

Mapping the Intracellular Temperature Dynamics with Organelle-Targeted Upconversion Nanoparticles

by Xiangjun Di

Thesis submitted in fulfilment of the requirements for
the degree of

Doctor of Philosophy

under the supervision of Prof. Dayong Jin, A/Prof. Jiajia Zhou,
Dr. Qian Peter Su,

University of Technology Sydney
Faculty of Science

10/11/2022

Certificate of Original Authorship

Certificate of Original Authorship

I, Xiangjun Di declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences, Faculty of Science, at the University of Technology Sydney.

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

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program and the China Scholarship Council Scholarship.

Production Note:

Signature: Signature removed prior to publication.

Date: 10/11/2022

Acknowledgements

Acknowledgements

I would like to express my deep and sincere gratitude to my principal supervisor, Prof. Dayong Jin, for supporting me in research training and academic writing. He taught me how to solve problems logically.

I would like to thank my co-supervisor Dr. Qian Su for his patience and guidance. He provided technical support in the microscope and data analysis. I would like to thank my co-supervisor A/Prof. Jiajia Zhou for the help with the materials. They have been the source of inspiration and a safe port to reach for help.

I would like to address special thanks to the help from Dr. Hao He and Dr. Chaohao Chen in the surface functionalization of nanoparticles, Dr. Jiayan Liao, Dr. Sheng Mei and Dr. Yunfei Shang in nanoparticles synthesis, Mr. Dejiang Wang in cell experiments, Mr. Milad Nonahal, Mr. Javad Safaei, Miss Ziqing Du and Mr. Lei Ding in nanoparticles characterization, Ms. Mahnaz Mddahfar, Ms. Lin Zhang and Prof. Martina Stenzel for providing the polymers.

I would like to thank all the members of the IBMD (past and present) who has provided technical support and suggestions. I enjoy working with these lovely people.

Last but never least, I would like to thank my supportive family. To my parents for being immensely supportive in my choices. To my husband, Mr. Dejiang Wang, for encouraging me all the time.

Finally, I would like to acknowledge the UTS and the China Scholarship Council (CSC) for providing the scholarship and research opportunities.

