Cancer In-vitro Diagnostics Using Upconversion Nanoprobe

By

Hao He

Institute for Biomedical Materials & Devices,

School of Mathematical and Physical Sciences, Faculty of Science

Supervisors:

Prof. Dayong Jin & Dr. Christopher B. Howard

This thesis is presented for the degree of Doctor of Philosophy

October 2019

Certificate of Original Authorship

I, Hao He, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences, Faculty of Science, 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 literatures used are indicated in the thesis.

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

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

Signature: Production Note:
Signature removed prior to publication.

Date: 03/10/2019

Acknowledgements

Completing my PhD study means a significant milestone in my life. Many people have given me help and encouragement over the past four years. Here I will express my sincere appreciation to them.

Firstly, I would like to thank my principle supervisor, distinguished Prof. Dayong Jin. I appreciate everything he has done to support me in the research training, and academic presentation and writing, especially providing me the opportunity to explore the nanomaterial world. Under his supervision, I learned how to solve problems and overcome challenges in a logical way. I also learnt from him how to communicate and collaborate with other researchers. His attitude to life will continue to inspire me.

I would also thank my co-supervisor: Dr. Christopher B. Howard. He provided me the opportunity to study in the Australian Institute for Bioengineering and Nanotechnology, University of Queensland and provided his professional advice on my project. I appreciate his efforts.

Besides my supervisors, I would like to thank my collaborators for contributing significantly to my PhD projects. Thanks to Dr. Shihui Wen and Ms Jiayan Liao for providing me with upconversion nanoparticles at a high constant quality, Dr. Fan Wang, Dr Zhiguang Zhou and Mr Baolei Liu for building up the optical systems, Dr Martina Jones and Ms Neetika Arora for teaching me the phage display technique, and Dr. Gungun Lin and Dr. Jiajia Zhou for improving the illustrations and writing in my publications.

Thanks to all my friends, A/Prof. Wei Ren, Dr. Ming Guan, Mr. Chao Mi, Ms. Yingzhu Zhou, Ms. Yinghui Chen. They provided my life in Australia with laughs and fun.

To my family, particularly my parents and wife, thanks for your love and support.

Finally, I would like to acknowledge the China Scholarship Council, the Faculty of Science, UTS, and Australian Research Council Integrated Device for End-User Analysis

at Low Levels Research Hub. They supported my tuition fees, my living expenses, and top-up scholarship.

Format of Thesis

This is a thesis by compilation with six chapters.

Chapter 1 is an introduction chapter, including a comprehensive literature review.

Chapters 2-5 are result chapters, including two published first author papers, one published co-author paper and two unpublished works.

Chapter 6 is a conclusion and perspective chapter.

List of Publications

Research papers:

[1] **Hao He**, Christopher B. Howard*, Yinghui Chen, Shihui Wen, Gungun Lin, Jiajia Zhou, Kristofer J. Thurecht and Dayong Jin*. Bispecific Antibody-Functionalized Upconversion Nanoprobe. Analytical Chemistry. 2018, 90 (5): 3024-3029.

[2] **Hao He**, Baolei Liu, Shihui Wen, Jiayan Liao, Gungun Lin, Jiajia Zhou, and Dayong Jin*. Quantitative Lateral Flow Strip Sensor Using Highly Doped Upconversion Nanoparticles. Analytical chemistry. 2018, 90 (21): 12356-12360.

[3] Fan Wang*, Shihui Wen, **Hao He**, Baoming Wang, Zhiguang Zhou, Olga Shimoni, and Dayong Jin*. Microscopic inspection and tracking of single upconversion nanoparticles in living cells. Light: Science & Applications. 2018, 7 (4): 18007.

[4] Yingzhu Zhou, Yinghui Chen, **Hao He**, Jiayan Liao, Hien TT Duong, Maryam Parviz*, and Dayong Jin*. A homogeneous DNA assay by recovering inhibited emission of rare earth ions-doped upconversion nanoparticles. Journal of Rare Earths. 2019, 37 (1): 11-18.

[5] Wei Ren, Yingzhu Zhou, Shihui Wen, **Hao He**, Gungun Lin, Deming Liu, and Dayong Jin*. DNA-mediated anisotropic silica coating of upconversion nanoparticles. Chemical Communications. 2018, 54 (52): 7183-7186.

Papers [1] - [5] are closely related to my PhD program.

Statement of Contribution of Authors

[1] **Hao He**, Christopher B. Howard*, Yinghui Chen, Shihui Wen, Gungun Lin, Jiajia Zhou, Kristofer J. Thurecht and Dayong Jin*. Bispecific Antibody-Functionalized Upconversion Nanoprobe. Analytical Chemistry. 2018, 90 (5): 3024-3029.

	H.H	C.H	Y.C	S.W	G.L	J.Z	K.T	D.J
Experiment Design	●	●						●
Sample Preparation	●	●		●				
Data Collection	●		●					
Analysis	●							●
Manuscript	●	●			●	●	●	●

The concept of this paper was developed by my supervisor Christopher B. Howard, and he prepared all the bispecific antibodies. I designed the experiments and conducted the majority of experimental work, including modification of UCNPs, characterization of upconversion nanoprobe, ELISA-like assay, cell labelling, data analysis, and manuscript writing.

[2] **Hao He**, Baolei Liu, Shihui Wen, Jiayan Liao, Gungun Lin, Jiajia Zhou, and Dayong Jin*. Quantitative Lateral Flow Strip Sensor Using Highly Doped Upconversion Nanoparticles. Analytical chemistry. 2018, 90 (21): 12356-12360.

