Zinc Ferrite Magnetofluorescent Nanoparticles for Killing Cancer Cells and Single-Particle-Level Cellular Imaging *ACS Appl. Nano Mater.* **2018**. *1*, 2496– 2502. DOI: 10.1021/acsanm.8b00545
4. **Pan, U. N.;** Sanpui, P.; Paul, A.; Chattopadhyay, A. Protein-Nanoparticle Agglomerates as Plasmonic-Magneto-Luminescent Multifunctional Nanocarrier for Imaging and Combination Therapy (*Under Revision ACS Appl. Bio Mater.)*
5. Khandelia, R.; Bhandari, S.; **Pan, U. N.;** Ghosh, S. S.; Chattopadhyay, A. Gold Nanocluster Embedded Albumin Nanoparticles for Two-Photon Imaging of Cancer Cells Accompanying Drug Delivery *Small* **2015,** *11,* 4075– 4081. DOI: 10.1002/smll.201500216

6. Bhandari, S.; Khandelia, R.; **Pan, U. N.;** Chattopadhyay, A. Surface Complexation-Based Biocompatible Magnetofluorescent Nanoprobe for Targeted Cellular Imaging *ACS Appl. Mater. Interfaces* **2015,** *7*, 17552– 17557. DOI: 10.1021/acsami.5b04022
7. Pramanik, S.; Bhandari, S.; Roy, S.; **Pan, U. N.;** Chattopadhyay, A. A White Light-Emitting Quantum Dot Complex for Single Particle Level Interaction with Dopamine Leading to Changes in Color and Blinking Profile *Small* **2018**,*14*, 1800323. DOI: 10.1002/smll.201800323
8. Basu, S.; Bhandari, S.; **Pan, U. N.;** Paul, A.; Chattopadhyay, A. Crystalline Nanoscale Assembly of Gold Clusters for Reversible Storage and Sensing of CO_2 via Modulation of Photoluminescence Intermittency *J. Mater. Chem. C* **2018**, *6*, 8205-8211. DOI:10.1039/C8TC02225A
9. Ahmad, K.; Pal, A.; **Pan, U. N.;** Chattopadhyay, A.; Paul, A. Synthesis of Single-Particle Level White-Light Emitting Carbon Dots via a One-Step Microwave Method *J. Mater. Chem. C*, **2018,** *6,* 6691-6697. DOI: 10.1039/c8tc01276h
10. Gayen, C.; Basu, S.; **Pan, U. N.;** Paul, A. Few Particle-Level Chromaticity Index-Based Discrimination of Biothiols Using Chemically Interactive Dual-Emitting Nanoprobe *ACS Omega* **2018**, *3*, 17220−17226. DOI: 10.1021/acsomega.8b02373

Conferences Attended

1. Presented a poster in DAE-BRNS 6th Interdisciplinary Symposium on Materials Chemistry, **ISMC-2016** held at Bhabha Atomic Research Centre, (BARC) Mumbai, India.
2. Presented an oral In **ChemConvene-2017** held at Indian Institute of Technology Guwahati, India.
3. Presented a poster in 5th International Conference on Advanced Nanomaterials and Nanotechnology, **ICANN- 2017** held at Indian Institute of Technology Guwahati, India.