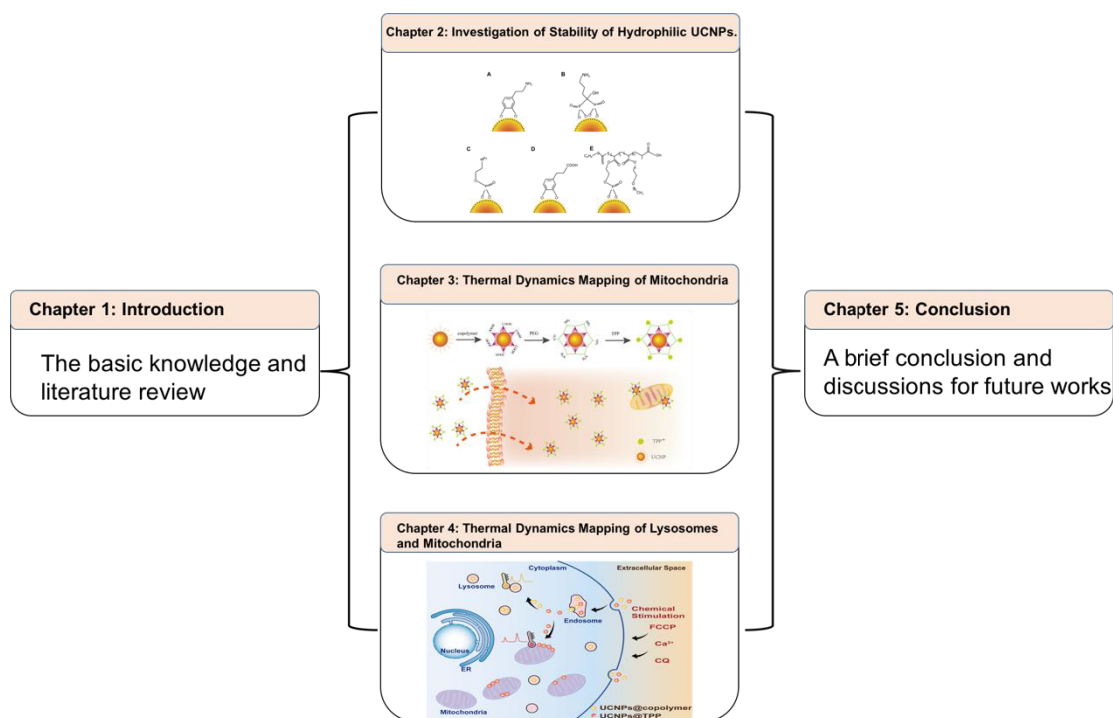
Format of Thesis

This thesis consists of five chapters.

Chapter 1 is the introduction, including the basic knowledge of mitochondria, intracellular luminescence thermometry, and surface functionalization of upconversion nanoparticles ($\text{NaYF}_4: 20\%\text{Yb}^{3+}, 2\%\text{Er}^{3+}$).

Chapter 2 is the result of surface modification to determine the stability and dispersity of UCNPs ($\text{NaYF}_4: 20\%\text{Yb}^{3+}, 2\%\text{Er}^{3+}$) capped with different ligands in the water. Chapters 3 and 4 focus on the intracellular temperature dynamics studied by organelle-targeted UCNPs ($\text{NaYF}_4: 20\%\text{Yb}^{3+}, 2\%\text{Er}^{3+}$).

Chapter 5 summarizes the key results of this thesis and further discusses the perspective of UCNPs-based thermometry in intracellular temperature mapping. This thesis flowchart is illustrated below.



List of Publications

Articles

- **Xiangjun Di**, Dejiang Wang, Jiajia Zhou, Yongtao Liu, Qian Peter Su*, Dayong Jin*, Cascade Targeting Upconversion Nanoparticles to map Spatiotemporal Thermodynamics of Mitochondria and Lysosomes. (*In preparation*).
- **Xiangjun Di**, Dejiang Wang, Jiajia Zhou, Lin Zhang, Martina Stenzel, Qian Peter Su*, Dayong Jin*, Quantitatively Monitoring in situ Mitochondrial Thermal Dynamics by Upconversion Nanoparticles. *Nano Letters*. 2021, 21, 4, 1651–1658.
- Baolei Liu, Chaohao Chen, **Xiangjun Di**, Jiayan Liao, Shihui Wen, Qian Peter Su, Xuchen Shan, Zai-Quan Xu, Lining Arnold Ju, Chao Mi, Fan Wang*, and Dayong Jin*, Upconversion Nonlinear Structured Illumination Microscopy, *Nano Letters* 20(7):4775-4781.
- Xuchen Shan, Fan Wang*, Dejiang Wang, Shihui Wen, Chaohao Chen, **Xiangjun Di**, Peng Nie, Jiayan Liao, Yongtao Liu, Lei Ding, Peter J. Reece* & Dayong Jin*, Optical tweezers beyond refractive index mismatch using highly doped upconversion nanoparticles, *Nature Nanotechnology* 16(5):1-7.
- Yuan Liu, Gungun Lin*, Guochen Bao, Ming Guan, Liu Yang, Yongtao Liu, Dejiang Wang, Xun Zhang, Jiayan Liao, Guocheng Fang, **Xiangjun Di**, Guan Huang, Jiajia Zhou, Yuen Yee Cheng, and Dayong Jin*, Stratified Disk Microrobots with Dynamic Maneuverability and Proton-Activatable Luminescence for in Vivo Imaging, *Acs Nano*, 2021, 15, 12, 19924–19937.

