

Contents

About the Author *xv*

Foreword *xvii*

Preface *xix*

- 1 Introduction of SELEX and Important SELEX Variants** *1*
Yiyang Dong, Zhuo Wang, Sai Wang, Yehui Wu, Yufan Ma, and Jiahui Liu
- 1.1 SELEX *1*
- 1.2 Negative SELEX and Its Analogs *3*
- 1.3 One-Round SELEX *5*
- 1.4 CE-SELEX *6*
- 1.5 Microfluidic SELEX *8*
- 1.6 Cell-SELEX *10*
- 1.7 *In Silico*-SELEX *12*
- 1.8 Post-SELEX and *In Chemico*-SELEX *14*
- 1.9 Auto-SELEX *17*
- 1.10 Primer-Free SELEX *17*
- 1.11 Genomic SELEX *18*
- 1.12 Photo-SELEX *19*
- 1.13 qPCR-SELEX *19*
- 1.14 Perspectives *20*
- References *21*
- 2 *In Chemico* Modification of Nucleotides for Better Recognition** *27*
Przemyslaw Jurek, Marta Matusiewicz, Maciej Mazurek, and Filip Jelen
- 2.1 Introduction *27*
- 2.1.1 Beyond ATGC *27*
- 2.1.2 The Scope of This Chapter *29*
- 2.2 Modified Functional Nucleic Acids *30*
- 2.2.1 The “Hows” *30*
- 2.2.1.1 Post-SELEX Optimization *30*
- 2.2.1.2 In-line Modifications *30*
- 2.2.2 The “Whys” *31*
- 2.2.2.1 The Hurdles *31*

2.2.2.2	The Gains	32
2.2.3	The “Ifs”	33
2.3	Backbone Modifications	35
2.3.1	2'-OH Modifications	36
2.3.2	Phosphodiester Bond Modifications	36
2.3.3	Xeno Nucleic Acids	38
2.3.3.1	TNA	39
2.3.3.2	FANAs	39
2.3.3.3	HNA, CeNA, LNA, ANA	39
2.3.3.4	Other Modifications	40
2.4	Nucleobase Modifications	40
2.4.1	General Information	40
2.4.2	Modified Aptamers and Catalysts	42
2.4.2.1	Introduction of Cationic Moieties	42
2.4.2.2	Catalysts with Protein-like Sidechains	43
2.4.2.3	Nucleobase-linked Nucleobases	44
2.4.2.4	Glycans Targeting with Boronic Acids	44
2.4.2.5	“Click Chemistry”-Based Versatile Approach	45
2.4.2.6	Nonenzymatic Selection – X-aptamers	45
2.4.2.7	Slow Off-rate Modified Aptamers	46
2.5	Aptamers with Expanded Genetic Alphabet	48
2.5.1	GACTZP Aptamers	48
2.5.2	Aptamers with a Hydrophobic Fifth Base	50
2.6	Summary	52
2.A	Appendix	52
	References	68
3	Immobilization of Aptamers on Substrates	85
	<i>Annalisa De Girolamo, Maureen McKeague, Michelangelo Pascale, Marina Cortese, and Maria C. DeRosa</i>	
3.1	Introduction	85
3.2	Methods for Immobilization of Aptamers	87
3.2.1	Physical Adsorption	87
3.2.2	Covalent Binding	88
3.2.2.1	Covalent Immobilization of Activated Aptamers on a Functionalized Surface	88
3.2.2.2	Covalent Immobilization of Modified Aptamers on Activated Surfaces	92
3.2.2.3	Covalent Immobilization by Entrapment	95
3.2.2.4	Covalent Immobilization by Electrografting	97
3.2.3	Self-assembled Monolayers	98
3.2.4	Avidin–Biotin Binding (Affinity Coupling)	100
3.2.5	Electrochemical Adsorption	101
3.2.6	Hybridization	101
3.3	Immobilization of Aptamers on Substrates for Diagnostic Applications	102
3.3.1	Flat Gold	102
3.3.1.1	Surface Plasmon Resonance Detection	109

