Mapping the Intracellular Temperature Dynamics with Organelle-Targeted Upconversion Nanoparticles

by Xiangjun Di

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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

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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.

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Signature: Production Note:
Signature removed prior to publication.

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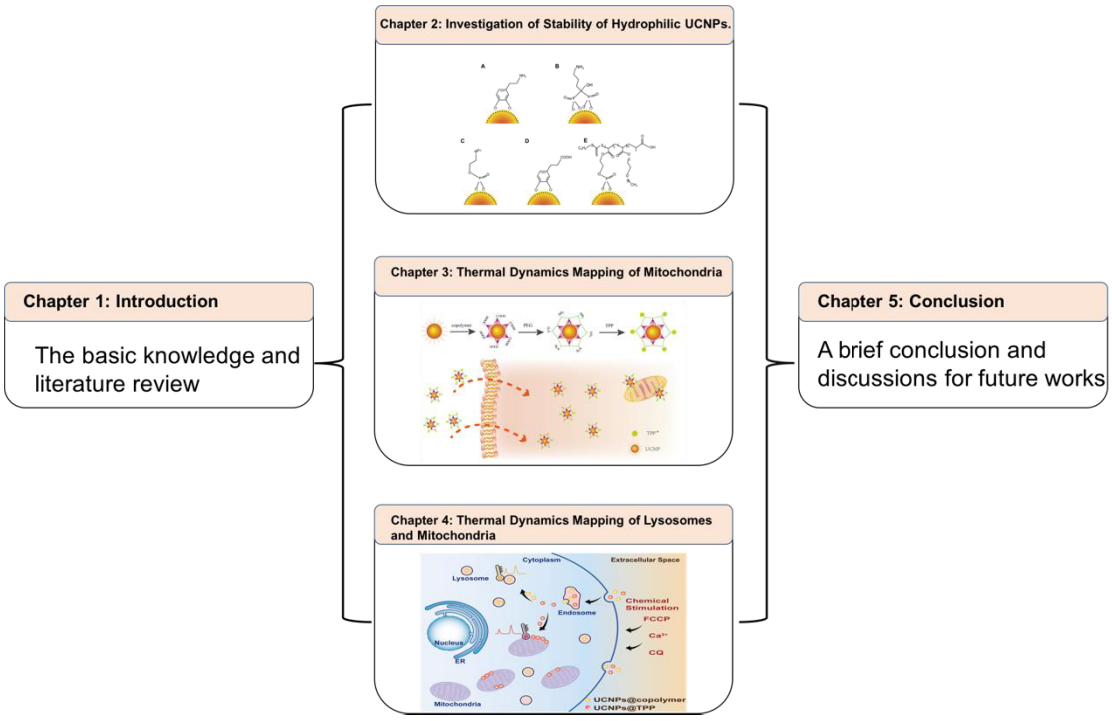
Format of Thesis

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 ($NaYF_4$: 20%Yb^{3+}, 2%Er^{3+}).

Chapter 2 is the result of surface modification to determine the stability and dispersity of UCNPs ($NaYF_4$: 20%Yb^{3+}, 2%Er^{3+}) capped with different ligands in the water. Chapters 3 and 4 focus on the intracellular temperature dynamics studied by organelle-targeted UCNPs ($NaYF_4$: 20%Yb^{3+}, 2%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

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

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

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	$PEGMEMA_{80}$-*b*-$EGMP_3$ 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	dynamin-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
$ErCl_3 \bullet 6H_2O$	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

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-(*N*-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

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
POLγ	DNA polymerase γ
POLγA	DNA polymerase γ catalytic subunit A
POLγB	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

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 $PEGMEMA_{80}$-*b*-$EGMP_3$ di-block copolymer

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
$YbCl_3$•$6H_2O$	ytterbium chloride hexahydrate
YCl_3•$6H_2O$	yttrium(III) chloride hexahydrate

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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 ($NaYF_4$: 20%Yb^{3+}, 2%Er^{3+}) 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

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.