Book Chapter

- **Xiangjun Di**†, Jinshan Qin†, Yujie Sun*, Qian Peter Su*, Visualize the Distribution and Dynamics of Mitochondrial DNA (mtDNA) nucleoids with Multiple Labelling Strategies. (*Book Chapter, in press*).
- Wanqing Du, **Xiangjun Di**, Qian Peter Su*, Studying Mitochondrial Network Formation by In Vivo and In Vitro Reconstitution Assay, *Methods in molecular biology (Clifton, N.J.)* 2276:333-341.

([1,2,6] are closely related to my PhD program)

Statement of Contribution of Authors

Statement of Contribution of Authors

Xiangjun Di, Dejiang Wang, Jiajia Zhou, Lin Zhang, Martina Stenzel, Qian Peter Su*, Dayong Jin*, Quantitatively Monitoring in situ Mitochondrial Thermal Dynamics by Upconversion Nanoparticles. **Nano Letters**. 2021, 21, 4, 1651–1658.

	X.D	D.W	J.Z	L.Z	M.S	Q.S	D.J
Experiment Design	■	■	■			■	■
Sample Preparation	■	■		■	■		
Data Collection	■						
Analysis	■	■	■			■	■
Manuscript	■	■	■			■	■

The concept of this paper was developed by my supervisor Prof. Dayong Jin and Dr. Qian Peter Su. I designed the experiments and conducted the experimental work, including synthesis and modification of UCNPs (NaYF_4 : 20% Yb^{3+} , 2% Er^{3+}), characterization of UCNPs (NaYF_4 : 20% Yb^{3+} , 2% Er^{3+}), cell labelling, data collection, data analysis, and manuscript writing.

Table of Symbols

Table of Symbols

S_R	relative temperature sensing sensitivity
δT	temperature uncertainty
C	constant
ΔE	energy gap
k	Boltzmann constant
T	Absolute temperature
κ	Thermal conductivity
L	size of a heat source
P	Power
ΔD	the shift in the transition frequency
α	temperature susceptibility

Abbreviations

Abbreviations

Abbreviations

ACR	accumulation rate
AEP	2-aminoethyl dihydrogen phosphate
AIE	aggregation-induced emission
ATP	adenosine 5'-triphosphate
ATPase	adenosine 5'-triphosphatase
BAK	Bcl-2 homologous antagonist/killer
BAX	Bcl-2-associated X protein
BrdU	bromodeoxyuridine
BSA	bovine serum albumin
C2C12 cells	mouse myoblast cell
Ca ²⁺	calcium ion
CCCP	carbonyl cyanide 3-chlorophenylhydrazone
CDs	carbon dots
cGAS	DNA sensor cyclic GMP-AMP synthase
CL	cardiolipin
copolymer	PEGMEMA ₈₀ - <i>b</i> -EGMP ₃ di-block copolymer
CQ	chloroquine
CTAB	cationic cetyltrimethylammonium bromide
cyt c	cytochrome complex
3,4-DHCA	3,4-Dihydroxyhydrocinnamic acid
DLS	dynamic light scattering
DMEM	Dulbecco's Modified Eagle Medium
DMF	N, N-Dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid

Abbreviations

Drp1	dynamamin-related protein 1
EDC	1-Ethyl-3-(3-dimethylamino-propyl)-carbodiimide
EdU	5-ethynyl-2'-deoxyuridine
EMCCD	Electron-multiplying CCD camera
ER	endoplasmic reticulum
Er	erbium
ErCl ₃ •6H ₂ O	erbium chloride hexahydrate
F	floride
FBS	fetal bovine serum
FCCP	carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone
FNDs	fluorescent nanodiamonds
FPs	fluorescent proteins
G1 phase	Gap 1 phase during the cell cycle
G2 phase	Gap 2 phase during the cell cycle
Gd	Gadolinium
GQDs	graphene quantum dots
GFP	green fluorescent proteins
GTPase	guanosine triphosphatase
H strand	heavy strand in mtDNA
HCl	Hydrochloric acid
HeLa	cervical cancer cells
HEPES	N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)
Ho	holmium
INF2	inverted-formin 2
IO ₄ ⁻	periodate