3.3.1.2	Electrochemical Detection	109
3.3.2	Solid Phase	111
3.3.2.1	Optical Detection	112
3.3.2.2	Sample Cleanup	114
3.3.3	Nanomaterials	115
3.4	Future Perspectives on New Substrates and New Immobilization Chemistries	116
3.5	Conclusions	117
	References	119
4	Characterization of Aptamer–Ligand Complexes	127
	<i>Rebeca Miranda-Castro, Noemí de-los-Santos-Álvarez, and María J. Lobo-Castañón</i>	
4.1	Introduction	127
4.2	Equilibrium Characterization: Thermodynamics	128
4.2.1	Basic Principles	128
4.2.2	Separation-Based Methods	133
4.2.2.1	Equilibrium Dialysis and Related Techniques	133
4.2.2.2	High-Performance Liquid Chromatography	135
4.2.2.3	Electrophoresis	136
4.2.3	Direct Methods	137
4.2.3.1	Isothermal Titration Calorimetry	138
4.2.3.2	Fluorescence-Based Methods	140
4.3	Kinetic Characterization	146
4.3.1	Heterogeneous Methods	148
4.3.1.1	Surface Plasmon Resonance	148
4.3.1.2	Electrochemical Impedance Spectroscopy	152
4.3.2	Homogeneous Methods	154
4.3.2.1	Rotating Droplet Electrochemistry	154
4.3.2.2	Capillary Electrophoresis	157
4.3.2.3	Nanopore-Based Studies	159
4.4	Concluding Remarks	162
	Acknowledgments	163
	References	164
5	Utilization of Aptamers for Sample Preparation in Analytical Methods	173
	<i>Zhiyong Yan and Yang Liu</i>	
5.1	Introduction	173
5.2	Substrate Materials Developed for Immobilization of Aptamers	175
5.3	Utilization of Aptamers for Sample Preparation in SPE	177
5.3.1	Aptamers Utilized in Affinity Column for SPE	181
5.3.2	Aptamers Utilized in Other SPE	182
5.4	Aptamers Utilized in SPME	182
5.4.1	Aptamers Utilized in Fiber SPME	183
5.4.2	Aptamers Utilized in SBSE	184
5.4.3	Aptamers Utilized in Other Formats of SPME	185

5.5	Aptamers Utilized in Other Affinity Chromatography	185
5.6	Aptamers Utilized in Microfluidic Separation System	187
5.7	Aptamers Utilized in Magnetic Separation System	189
5.7.1	Aptamers Utilized in Magnetic Solid-Phase Extraction (MSPE)	190
5.7.2	Aptamers Utilized in Other Magnetic Separation Formats	190
5.8	Aptamers Utilized in CE	191
5.9	Aptamers Utilized in Other Sample Separation Methods	192
5.10	Conclusion and Outlook	192
	References	192
6	Development of Aptamer-Based Colorimetric Analytical Methods	205
	<i>Subash C.B. Gopinath, Thangavel Lakshmi Priya, M.K. Md Arshad, and Chun Hong Voon</i>	
6.1	Introduction	205
6.2	Aptamer Generation for Colorimetric Assay	206
6.3	Aptasensor	206
6.4	Aptamer-AuNP-Based Colorimetric Assays	207
6.5	Applications of AuNP-Aptamer-Based Colorimetric Assays	211
6.6	Conclusions	213
	References	213
7	Enzyme-Linked Aptamer Assay (ELAA)	219
	<i>Yiyang Dong and Sai Wang</i>	
7.1	Introduction	219
7.2	Enzyme-Linked Immunosorbent Assay	219
7.3	Analytical Merits of Aptamer vs Antibody	221
7.4	Enzyme-Linked Aptamer Assay (ELAA)	223
7.5	Comparison of Direct-Competitive ELAA (<i>dc</i> -ELAA), Indirect-Competitive ELAA (<i>ic</i> -ELAA), and ELISA	225
7.6	Conclusion	226
	References	227
8	Development of Aptamer-Based Fluorescence Sensors	229
	<i>Seyed M. Taghdisi, Rezvan Yazdian-Robati, Mona Alibolandi, Mohammad Ramezani, and Khalil Abnous</i>	
8.1	Introduction	229
8.2	Fluorescent-Dye-Based Aptasensors	230
8.3	Nanoparticle-Based Aptasensors	231
8.3.1	Fluorescent Aptasensors Based on Gold Nanoparticles	231
8.3.2	Fluorescent Aptasensors Based on Carbon Nanomaterials	234
8.3.3	Fluorescent Aptasensors Based on Silica Nanoparticles	236
8.3.4	Fluorescent Aptasensors Based on Silver Nanoparticles	238
8.3.5	Fluorescent Aptasensors Based on DNA Structures	239
8.3.5.1	Fluorescent Aptasensors Based on DNA Nanostructures	239
8.3.5.2	Fluorescent Aptasensors Based on Triple-Helix Molecular Switch (THMS)	240