	H.H	B.L	S.W	J.L	G.L	J.Z	D.J
Experiment Design	●						●
Sample Preparation	●		●	●			
Data Collection	●	●					
Analysis	●						●
Manuscript	●				●	●	●

This project has been primarily carried out by myself, which reflects my independence in conducting research from concept development, experimental design, sample preparations, data collections and analysis, to the final stage of publication preparation.

[3] Fan Wang*, Shihui Wen, **Hao He**, Baoming Wang, Zhiguang Zhou, Olga Shimoni, and Dayong Jin*. Microscopic inspection and tracking of single upconversion nanoparticles in living cells. Light: Science & Applications. 2018, 7 (4): 18007.

	F.W	S.W	H.H	B.W	Z.Z	O.S	D.J
Experiment Design	●	●	●			●	●
Sample Preparation	●	●	●	●			
Data Collection	●	●	●	●	●		
Analysis	●	●	●	●	●		●
Manuscript	●					●	●

This co-authored paper that I designed and did most of the cell experiments has inspired me. I used the system in this paper and developed it into an in-vitro detection platform.

List of Acronyms (in alphabetic order)

ABTS	2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt
AGRF	Australia genome research facility
APTES	(3-aminopropyl) triethoxysilane
AuNPs	Gold nanoparticles
BSA	Bovine serum albumin
BsAb	Bispecific antibody
CDs	Carbon dots
CT	Computed tomography
CTAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribonucleic acid
DSNPs	Downshifting nanoparticles
ECL	Electrochemiluminescence
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
ELISA	Enzyme-linked immunosorbent assay
EphA2	Ephrin type-A receptor 2
ESA	Excited state absorption
ETU	Energy transfer upconversion
FISH	Fluorescent in situ-hybridization
FRET	Förster resonance energy transfer
GOx	Glucose oxidase
GQDs	Graphene quantum dots
HRP	Horseradish peroxide

ITO	Indium tin oxide
IVD	In-vitro diagnostics
LFS	Lateral flow strip
LOD	Limit of detection
LRET	Luminescence resonance energy transfer
LSPR	Localized surface plasmon resonance
MGNP	Magnetic glycol-nanoparticle
mPEG	Methoxy polyethylene glycol
MRI	Magnetic resonance imaging
MRS	Magnetic relaxation switch
NGS	Next Generation Sequencing
NHS	N-hydroxysuccinimide
NIR	Near-infrared
NSE	Neuron specific enolase
OA	Oleic acid
PAA	Polyacrylic acid
PAMAM	Poly(amidoamine)
PBB15	Polybrominated biphenyl
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PET	Positron emission tomography
PMHC18	Poly(maleic anhydride-alt-1-octadecene)
POC	Point of Care

PSA	Prostate specific antigen
PVP	Polyvinylpyrrolidone
QDs	Quantum dots
RENPs	Rare earth doped nanoparticles
RNA	Ribonucleic acid
scFv	Single-chain variable fragment
SDS	Sodium dodecyl sulfate
SERS	Surface enhanced Raman spectroscopy
SMCC	Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate
SPR	Surface plasmon resonance
TBA	Thrombin binding aptamer
TEOS	Tetraethyl orthosilicate
THF	Tetrahydrofuran
TMB	3,3’,5,5’-Tetramethylbenzidine
UCNPs	Upconversion nanoparticles
ULISA	Upconversion-linked immunosorbent assay

Abstract

Developing sensitive, specific and fast assays to detect and quantify cancer biomarkers at low concentration permit early stage diagnosis of cancer and to improve the survival rate. Many kinds of nanomaterials have been applied to break the limitations of conventional bioassays so as to offer high sensitivity, simplicity and at lower cost. The current development of nanoparticle based in-vitro diagnostics tests, such as paper-based testing strips using gold nanoparticles, remain as being indicative rather than being quantitative. To fill the gap between lab based quantitative assays and techniques for point of care testing, smartphones will play an essential role. This takes the advantage of build-in optics and electronics suitable for data acquisition, quantification and communication. But this still requires the biochemistry methods to be more stable, selective and quantitative, particularly suitable for small volume of samples after minimum level of preparations.

This thesis explores a new class of fluorescent probes and detection methods to bridge this gap. The new probes are rare earth doped upconversion nanoparticles (UCNPs) with high fluorescent intensity and negligible background noise. To improve the stability and selectivity of UCNPs, two techniques have been explored. Phage display has been employed to select antibodies that can bind to UCNPs surface forming more stable and biocompatible upconversion nanoprobe; bispecific antibodies are used to avoid chemical reaction in the bioconjugation step and deactivation of antibody bioconjugated to UCNPs so that the upconversion nanoprobes can have higher binding efficiency to the cancer biomarker. To meet the requirements of simple and quantitative point of care testing, highly rare earth doped UCNPs with hundreds to thousands of emitters have been introduced to essentially amplify the signal strength so that smartphone can read and quantify the prostate cancer biomarkers resulted in a paper based strip sensor; single nanoparticle image system has been applied to reach a single molecule level sensitivity for detecting prostate cancer biomarkers in sub-microliter samples.

Key words: in-vitro diagnosis, cancer detection, upconversion nanoparticles, phage display, bispecific antibody, lateral flow strip, single-molecule detection.

Table of Contents