Abbreviations

L strand	light strand in mtDNA
LD	lipid droplets
Ln	Lanthanide
Lu	lutetium
M phase	mitosis phase during the cell cycle
MCF-7	Michigan Cancer Foundation-7
MES	2-(<i>N</i> -morpholino)ethanesulfonic acid
MFF	tail-anchored proteins mitochondrial fission factor
Mfn1	mitofusin 1
Mfn2	mitofusin 2
MiDs	mitochondrial dynamics proteins
MnO ₂	Manganese(IV) oxide
MnO ₄ ⁻	permanganate
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
mtDNA	mitochondrial DNA
mtSSB	mitochondrial single-stranded DNA-binding protein
n	refractive index
Na	sodium
NADH	nicotinamide adenine dinucleotide (reduced)
Nd	neodymium
N-GQDs	nitrogen-doped graphene quantum dots
NHS	N-Hydroxysuccinimide
NIR	near-infrared
NOBF ₄	nitrosonium tetrafluoroborate

Abbreviations

NV ⁻	negatively charged nitrogen-vacancy
OA	oleic acid
ODMR	optically detected magnetic resonance
O _H and O _L	origin sites for replication in each strand of mtDNA
Opa1	optic atrophy 1
PAA	Poly (acrylic acid)
PBS	phosphate-buffered saline
PDA	poly(dopamine)
PEG	4Arm-PEG-NH ₂
PFA	Paraformaldehyde
PLL	poly-l-lysine
PMA	phorbol 12-myristate-13-acetate
PMHC18	poly (maleic anhydride-alt-1-octadecene)
PMT	photomultiplier tube
POLRMT	mitochondrial RNA polymerase
POL γ	DNA polymerase γ
POL γ A	DNA polymerase γ catalytic subunit A
POL γ B	DNA polymerase γ catalytic subunit B
PS	penicillin-streptomycin
QDs	quantum dots
RAFT	reversible addition fragmentation chain transfer
RGQDs	reduced graphene quantum dots
RMSD	root-mean-square deviation
RNA	ribonucleic acid
ROS	reactive oxygen species

Abbreviations

rRNAs	Ribosomal RNA
RSS	Residual Sum of Squares
S phase	DNA synthesis during the cell cycle
SA	streptavidin
sacro/ER	sarcoplasmic reticulum
SDS	anionic sodium dodecyl sulfate
SEM	scanning electron microscopy
siRNA	Small interfering RNA
STED	stimulated emission depletion
STING	stimulator of interferon genes
TEM	transmission electron microscopy
TEOS	tetraethyl orthosilicates
TFAM	mitochondrial transcription factor A
TFEB	transcription factor EB
THF	tetrahydrofuran
TIRF	total internal reflection fluorescence
TPP	triphenylphosphonium/(3-carboxypropyl)triphenylphosphonium bromide
tRNAs	Transfer RNA
TWINKLE	DNA helicase
UCNPs	upconversion nanoparticles
UCNPs@AEP	UCNPs coated with AEP
UCNPs@Alendronate	UCNPs coated with alendronate
UCNPs@copolymer (LysoDots)	UCNPs coated with PEGMEMA ₈₀ - <i>b</i> -EGMP ₃ di-block copolymer

