UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Biofunctionalization of upconversion nanoparticles for intracellular labeling and imaging

by Tesfaye Eshete Asrat

Thesis submitted in fulfilment of the requirements for the degree of

PhD thesis: Science

under the supervision of

Distinguished Professor Dayong Jin

A/Professor Jiajia Zhou; Dr. Qiang Fu; Dr. Peter Su; and Dr. Jiayan Liao

University of Technology Sydney

Faculty of Science

25 September 2022

Certificate of original authorship

I, Tesfaye Eshete Asrat, declare that this thesis is submitted in fulfillment of the requirements for the award of Ph.D. in Science in the school of mathematical and physical sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This research is supported by the Australian Government Research Training Program.

Signature:

Production Note:
Signature removed prior to publication.

25 September 2022

Acknowledgment

I am pleased to use this opportunity to thank my supervisors, family, and friends for their unlimited help during the study period. I thank distinguished Professor Dayong Jin, for the Ph.D. opportunity and his inspiration, advice, and support throughout the study period. The end-user and industry-oriented research projects at IBMD are great learning opportunities, and I gained vast experience in how scientific research could be used to address real-life challenges. Besides the comprehensive academic and research experience I acquired during my stay, I have gained many personal and professional development lessons from him.

I want to express my deep gratitude to A/Professor Olga Shimoni, my former principal supervisor, for her support and advice at the start of the Ph.D. project. Her guidance and support helped me a lot, especially in my first year of study. I am very thankful to all my co-supervisors A/Professor Jiajia Zhou, Dr. Qiang Fu, Dr. Peter Su, and Dr. Jiayan Liao. I am grateful for A/Professor Jiajia Zhou's continued advice and support during the experimental works and thesis writing. I am thankful for Dr. Qiang Fu's advice on polymer synthesis and characterization. I greatly appreciate Dr. Peter Su's valuable advice and guidance in cell labeling experiments and microscopy techniques. I thank Dr. Jiayan Liao because she provided me with extensive advice during experimental works and the thesis write-up. I thank all members of IBMD for their help and support during my study period. I am grateful to the chemical technology, MAU, and biological science laboratory technical officers, for the lab inductions and training and their immediate helps whenever I need their support.

To my father, Eshete Asrat, and my mother, Menbere Gebryie, for their sacrifices to raise and educate my siblings and me in challenging situations. All that I have achieved so far is due to their unlimited help. Tizta B. Woldekidan, who is not only my life partner but also secures my family, helped me a lot in managing the home and our children. I thank my daughter Emnet and my sons Natan and Dagim for their patience in staying alone in my home country during the study, including the COVID-19 lockdown period. To all my siblings, especially Zelalem E., Bahru M., Zewge A., and Dejene B., for helping my parents and family; and to my friends A/Professor Yedilfana S., Dr. Amanuel B., Dr. Tessema F., and Dr. Wassie T. for sharing their experiences, and Mr. Anthony Sarabi. for proofreading. I thank the Ethiopian community at Sydney Debre Tsion St Mary church for their encouragement and support. I am grateful to Addis Ababa Science

and Technology University for the study leave, Alcolizer technology, and the APRIntern for the internship. I thank MaPS and GRS-UTS for the IRS and UTSP's scholarships. In a nutshell, I am very grateful to all involved in my Ph.D. study journey, and lastly, I acknowledge my everyday aspiring UTS's motto, 'THINK.CHANGE.DO'.

Table of Contents

List of Figures

List of tables

List of schematic illustrations

Acronym

UCNPs	Up conversion nanoparticles
UC	Up conversion
DS	Down shifting
OA	Oleic acid
ODE	1-Octadecene
DLS	Dynamic light scattering
RESOLFT	Reversible Saturable optical fluorescence transitions
VIS	Visible
CT	Computed tomography
IVM	Intravital microscopy
NIR	Near infrared
XRD	X-ray Powder Diffraction
FDA	Food and drug administration
SNR	Signal to Noise Ratio
SR	Super-resolution
LED	Light emitting diode
STED	Stimulated emission depletion
STORM	Stochastic optical reconstruction microscopy
SMLM	Single-molecule super-resolution imaging
TIRF	Total internal reflection fluorescence
ATR-FTIR	Total internal reflectance
NIR	Near-infrared radiation (light)
DMEM	Dulbecco's modified eagle medium
MAMs	More activated monomers
LAMs	Less activated monomers
RDRP	Reverse deactivation radical polymerization
RAFT	Reversible addition-fragmentation chain transfer polymerization
MADIX	Macromolecular design via the interchange of xanthates
NMP	Nitroxide-mediated polymerization
DP	Degree of polymerization
DMF	dimethylformamide
PEG	Poly (ethylene glycol)
CRP	Controlled radical polymerization
OEGMA	Oligo (ethylene glycol) methyl methacrylate)
OEGMA-500:	Oligo (ethylene glycol) methyl methacrylate) (MW 500 g/mol)
OEGMA-480	Oligo (ethylene glycol) methyl methacrylate) (MW 480 g/mol)
OEGMA-300	Oligo (ethylene glycol) methyl methacrylate) (MW 300 g/mol)
TGA	Thermogravimetric analysis
MW	Molecular weight
HSAB	Hard-soft acid-base
MOEP	2-(methacryloyloxy)ethyl phosphate