8.4	Conclusion	241
	Acknowledgment	241
	Suggested Websites	242
	References	242
9	Development of Aptamer-Based Electrochemical Methods	247
	<i>Jian-guo Xu, Li Yao, Lin Cheng, Chao Yan, and Wei Chen</i>	
9.1	Introduction	247
9.2	Classification of Electrochemical Aptasensors	247
9.3	Amperometric Aptasensors	248
9.3.1	Covalent Labels	248
9.3.1.1	Enzyme Labels	248
9.3.1.2	Other Covalently Linked Redox Species	250
9.3.2	Non-covalent Labels	256
9.3.2.1	Intercalated Redox Species	256
9.3.2.2	Cationic Redox Species	260
9.3.3	Label-Free Aptasensors	263
9.4	Potentiometric Aptasensors	265
9.5	Impedimetric Aptasensors	266
9.6	Electrochemiluminescence Aptasensors	268
9.7	Conclusion	268
	References	269
10	Development of Aptamer-Based Lateral Flow Assay Methods	273
	<i>Miriam Jauset-Rubio, Mohammad S. El-Shahawi, Abdulaziz S. Bashammakh, Abdulrahman O. Alyoubi, and Ciara K. O'Sullivan</i>	
10.1	Introduction	273
10.2	Development of Aptamer-Based Lateral Flow Assay – Strategy	275
10.2.1	Analogies and Differences Compared to Lateral flow Immunoassays (LFIA) s)	275
10.2.2	Fundamental Assay Considerations	276
10.2.3	Fundamental Analytical Considerations	277
10.3	Lateral Flow Aptamer Assays	278
10.3.1	Sandwich Assay	278
10.3.2	Competitive Assay	281
10.3.3	Signal Amplification	283
10.4	Summary and Perspectives	291
	References	294
11	Development of Aptamer-Based Non-labeling Methods	301
	<i>Huajie Gu, Liling Hao, and Zhouping Wang</i>	
11.1	Introduction	301
11.2	Surface Plasmon Resonance (SPR)-Based Aptasensor	302
11.2.1	Introduction	302

