Lanthanide Nanoparticles for Improving the Sensitivity of Mass Cytometry at the Single-Cell Level

By Mahnaz Maddahfar

Thesis submitted in fulfilment of the requirements for
the degree of **Doctor of Philosophy**

Under the supervision of Prof. Dayong Jin, Prof. Barbara Fazekas de St Groth, Prof. Martina Stenzel, Dr. Shihui Wen.

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, *Mahnaz Maddahfar,* declare that this thesis is submitted in fulfilment of the requirements for the award of *Doctor of Philosophy*, in the *School of Mathematics and Physical Science, Faculty of Science* 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 document has not been submitted for qualifications at any other academic institution.

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Completing my PhD in nanobiotechnology is undoubtedly the biggest achievement in my whole life. PhD, Doctor of Philosophy, means the study of the fundamental nature of knowledge, reality, and existence. From my point of view, PhD, as the name implies, includes not only deep learning and deep passion for problem-solving but also how to communicate with a multidisciplinary team (enhancing the spirit of collaboration), how to be patient and resilient during the ups and downs of life, and how to strengthen the spirit of persistence to achieve an ultimate goal. After migrating to Australia with my husband in 2018 and starting my PhD journey, never even one percent did I picture myself obtaining a PhD in nanobiotechnology overseas because of language problem, lack of knowledge, having different background etc. Every single moment I look back, I sincerely realise that I would not have completed my journey without all the people in my life, including my husband, my family, my supervisors, my colleagues, my friends, who have supported me in one way or another. I would like to thank everyone who paved the hard way of my PhD.

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Format of thesis

This is a thesis by a compilation with five chapters.

Chapter 1 includes a comprehensive study of a PhD project called a literature review.

Chapter 2 includes Materials and methods.

Chapters 3 and 4 are experimental, results, and discussion sections, including 1 published paper and 1 unpublished work.

Chapter 5 includes the conclusions, and future perspectives.

Chapter 1

Significance of biomarker research

↓

Pros and cons of current detection techniques

↓

Significance of mass cytometry assay

Challenges

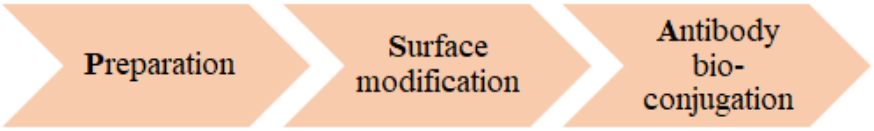
↓

❖ Sensitivity

↓

Lanthanide nanoparticles in enhancing the sensitivity

↓



Chapter 2

Materials and Methods

Chapter 3

Surface chemistry and polymers

Chapter 4

Site-specific labelling of antibody (IgG) by LnNPs

Chapter 5

Conclusions and Future perspectives

List of publications, conferences, and awards

Research papers

[1] **Mahnaz Maddahfar**, Shihui Wen, Seyed Mostafa Hosseinpour Mashkani, Lin Zhang, Olga Shimoni, Martina Stenzel, Jiajia Zhou, Barbara Fazekas de St Groth*, Dayong Jin*. Stable and high-efficient antibody-nanoparticles conjugation. Bioconjugate Chemistry (published). https://doi.org/10.1021/acs.bioconjchem.1c00192

[2] **Mahnaz Maddahfar**, Barbara Fazekas de St Groth*, Seyed Mostafa Hosseinpour Mashkani, Shihui Wen , Helen McGuire, Nima Sayyadi, , Martina Stenzel, Dayong Jin*. Functionalising lanthanide nanoparticles in flow cytometry and mass cytometry application: A comparison of strategies. (in preparation)

[3] Zayakhuu Gerelkhuu, Haribalan Perumalsamy, **Mahnaz Maddahfar**, Dayong Jin, Jaewoo Song, and Tae Hyun Yoon*. A study on peripheral blood mononuclear cell and upconversion nanoparticles using single-cell mass cytometry. (Submitted to Environmental Science: Nano).