AMPS	2-acrylamide-2-methylpropane sulphonic acid
MAA	Methyl methacrylic acid
CTCPA	4-((((2-carboxyethyl) thio) carbonothioyl) thio)-4-cyanopentanoic acid
CDTPA	Cyano-4-(((dodecylthio)carbonothioyl) thio) pentanoic acid
OESPX	O-Ethyl S-Phthalimidyl methyl xanthate
CPADB	4-Cyano-4-(phenyl carbonothioylthio) pentanoic acid
PAA	Poly (acrylic acid)
ATRP	Atom transfer reversible polymerization
GPC	Gel permeation chromatography
SEC	Size exclusion chromatography
NMR	Nuclear magnetic resonance
DLS:	Dynamic light scattering
CTA	Chain Transfer Agent
MPC	2-(Methacryloyloxy) ethyl Phosphorylcholine
GNP	Grafted nanoparticles
FRET	Fluorescence Resonance Energy Transfer
NP	Nanoparticles
SIM	Structured illumination microscope
CLSM	Confocal laser scanning microscope
MRI	Magnetic resonance imaging
SPECT	Single-photon emission computerized tomography
PDI	Polydispersity index
PET	Positron emission tomography
NP	Nanoparticle (s)
GNP(s)	Grafted Nano Particle (s)
NA	Numerical aperture
NPC	Nuclear pore complex
F-actin	Actin filament
DMEM	Dulbecco's Modified Eagle Medium
PBS	Phosphate Buffered Saline
MTT	3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
PFA	Paraformaldehyde
TAT	Trans-activator of transcription
HIV	Human immunodeficiency virus
FITC	Fluorescein isothiocyanate
CPPs	Cell penetrating peptide
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
NHS	N-hydroxysuccinimide
LFA	Lateral flow immunoassay
NA	Avodadro number
TEM:	Transmission electron microscopy

Abstract

Lanthanide-doped upconversion nanoparticles (UCNPs) are emerging as the next-generation agent for intracellular fluorescent labeling and imaging. To label the subcellular structures using UCNPs, vast opportunities and immense potential lay in their surface functionalization and subsequent bioconjugations. The surface stability and reactivity of UCNPs determine their specific interactions with target molecules, and it enables control of the degree of non-specific bindings to the surroundings. The targetability of UCNPs could be optimized by molecule-specific moiety via conjugating to the grafted polymers on the surface of nanoparticles. As the surface of UCNPs is highly positively charged, due to the exposed lanthanide ions at the lattice termination sites, the nanocrystal surfaces allow the tethering of polymers with negative charges. Therefore, the design and tethering polymers is the key factor in producing functional inorganic nanoparticles with a desirable surface property.

Throughout the Ph.D. study, a new understanding of the roles of polymers in functionalizing UCNPs has been achieved by systematic investigations of multiple RAFT copolymers. RAFT copolymers play a crucial role in controlling surface features and reactivities of UCNPs by manipulating physicochemical properties. The UCNP's surface coupling efficiency could be enhanced using highly reactive triblock RAFT copolymers containing methacrylic acid (MAA). Through increasing surface carboxylic acids density and by enabling an extended surface reactive site, advances in reactivity and dispersibility of UCNPs could be achieved. The surface graft copolymer composition determines the amphiphilicity, dispersibility, and stability of UCNPs. The concept of double copolymer surface grafting using stepwise co-grafting has been implemented to attain high control of surface composition. Efficient immobilization of antibodies and peptides to UCNPs enables the targeting and imaging of single biomolecules and intracellular structures. The performed intracellular labeling and imaging experiments prove that the functionalized UCNPs are suitable for detailed intracellular labeling and investigations. This thesis, therefore, contributes to developing the next-generation super-resolution probes for single-molecule tracking and live cell imaging applications. Moreover, besides visualization of structural features and dynamics of molecular-level phenomena, the functionalized nanoparticles could be implemented as a nano-theranostic tool for personalized nanomedicine.

Key words: Nanomedicine, Nano(bio)technology, RAFT polymers, Nanoprobes, Optical imaging