

Contents

Acknowledgements	5
Abstract.....	7
Résumé.....	9
Contents	11
List of figures.....	14
List of Tables	15
Motivation and objectives	17
Chapter 1. Introduction	21
1. Information about life is encoded in a genome.....	21
1.1 From gene to protein.....	21
1.1.1 Transcription	23
1.1.2 Transcription factors	24
1.2 TF binding specificity.....	26
1.2.1 Methods for identification of TF binding specificity.....	28
1.2.1.1 Low-throughput methods	29
1.2.1.2 Medium and high-throughput methods	31
Chapter 2. Cooperative DNA binding by heterodimers.....	35
2.1 Introduction.....	36
2.2 Results.....	38
2.2.1 Dimerization with RXR α is crucial for PPAR γ in acquiring the DNA binding specificity.....	38
2.2.2 Biophysical characterization of monomeric PPAR γ and RXR α binding to the PPRE element	41
2.2.3 PPAR γ binds to the PAL3 element as a monomer	43
2.2.4 Mechanistic model of the dimer formation on DNA	47

2.2.5 Influence of co-factors and ligands on PPAR γ :RXR α DNA binding <i>in vitro</i>	53
2.3 Discussion	57
2.4 Methods	60
2.5 Supplementary figures	62
Chapter 3. MITOMI-seq	69
3.1 Introduction	70
3.1.1 Dimerization is an important regulatory mechanism of gene expression	70
3.1.2 Microfluidics	71
3.1.3 Mechanically Induced Trapping of Molecular Interactions (MITOMI)	72
3.2 MITOMI followed by HT sequencing (MITOMI-seq)	75
3.2.1 Method development	75
3.2.2 64-unit device	77
3.2.3 Chip fabrication	78
3.2.4 PDMS dispenser	80
3.2.5 MITOMI-seq: principle	82
3.2.6 Random library design	82
3.2.6.1 “Short” DNA library	82
3.2.6.2 Extended DNA library	87
3.2.7 MITOMI-seq: current procedure	87
3.3 Results	90
3.3.1 MITOMI-seq identifies DNA binding motifs of TF monomers and dimers	90
3.3.2 Comparison of MITOMI-seq, PBM and HT-SELEX platforms: NF κ B1, a case study	94
3.3.3 Comparison of MITOMI-seq data and MITOMI affinity data	99
3.4 Discussion	101
3.5 Methods	106
3.6 Supplementary tables and figures	110
Conclusions and outlook	113
Bibliography	117

Curriculum vitae.....127