[4] Yinghui Chen, Olga Shimoni, Guan Huang, Shihui Wen, Jiayan Liao, Hien Duong, **Mahnaz Maddahfar,** Qian Su, David Ortega, Yanling Lu, Douglas Campbell, Bradley Walsh, Dayong Jin*. Upconversion nanoparticle-assisted single-molecule assay for detecting circulating antigens of aggressive prostate cancer https://doi.org/10.1002/cyto.a.24504

[5] Guan Huang, Ying Zhu, Shihui Wen, Haoqi Mei, Yongtao Liu, Dejiang Wang, **Mahnaz Maddahfar**, Qian Peter Su, Gungun Lin*, Yinghui Chen*, Dayong Jin*. Single small extracellular vesicle (sEV) quantification by upconversion nanoparticles. https://doi.org/10.1021/acs.nanolett.2c00724

[6] Xiangjun Di, Qian Peter Su*, Dejiang Wang, Yongtao Liu, **Mahnaz Maddahfar**, Jiajia Zhou, Dayong Jin. Spatiotemporally mapping temperature dynamics of lysosomes and mitochondria using cascade organelle-targeting upconversion nanoparticles. https://doi.org/10.1073/pnas.2207402119

[7] Lei Ding, Xuchen Shan, Dejiang Wang, Baolei Liu*, Ziqing Du, Xiangjun Di, Chaohao Chen*, **Mahnaz Maddahfar**, Ling Zhang, Yuzhi Shi, Peter Reece, Benjamin Halkon, Igor Aharonovich, Xiaoxue Xu*, Fan Wang*. Lanthanide Ion Resonance-Driven Rayleigh Scattering of Nanoparticles for Dual-Modality Interferometric Scattering Microscopy. https://doi.org/10.1002/advs.202203354

Conferences:

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[3] **Mahnaz Maddahfar***. Lanthanide Nanoparticles for improving the sensitivity of Mass Cytometry at the Single-cell level. Biomedical Shark Tank, Institute for Biomedical Materials and Devices (IBMD- Oral presentation). 17 February 2020 at Sydney, Australia.

[4] **Mahnaz Maddahfar*** Dayong Jin, Barbara Fazekas de St Groth. Nanotechnology in mass cytometry assays. Australian Cytometry Society (ACS- Oral presentation). 7th-10th November 2021 at Sydney, Australia.

[5] **Mahnaz Maddahfar*** Dayong Jin, Barbara Fazekas de St Groth. Lanthanide Nanoparticles for improving the sensitivity of mass cytometry at the single-cell level. 4th World Congress on Materials Science and Engineering (WCMSE- Oral presentation). November 16th -17th, 2021 at Miami, USA

[6] **Mahnaz Maddahfar*** Dayong Jin, Barbara Fazekas de St Groth. Lanthanide nanoparticles in Mass cytometry. 3rd International Conference on Advanced Materials Science and Nanotechnology. (Oral presentation). August 18th-19th, 2022, Singapore.

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Statement of Contribution of Authors

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	M.M	S.W	S.M.H.M	L.Z	O.S	M.S	J.Z	B.F	D.J
Experimental Design	X		X					X	X
Sample Preparation	X	X	X					X	
Data Collection	X			X				X	
Analysis Data	X	X		X	X	X		X	
Writing Manuscript	X		X						
Drawing Graphs	X			X			X	X	
Review &Editing	X	X	X	X		X	X	X	X

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	M.M	B.F	S.M.H.M	S.W	H.M	N.S	M.S	D.J
Experimental Design	X	X				X		
Sample Preparation	X	X	X	X	X			
Data Collection	X	X						
Analysis Data	X	X						
Writing Manuscript	X	X						
Drawing Graphs	X	X	X					
Review &Editing	X	X	X	X	X		X	X

List of Acronyms (in alphabetic order)

ADH	Adipic acid dihydrazide
AIBN	2,20-azobisisobutyronitrile
AuNPs	Gold nanoparticles
BTPA	2-(n-butyltrithiocarbonate)-propionic acid
CT	Computed tomography
CIT	Citrate
CYTOF	Cytometry time of flight
DDA	1,10-decanedicarbocylic
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
DNP	Diameter of nanoparticles
DMSA	3-dimercaptosuccinic acid
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTPA	Diethylenetriaminepentaacetic acid
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
ELISA	Enzyme-linked immunosorbent assay
FA	Folic acid
FT-IR	Fourier-transform infrared spectroscopy
FSC	Forward scattering channel
GPC	Gel permeation chromatography
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
HRTEM	High-resolution transmission electron microscope
HFS	HEPES fetal serum