Abbreviations

UCNPs@3,4-DHCA	UCNPs coated with 3,4-DHCA
UCNPs@SA	UCNPs@copolymer conjugated with SA
UCNPs@SiO ₂	UCNPs coated with silica shell
UCNPs@dSiO ₂	UCNPs coated with dense silica shell
UCNPs@mSiO ₂	UCNPs coated with mesoporous silica shell
UCNPs@OA	UCNPs coated with OA
UCNPs@PEG	UCNPs@copolymer linked with 4Arm-PEG-NH ₂
UCNPs@PDA	UCNPs coated with polydopamine
UCNPs@PEG@TPP	UCNPs@PEG conjugated with TPP
UCNPs@PLL	UCNPs coated with PLL
UCNPs@PLL@TPP (MitoDots)	UCNPs@PLL conjugated with TPP
UCNPs@SiO ₂	UCNPs coated with silica shell
UV	Ultraviolet
WT-1 cells	mouse brown preadipocyte cell line
Y	yttrium
Yb	ytterbium
YbCl ₃ •6H ₂ O	ytterbium chloride hexahydrate
YCl ₃ •6H ₂ O	yttrium(III) chloride hexahydrate

Table of Contents

Table of Contents

Certificate of Original Authorship	ii
Acknowledgements	iii
Format of Thesis	iv
List of Publications	v
Statement of Contribution of Authors	vi
Table of Symbols	vii
Abbreviations	viii
Table of Contents	xiv
List of Figures	xviii
List of Tables	xxii
Abstract	xxiii
Chapter 1 Introduction	1
1.1 Mitochondrial Form and Function	2
1.1.1 Basic Background of Mitochondria	2
1.1.2 Mitochondrial architecture and function	3
1.1.3 Mitochondrial dynamics	5
1.1.4 Mitochondrial DNA (mtDNA) and dysfunction	13
1.2 Intracellular Luminescence Thermometers	15
1.2.1 Mechanism of intracellular luminescence thermometers	16
1.2.2 Development of intracellular luminescence thermometers	19
1.2.3 Intracellular temperature dynamics	26
1.2.4 Validations of Intracellular Thermometry	29
1.2.5 Physiological Meaning of Intracellular Thermometry	30
1.3 Surface Functionalization of Upconversion Nanoparticles	

Table of Contents

1.3.1 Generating a hydrophilic surface on UCNPs	32
1.3.2 Bioconjugation	36
1.3.3 Approaches to targeting mitochondria	37
1.4 Aims and Outline	41
Chapter 2 Investigation of Colloidal Stability of Hydrophilic Upconversion Nanoparticles	44
2.1 Background	44
2.2 Methodology	46
2.2.1 Materials	46
2.2.2 Synthesis of UCNPs (NaYF ₄ : 20%Yb ³⁺ , 2%Er ³⁺)	47
2.2.3 Coating UCNPs (NaYF ₄ : 20%Yb ³⁺ , 2%Er ³⁺) with PDA	47
2.2.4 Coating UCNPs (NaYF ₄ : 20%Yb ³⁺ , 2%Er ³⁺) with Alendronate	48
2.2.5 Coating UCNPs (NaYF ₄ : 20%Yb ³⁺ , 2%Er ³⁺) with AEP/ 3,4-DHCA/Copolymer	48
2.2.6 Characterization	48
2.2.7 Bioconjugation of streptavidin with UCNPs@copolymer	48
2.2.8 Cell Culture	49
2.2.9 Immunostaining of microtubules with UCNPs	49
2.3 Results and Discussion	49
2.3.1 Synthesis and Characterization of UCNPs@OA	49
2.3.2 Characterization of Hydrophilic UCNPs (NaYF ₄ : 20%Yb ³⁺ , 2%Er ³⁺)	52
2.3.3 Microtubules Labelling	56
2.4 Conclusion and Discussion	59
Chapter 3 Quantitatively Monitoring in situ Mitochondrial Temperature Dynamics by Upconversion Nanoparticles	61

