香港浸會大學
HONG KONG BAPTIST UNIVERSITY

HKBU Scholars

DOCTORAL THESIS

Measuring binding kinetics of ligands with tethered receptors by fluorescence polarization complemented with total internal reflection fluorescence microscopy

Kwok, Ka Cheung

Date of Award:
2010

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

Download date: 19 Apr, 2024

Measuring Binding Kinetics of Ligands with Tethered Receptors by Fluorescence Polarization Complemented with Total Internal Reflection Fluorescence Microscopy

KWOK Ka Cheung

A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal supervisor: Prof CHEUNG Nai Ho

Hong Kong Baptist University

July 2010

Abstract

The study of the binding between estrogen receptors (ER) and their ligands *in vitro* has long been of interest mainly because of its application in anti-estrogen drug discovery for breast cancer treatment as well as in the screening of environmental contaminants for endocrine disruptors. Binding strength was conventionally quantified in terms of equilibrium dissociation constant (K_D). Recently, emphasis is shifting towards kinetics rate constants, and off-rate (k_{off}) in particular. This thesis reported a novel method to measure such binding kinetics based on fluorescence polarization complemented with total internal reflection fluorescence (FP-TIRF). It used tethered receptors in a flow cell format. For the first time, the kinetics rate constants of the binding of full-length human recombinant ERα with its standard ligands were measured. k_{off} was found to range from 1.3×10^{-3} to 2.3×10^{-3} s^{-1}. k_{on} ranged from 0.3×10^{5} to 11×10^{5} M^{-1} s^{-1}. The method could also be used to screen potential ligands. Motivated by recent findings that ginsenosides might be functional ligands of nuclear receptors, eleven ginsenosides were scanned for binding with ERα and peroxisome proliferator-activated receptor gamma (PPARγ). None of the ginsenosides showed significant binding to ERα, but Rb1 and 20(S)-Rg3 exhibited significant specific binding with PPARγ.

Table of Contents

Declaration …….. i

Abstract …….. ii

Acknowledgments ……..…….. iii

Table of Contents ……..…..... iv

List of Tables ……...….... viii

List of Figures ……... ix

List of Abbreviations …….. xiv

Chapter 1 Introduction ……... 1

Chapter 2 Background ……... 4

2.1 Receptor ligand interaction ……. 4

2.1.1 Nuclear Receptor superfamily ……... 4

2.1.2 Estrogen receptor structure and function ……... 5

2.1.3 Breast cancer and drug discovery …… 10

2.1.4 Endocrine disruptors and contaminant screening …… 12

2.1.5 Peroxisome proliferator-activated receptors …… 13

2.1.6 Ginsenosides …… 14

2.2 *In-vitro* binding assays …….. 16

2.2.1 Mass difference assays ……. 16

2.2.2 Surface plasmon resonance (SPR) ……...… 16

2.2.3 Fluorescence assays ……..… 18

2.2.4 Total internal reflection fluorescence (TIRF) ……. 19

2.2.5 Total internal reflection with fluorescence correlation spectroscopy (TIR-FCS) ……..……...… 20

2.2.6 Total internal reflection with fluorescence photobleaching recovery (TIR-FPR) …… 21

2.2.7 Total internal reflection with reflectance interferometry (TIR-RIf) ……..…… 22

2.2.8 Fluorescence polarization (FP) …… 23

2.2.9 Free probes versus tethered probes …….. 24

Chapter 3 Methods ……..…….……. 26

3.1 FP-TIRF setup ……..…….. 26

3.2 Setup optimization and calibration ……... 27

3.3 Flow cell fabrication and fluidics ……..… 29

3.3.1 Flow cell fabrication ……..……...… 30

3.3.2 Fluidics ……..…… 31

3.4 Channel surface preparation ……..…… 32

3.4.1 Coating with PEG ……..……... 32

3.4.2 Immobilization of primary and secondary antibody …… 33

3.4.3 Receptor preparation and tethering …….. 34

3.5 Ligand preparation ……..……... 36

3.6 Program for real-time data capture and display …… 36

3.7 Data runs ……..……..…. 38

3.7.1 Kinetics data capture for surface tethered receptors …… 38

3.7.2 Kinetics data capture for free receptors and ligands in solution .. 39

3.7.3 Screening ginsenosides for binding with nuclear receptors ……. 40

3.8 Data analysis – From fluorescence anisotropy to bound fraction ……. 41

3.9 Kinetics modeling ……..…… 43

3.10 Rapid mixing assumption ……..…..…….…… 44

Chapter 4 Results and discussion ……..…… 46

4.1 Characterization of the FP-TIRF setup …….. 47

4.1.1 Correcting for // and ⊥ arm imbalance …… 47

4.1.2 Monitoring [F] ……..…… 48

4.1.3 Consistency of FP and TIRF …… 48

4.2 Characterization of each tether link …….. 51

4.2.1 Strength and specificity of tether ……. 52

4.2.2 Tethering preserved receptor function ……...…. 57

4.2.3 Tethering preserved the random orientation of μ …..... 59

4.3 Binding kinetics of the fluorescent ligand with ERα …… 62

4.4 Justify rapid mixing assumption ……... 68

4.5 Binding kinetics of nonfluorescent ligands with ERα ……. 72

4.6 Positive and negative controls …….. 78

4.7 Screening ginsenosides for ERα binding …….. 80

4.8 Screening ginsenosides for PPARγ binding …….. 82

4.9 Binding affinity and chemical structure …… 85

Chapter 5 Conclusion …….…….. 91

List of References …….……... 94

Curriculum Vitae …….……...… 100