ICP-MS	Inductively coupled plasma-mass spectrometry
LnNPs	Lanthanide nanoparticles
mAbs	Monocolonal antibody
MAEP	Monoacryloxyethyl phosphate
MCPs	Metal-chelating polymers
MC	Mass cytometry
MES	2-(N-morpholino)ethanesulfonic acid
MF	Melamine formaldehyde
MPA	Mercaptopropionic acid
MFI	Mean fluorescent intensity
MRI	Magnetic resonance imaging
MSA	Mercaptosuccinic acid
MWCO	Molecular weight cut-off
MUA	Mercaptoundecanoic
NHS	N-hydroxysuccinimide
NIH	National institutes of health
NIR	Near infrared
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
OA	Oleic acid
ODE	1-octadecene
OEGMEA	Oligo (ethylene glycol) methylether acrylate
OM	Oleyl amine
PAA	Poly (acrylic acid)

PAH	Poly (allylamine hydrochloride)
PAMAM	Poly (amido amine)
PBMC	Peripheral blood mononuclear cell
PDI	Poly dispersity index
PE	Phycierythrin
PEG	Polyethylene glycol
PFA	Paraformaldehyde
PSA	Prostate specific antigen
PVP	Poly(vinylpyrrolidone)
PG	Bis-phosphono glycine
QDs	Quantum dots
RAFT	Reversible addition-fragmental chain-transfer polymerization
RIA	Radioimmunoassay
RNA	Ribonucleic acid
ScFv	Single-chain variable fragment
SEC	Size exclusion chromatography
SERS	Surface-enhanced raman scattering
SSC	Side scattering channel
SNP	Specific surface area
Sulfo-NHS	N-hydroxysulfosuccinimide
TCEP	Tris(2-carboxyethyl)phosphine
THF	Tetrahydrofuran
TEOS	Tetraethyl silicate
TEM	Transmission electron microscopy

TGA	Thermal gravimetric analysis
UCNPs	Up-conversion nanoparticles
UV	Ultraviolent
XEDS	X-ray energy dispersive spectroscopy

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Abstract

Early detection of cancer increases the possibility of successful treatment which often requires the multiplexed detection of a panel of biomarkers of molecules and single cells. Mass cytometry (CyTOF), combining the powers of flow cytometry and mass spectrometry provides simultaneous measurement of over 40 cellular parameters at single-cell resolution, significantly augmenting the ability of cytometry to evaluate complex cellular systems and processes. This technology is based on isotopically-labelled antibodies as tags and mass spectrometry time-of-flight to distinguish the individual isotope labels on single cells. However, metal chelating polymers, currently used in CyTOF, have been found insufficient in detecting low abundance biomarkers, as the number of metal atoms per tag is too low to detect biomarker expression at levels of 10^2 to 10^4 per cell.

This thesis aims to address the issue of the low sensitivity of mass cytometry by developing lanthanide nanoparticles as cellular barcoding mass-tags, as individual nanoparticles can be doped with a considerable number of elemental atoms, typically in the range of 10^4-10^6 lanthanide ions per nanoparticle. As the key to producing bio-specific nanoparticles lies in the surface functionalisation of LnNPs and their subsequent conjugation to antibodies, the first focus of this thesis is on the design and synthesis of a well-defined diblock copolymer with tuneable size composed of monoacryloxyethyl phosphate block and oligo(ethylene glycol) methyl ether acrylate block through the RAFT polymerisation technique. Systematic insight into the effect of the chain length of POEGMEA on the long-term colloidal stability and antibody-conjugation efficiency of nanoparticles has been provided.

Next, I explored two novel bioconjugation strategies to couple anti-B220 antibody to LnNPs: a) Carbodiimide chemistry in which carboxylate groups of polymer capped LnNPs target lysine sidechains of the antibody, b) Schiff-base interaction in which hydrazide functionalised LnNPs target aldehyde groups in the Fc region of oxidised IgG antibody. Both conjugation strategies were applied to assess the sensitivity and specificity of the LnNP-coupled antibody as a ligand-specific probe for mass cytometry assays. Random orientation of antibodies on the surface of polymer-LnNP and failure to exclude free LnNPs from the coupled ones caused the carbodiimide strategy to generate significant background in CyTOF, making it difficult to distinguish signal. However, the

combination of Schiff-based chemistry to orient coupling of IgG antibodies to LnNPs and the use of a blocking reagent to allow separation of free versus conjugated nanoparticles increased conjugation efficacy and significantly improved signal to noise ratio in mass cytometry assays.