Table of Contents

3.1 Abstract	61
3.2 Background	61
3.3 Materials and Methods	65
3.3.1 Reagents	65
3.3.2 Methods	65
3.4 Results and Discussion	69
3.4.1 Design of UCNPs@PEG@TPP	69
3.4.2 Thermal responsive properties of UCNPs@PEG@TPP <i>in vitro</i>	72
3.4.3 UCNPs@PEG@TPP work as subcellular thermosensors in HeLa cells	75
3.4.4 Visualization of mitochondrial thermal dynamics in HeLa cells	78
3.5 Conclusion	80
Chapter 4 Spatiotemporally Mapping Temperature dynamics of Lysosomes and Mitochondria using Cascade Organelle-targeting Upconversion Nanoparticles	
4.1 Motivation	82
4.2 Abstract	82
4.3 Background	83
4.4 Materials and Methods	86
4.4.1 Materials.	86
4.4.2 Synthesis of hydrophilic upconversion nanoparticles	87
4.4.3 Bioconjugation of upconversion nanoparticles with (3- carboxypropyl)triphenylphosphonium bromide (TPP)	88
4.4.4 Characterization	88
4.4.5 Luminescence Stability of UCNPs@TPP Against Environmental Parameters	89

Table of Contents

4.4.6 Colocalization of UCNPs with MitoTracker or/and LysoTracker.....	89
4.4.7 Time-dependent Localization/Accumulation of LysoDots and MitoDots	90
4.4.8 Relative temperature sensing sensitivity and uncertainty.	90
4.4.9 <i>In situ</i> Calibration Curves and Temperature Sensing of Lysosomes and Mitochondria	90
4.4.10 Statistical Analysis.	91
4.5 Results and Discussion	91
4.5.1 Design, Synthesis and Characterization of Organelle-Targeted Thermometers	91
4.5.2 <i>in vitro</i> Thermo-responsive Properties of LysoDots and MitoDots	96
4.5.3 Specificities of the Cascade Organelle-targeting and Accumulation of LysoDots and MitoDots	99
4.5.4 Mapping the Thermodynamics of Lysosomes and Mitochondria Under Chemical Stimulations	103
4.6 Conclusion	106
Chapter 5 Conclusions and Future works	109
5.1 Conclusions	109
5.2 Future works	111
5.2.1 Materials design and challenges	111
5.2.2 Instrumentation development	112
5.2.3 Biomedical applications	112
References	123

List of Figures

<i>Figure 1- 1 The architecture of mitochondria.</i>	3
<i>Figure 1-2 Overview of mitochondrial dynamics.</i>	6
<i>Figure 1-3 Schematic representation of the cell cycle in the eukaryotic cell.</i>	7
<i>Figure 1-4 Schematic representation of the multi-step processes required for mitochondria division.</i>	8
<i>Figure 1-5 Schematic representation of the multi-step processes required for mitochondria fusion.</i>	9
<i>Figure 1-6 Schematic of mitochondria–organelle contacts and functions.</i>	10
<i>Figure 1-7 Replication of the human mitochondrial genome [83].</i>	14
<i>Figure 1-8 Schematic representation of the typical temperature-sensing strategies.</i> .	16
<i>Figure 1-9 Schematic drawing of intracellular luminescence thermometers.</i>	20
<i>Figure 1-10 Real-time monitoring of mitochondrial-temperature changes in PMA-stimulated MCF-7 cells.</i>	27
<i>Figure 1-11 Real-time monitoring of ER-temperature changes in FCCP-stimulated WT-1 cells.</i>	28
<i>Figure 1-12 Temperature mapping of nucleus and cytoplasm in living COS7 cells.</i> .	29
<i>Figure 1-13 The diagram of common strategies for surface modification of UCNPs (NaYF₄: 20%Yb³⁺, 2%Er³⁺) coated with oleic acid.</i>	32
<i>Figure 1-14 Schematic illustration of the bioconjugation of UCNPs.</i>	36
<i>Figure 1-15 Structure of TPP-based mitochondria targeting molecules.</i>	39
<i>Figure 1-16 Comparison of the mitochondria to cytosol accumulation rate (ACR) for TPP⁺-linked carboxylic acid and amines, calculated equilibrium ACR values as a function of pK_a of acids and bases.</i>	39
<i>Figure 1-17 Examples of Heterocyclic Cations Used as Mitochondria-Targeting Moieties.</i>	40

