

DOCTORAL THESIS

Analysis of biomolecules by total internal reflection fluorescence microscopy

Chan, Ho Man

Date of Award:
2011

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**Analysis of Biomolecules by
Total Internal Reflection Fluorescence Microscopy**

CHAN Ho Man

A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Dr. LI Hung Wing

Hong Kong Baptist University

August 2011

ABSTRACT

Total internal reflection fluorescence microscopy (TIRFM) has been widely applied in the study of biological samples. Unlike the costly, sample-intensive and pretreatment-required conventional bioanalytical assays, TIRFM offers a simple but ultrasensitive detection platform for the study of biomolecules in single-molecule level. This thesis presented the principle and application of TIRFM systems for the analysis of single biomolecules. Four research works on the detection, quantification, observation and manipulation of biomolecules with the aid of TIRFM were described herein respectively.

In Chapter 2, a pretreatment-free miRNA detection assay in single-molecule level with TIRFM was presented. MicroRNAs express differently in normal and cancerous tissues and thus are regarded as potent cancer biomarkers for early diagnosis. However, the short length and low abundance of miRNAs have brought challenges to the established detection assay in terms of sensitivity and selectivity. The established TIRFM-based assay was applied in the quantification of miRNAs among cancerous and normal cell lines respectively. The results agreed very well with those from the prevalent real-time polymerase chain reaction analysis, showing that the assay is of high potential for applications in miRNA expression profiling and early cancer diagnosis.

In Chapter 3, the first application of Group 9 metal complexes (*i.e.* iridium (III) and rhodium (III)) as inhibitors of amyloid fibrillogenesis and as luminescent probes for beta-amyloid (1–40) ($A\beta_{1-40}$) peptide was reported. These complexes contained aromatic co-ligands to interact with the hydrophobic residues around the *N*-terminal domain of the $A\beta_{1-40}$ peptide, as well as solvato co-ligands to allow coordinative bond formation with histidine residues. TIRFM was applied to monitor and estimate the growth of $A\beta$ fibrils in the presence of metal complexes. We demonstrated that these complexes could inhibit $A\beta_{1-40}$ peptide aggregation *in vitro*, with potency superior to previous metal-based inhibitors reported. Furthermore, we have demonstrated the first example of luminescent detection of $A\beta_{1-40}$ peptides by transition metal complexes.

In Chapter 4, the effect of nanoparticles (NPs) of various sizes and surface functionalities on the self-assembling fibrillation of $A\beta_{1-40}$ was investigated with TIRFM. Dramatically, slight change in sizes of functionalized quantum dots (QD) showed obvious contrary effect on beta-amyloid fibrillation even though the concentration of QD was 1 / 5000 of the peptide. We proposed that the dramatic effect was induced by the critical alteration of the charge-to-surface area ratio of the NPs and the interaction between QDs and $A\beta$ of approximate sizes. On the other hand, gold nanoparticles (AuNPs) with different functionality and surface charges further extended the current study. There was no significant variation in the fibrillation rate and fibril lengths for $A\beta$ growing both in the presence and absence AuNP and hence we attributed that significant increase in colloidal surface area of AuNP abated the charge effect and thus reduces the interactions between $A\beta$ and nanoparticles.

In Chapter 5, a one-dimensional nanofibrillar array formed by the co-assembly of native and biotin-functionalized beta-amyloid (A β) peptide was developed for biomolecule sensing. With the presence of biotin moiety, a variety of biomolecular probes can be conjugated onto the nanofibrils and thus converting the protein assembly into a miniature biosensor. In this work, DNA probes were immobilized onto the fibril for the detection of complementary DNA sequences. The as-developed 'DNA-nanoarray' achieved a detection limit at sub-attomole level. This highly sensitive yet simple assay required trace amount of sample consumption ($< 10 \mu\text{L}$) and was pretreatment-free. In addition, we reported the preparation of alternate-segmented amyloid nanofibrils with multifunctionality. The fibrils hereby served as an encoded template that can be visualized with various fluorescence labeling dyes for barcode recognition purpose, and hence multiplex detection of biomolecules was achieved. Regarding that each protein nanofibril represented a single detection platform, a large number of single fibrils were simultaneously monitored with the dual-colour TIRFM in a high-throughput manner.

TABLE OF CONTENTS

DECLARATION	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF SYMBOLS	xx
LIST OF ABBREVIATIONS	xxi
CHAPTER 1. INTRODUCTION	1
1.1 Single Molecule Detection	1
Privileges of Single Molecule Detection	1
Improving Signal-to-Noise Ratio in SMD	1
Applications of SMD in Biomolecules Studies	5
1.2 Total Internal Reflection Fluorescence Microscopy	7
Principle of TIRFM.....	7
Application of TIRFM in Detection of Single Biomolecules	10
1.3 References	11
CHAPTER 2. DIRECT QUANTIFICATION OF SINGLE MOLECULES OF MICRORNA BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY	13
2.1 Introduction	13

2.2	Experimental Section	17
2.3	Results and Discussion.....	27
2.4	Conclusions	46
2.5	References.....	47
CHAPTER 3. BETA-AMYLOID (1–40) FIBRILLATION AS POTENTIAL		
THERAPEUTIC AGENTS FOR ALZHEIMER’S DISEASE.....51		
3.1	Introduction.....	51
3.2	Experimental Section	55
3.3	Results and Discussion.....	62
3.4	Conclusions	80
3.5	References.....	81
CHAPTER 4. INFLUENCE OF SURFACE FUNCTIONALIZED		
NANOPARTICLES OF VARIOUS SIZES ON BETA-AMYLOID (1–40)		
FIBRILLATION.....85		
4.1	Introduction.....	85
4.2	Experimental Section	89
4.3	Results and Discussion.....	93
4.4	Conclusions	114
4.5	References.....	115
CHAPTER 5. MULTIFUNCTIONAL ENCODED SELF-ASSEMBLING		
PROTEIN NANOFIBRILS AS PLATFORMS FOR HIGH-THROUGHPUT		
AND MULTIPLEXED DETECTION OF BIOMOLECULES117		
5.1	Introduction.....	117
5.2	Experimental Section	120
5.3	Results and Discussion.....	125

5.4 Conclusions	146
5.5 References.....	147
CHAPTER 6. GENERAL CONCLUSIONS.....	150
APPENDIX I.....	152
LIST OF PUBLICATIONS	154
ACADEMIC ACHIEVEMENTS	157
CURRICULUM VITAE	158