11.2.2	The Principle of SPR Technique	302
11.2.3	The Classification of SPR Biosensors	303
11.2.3.1	SPR Biosensors Based on Angular Modulation	303
11.2.3.2	SPR Biosensors Based on Wavelength Modulation	304
11.2.3.3	SPR Biosensors Based on Amplitude Modulation	304
11.2.3.4	SPR Biosensors Based on Phase Modulation	304
11.2.4	The Application of Aptamer-Based SPR Technique	304
11.2.4.1	Determination of the Affinity of Aptamers	305
11.2.4.2	Detection Analyte Concentrations	305
11.2.5	Summary and Prospects of SPR Aptasensors	310
11.3	Quartz Crystal Microbalance (QCM)-Based Aptasensor	311
11.3.1	Introduction	311
11.3.2	The Principle of QCM Technique	311
11.3.3	The Application of Aptamer-Based QCM Technique	312
11.3.3.1	Determination of the Affinity of Aptamers	312
11.3.3.2	Detection of Analyte Concentrations	313
11.3.4	Summary and Prospect of QCM Aptasensors	318
11.4	Isothermal Titration Calorimetry (ITC)	319
11.4.1	Introduction	319
11.4.2	The Principle of ITC Technique	319
11.4.3	Thermodynamic Parameters Obtained from ITC Experiment	320
11.4.4	Application of ITC in Association Between Aptamer and Target	322
11.4.4.1	Interaction Between the Aptamer Domain of the Purine Riboswitch and Ligands	322
11.4.4.2	Interaction Between the Cocaine-Binding Aptamer and Quinine	324
11.4.4.3	Affinity Test by ITC After Systemic Evolution of Ligands by EXponential Enrichment (SELEX)	327
11.4.5	Summary	329
11.5	MicroScale Thermophoresis (MST)	329
11.5.1	Introduction	329
11.5.2	The Principle of MST Technique	330
11.5.3	Application of MST in Association Between Aptamer and Target	332
11.5.3.1	Interaction Between Steroid Hormones and Aptamers	332
11.5.3.2	Affinity Test by MST After Systemic Evolution of Ligands by EXponential Enrichment (SELEX)	333
11.5.4	Summary	335
	References	335
12	Challenges of SELEX and Demerits of Aptamer-Based Methods	345
	<i>Haiyun Liu and Jinghua Yu</i>	
12.1	Introduction	345
12.2	Challenges of SELEX	347
12.2.1	Aptamer Degradation	347
12.2.2	Purification	348
12.2.3	Binding Affinity (K_d)	348
12.2.4	Target Immobilization	349

12.2.5	Cross-Reactivity	350
12.2.6	Time and Cost	350
12.2.7	Interaction of Aptamers with Intracellular Targets	351
12.2.8	Bioinformatics Tools	352
12.3	Demerits of Aptamer-Based Methods	352
12.3.1	Sensitivity	352
12.3.2	Selectivity and Specificity	354
12.3.3	Reproducibility	355
12.3.4	Calibration and Uncertainty	355
12.3.5	Regeneration	355
12.3.6	Immobilization of Aptamers	356
12.4	Summary and Perspectives	356
	References	357
13	State of the Art and Emerging Applications	365
	<i>Lin-Chi Chen, Jui-Hong Weng, and Pei-Wei Lee</i>	
13.1	Introduction	365
13.2	Frontiers of Analytical Aptamer Selection and Probe Design	368
13.2.1	Biochip-Based Aptamer Selection	368
13.2.2	SELEX with Next-Generation Sequencing (NGS)	372
13.2.3	Aptamer Optimization and Specialized Selection	373
13.2.4	<i>In Silico</i> Aptamer Design	376
13.3	Novel Aptasensing Platforms – From Assays and Sensors to Instrumental Analyses	378
13.3.1	Aptamer Assays	378
13.3.2	Aptasensors	380
13.3.3	Aptamer Chips	382
13.3.4	Cell-Based Aptasensing	384
13.4	Emerging Applications of Aptamer Diagnostics	385
13.4.1	Human Disease Diagnosis	386
13.4.2	Food/Environmental Monitoring – Mycotoxins, Pesticides, Heavy Metal Ions	387
13.4.3	Therapeutic Drug Assessment – Organ-on-a-Chip	387
13.4.4	New Molecular Biology Applications – CRISPR/Cas9, Stem Cells, IHC	388
13.5	Concluding Remarks – Frontiers of Frontiers	389
	Acknowledgments	389
	References	390
	Index	397