List of Figures

Figure 2-1 Schematic of UCNP_s (NaYF₄: 20%Yb³⁺, 2%Er³⁺) with five different surface modifications.	44
Figure 2-2 TEM images of UCNP_s (NaYF₄: 20%Yb³⁺, 2%Er³⁺) capped with OA.	50
Figure 2-3 TEM images of as-synthesized UCNP_s (NaYF₄: 20%Yb³⁺, 2%Er³⁺).	51
Figure 2-4 TEM images of five ligands capped UCNP_s.	53
Figure 2-5 Images of UCNP_s@PDA prepared after 1 hour (A) and UCNP_s@PDA prepared after 24 hours (B), where precipitant was observed at the bottom of the tube.	53
Figure 2-6 Images of UCNP_s@PDA incubated with HeLa cells for 6 hours.	54
Figure 2-7 Images of UCNP_s@copolymer (up) and UCNP_s@3,4-DHCA (bottom) incubated with HeLa cells for 6 hours.	56
Figure 2-8 Schematic of microtubules labelling.	58
Figure 2-9 TEM images of UCNP_s@OA (left), UCNP_s@copolymer (middle), and UCNP_s@SA (right).	58
Figure 2- 10 Images of microtubules labelled by UCNP_s@SA.	59
Figure 3-1The conjugation steps and selective accumulation of UCNP_s@PEG@TPP to the mitochondria through incubation.	70
Figure 3-2 Characterization of UCNP_s thermo-sensors.	71
Figure 3-3 Characterization of UCNP_s thermosensors by ATR-FTIR and DLS.	72
Figure 3-4 Thermoresponsive properties of UCNP_s@PEG@TPP in vitro.	73
Figure 3-5 The phototoxicity of 980 nm laser.	74
Figure 3-6 The cytotoxicity of UCNP_s.	75
Figure 3-7 Temperature-dependent fluorescence characteristics of UCNP_s@PEG@TPP targeted to in situ mitochondria in HeLa cells.	76
Figure 3-8 Intracellular co-localization of UCNP_s with LysoTracker.	77

List of Figures

<i>Figure 3-9 Data analysis of peak intensity calculation.</i>	78
<i>Figure 3-10 Visualization of mitochondrial thermal dynamics in HeLa cells response to nutrient and chemical stimulations.</i>	80
<i>Figure 4-1 Schematic diagram of cascade organelle-targeted thermometers platform based on upconversion nanoparticles (UCNPs)</i>	86
<i>Figure 4-2 Scanning confocal system for single UCNP (NaYF₄: 20%Yb³⁺, 2%Er³⁺) emission measurements.</i>	89
<i>Figure 4-3 The long-term bio-stability of UCNPs in aqueous solution.</i>	93
<i>Figure 4-4 Surface modification strategy and characterization of UCNPs (NaYF₄: 20%Yb³⁺, 2%Er³⁺) to produce efficient organelle-targeting thermometers.</i>	94
<i>Figure 4-5 The ATR-FTIR spectra of prepared UCNPs@OA, UCNPs@copolymer, UCNPs@PLL and UCNPs@PLL@TPP.</i>	95
<i>Figure 4-6 Luminescence emission images from individual UCNP coated with OA, copolymer, PLL, and PEG under 980 nm excitation.</i>	95
<i>Figure 4-7 UV-Vis absorption standard curve of TPP (268 nm) at various concentrations.</i>	96
<i>Figure 4-8 Stable and efficient LysoDots and MitoDots.</i>	98
<i>Figure 4-9 Organelle-specific accumulations of LysoDots and MitoDots.</i>	101
<i>Figure 4-10 Cascade-targeting evaluations and time-dependent accumulation of LysoDots and MitoDots in living HeLa cells.</i>	102
<i>Figure 4-11 Distinct lysosomal and mitochondrial temperature dynamics in response to chemical stimulations in living HeLa cells.</i>	105
<i>Figure 5-1 DNA synthesis labelled by EdU.</i>	116
<i>Figure 5-2 mtDNA synthesis labelled by EdU.</i>	116
<i>Figure 5-3 A typical result from a failure experiment suing BrdU to label the mtDNA synthesis.</i>	1

List of Figures

<i>Figure 5-4 DNA synthesis labelled by BrdU with different antibodies.</i>	<i>118</i>
<i>Figure 5-5 A typical successful labelling result of the mtDNA synthesis by BrdU. ..</i>	<i>119</i>
<i>Figure 5-6 mtDNA synthesis labelled by BrdU.</i>	<i>119</i>
<i>Figure 5-7 Dual labelling results of DNA synthesis by EdU and BrdU across the different phases of cell cycle.</i>	<i>120</i>
<i>Figure 5-8 Illustration of human mtDNA replication patterns..</i>	<i>122</i>

List of Tables

List of Tables

Table 2-1 Quantitative analysis of newly synthesized UCNPs@OA.	52
Table 2- 2 The size and Zeta potential of five ligands capped UCNPs.	56
Table 2- 3 The DLS results for UCNPs with different modification.	58
Table 4-1 Mitochondrial temperature variation summary by the intracellular thermometer	107

Abstract

Abstract

Temperature plays a key role in regulating intracellular activities. Accurate measurements of temperature inside living cells at the nanoscale can tell if the cells are under their healthy physiological status or in dysfunctional diseases. As the energy factory and metabolism center, mitochondria constantly release heat during ATP production, which greatly impacts the intracellular organelles' temperature. A direct visualization platform with probes that can sense the *in situ* temperature dynamics of mitochondria, and map the temperature-related interactions among intercellular organelles, will facilitate our understanding of mitochondria-related diseases towards better therapy.

Upconversion nanoparticles (UCNPs), being excited by near-infrared (NIR) light to generate visible light, have been widely applied in the fields of single-molecule bioassays, super-resolution microscopy, and recently non-contact thermometers, due to their unique optical properties, including their exceptional photo-stability against photo-bleaching or photo-blinking, tunable multi-wavelength emissions for multiplexing assays, anti-Stokes' emissions to suppress autofluorescence background, near infrared excitation and emissions allowing deep-tissue penetration depth, and most importantly, temperature-dependent ratiometric luminescence for thermometry application. However, the chemical stability of hydrophilic UCNPs has limited their developments in biomedical applications.

To obtain the hydrophilic UCNPs (NaYF₄: 20%Yb³⁺, 2%Er³⁺) with excellent stability and dispersibility in aqueous physiological buffers, five different functionalization strategies have been systematically evaluated (chapter 2). To study the temperature dynamics of mitochondria, I developed a mitochondria-targeting UCNPs-based thermometer with a sensing sensitivity of 3.2% K⁻¹ to monitor the temperature variations through the chemical stimulations. The cells displayed distinct response time and temperature dynamic profiles (chapter 3).

To further study the interaction between lysosomes and mitochondria, I updated the design of UCNPs-based thermometer by optimizing the surface functionalization of UCNPs, which resulted in an enhanced reliability with the relative temperature sensing sensitivity of 2.7% K⁻¹ and temperature uncertainty of 0.8 K in HeLa cells. The new

Abstract

probes can cascade target lysosomes and mitochondria, respectively (chapter 4).

Chapter 5 concludes this thesis by providing a thermometry platform to study the temperature dynamics of mitochondria and organelles' functional interactions under the physiological or pathological status. Combined with other state-of-the-art technologies, such as sequential labelling of mtDNA, super-resolution imaging, UCNPs-based thermometer will become a powerful multimodal probe for imaging, sensing, and therapy.