Novel Fingermark Detection Methods Using Biomolecular Recognition

by

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Certificate of Authorship and Originality

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

Rolanda Lam
February 17, 2018
Dedicated to Somebody I Loved Dearly –
You will forever be in my thoughts.
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Table of Contents

CERTIFICATE OF AUTHORSHIP AND ORIGINALITY ........................................ II

ACKNOWLEDGEMENTS ........................................................................................ IV

TABLE OF CONTENTS ............................................................................................ VI

LIST OF FIGURES .................................................................................................. XI

LIST OF TABLES ................................................................................................... XXI

LIST OF ABBREVIATIONS .................................................................................. XXIII

ABSTRACT ........................................................................................................... XXVI

PUBLICATIONS AND PRESENTATIONS ................................................... XXVIII

CHAPTER 1 INTRODUCTION ........................................................................... 32

1.1 FINGERPRINTS ............................................................................................ 32

1.1.1 Identification ........................................................................................... 32

1.1.2 Formation of Friction Ridge Skin ........................................................... 35

1.2 FINGERMARK DETECTION METHODS ....................................................... 36

1.2.1 Types of Fingermarks .............................................................................. 36

1.2.1.1 Fingermark Residue Composition ................................................... 37

1.2.1.2 Substrate Characteristics .................................................................. 38

1.2.1.3 Factors Affecting Fingermark Deposition and Composition .......... 39

1.2.2 Current Latent Fingermark Detection Methods .................................... 40

1.2.3 Current Blood-Contaminated Fingermark Detection Methods .......... 42

1.2.4 Current Limitations .................................................................................. 43

1.3 PROJECT AIM AND OBJECTIVES .............................................................. 43

CHAPTER 2 OPTIMISATION AND VALIDATION OF MULTI-TARGET
BIOMOLECULAR REAGENTS .............................................................................. 46

2.1 INTRODUCTION ............................................................................................ 46
Table of Contents

2.2 IMMUNODETECTION ................................................................. 47
  2.2.1 Antibodies in Forensic Science ........................................... 47
    2.2.1.1 Antibodies in Fingermark Detection ............................ 47

2.3 OBJECTIVES ........................................................................ 54

2.4 EXPERIMENTAL DESIGN ..................................................... 55
  2.4.1 Materials and Instrumentation ........................................ 58
  2.4.2 Methods ........................................................................ 61
    2.4.2.1 Reagent Preparation .................................................. 61
    2.4.2.2 Sample Preparation .................................................... 64
    2.4.2.3 Sample Processing ...................................................... 65
    2.4.2.4 Fingermark Evaluation .............................................. 66
    2.4.2.5 Optimisation of Multiplex Solutions ............................ 69
    2.4.2.6 Direct Comparison to Routine Fingermark Enhancement Techniques ................................................. 72
    2.4.2.7 Compatibility with Routine Technique Sequences .......... 74

2.5 RESULTS AND DISCUSSION ................................................. 78
  2.5.1 Optimisation of Multiplex Solution .................................... 78
    2.5.1.1 Determination of Optimal Processing Time .................. 78
    2.5.1.2 Investigation of Optimal Multiplex Conditions ............... 82
  2.5.2 Direct Comparison to Routine Fingermark Enhancement Techniques ......................................................... 90
  2.5.3 Compatibility with Routine Technique Sequences .............. 95
    2.5.3.1 Assessment of Routine Technique Effects on Multiplex Solution Performance ............................................. 95
    2.5.3.2 Impact of Routine Dyestain on Multiplex Solution Luminescence . 96
    2.5.3.3 Effect of Multiplex Solutions on Routine Technique Sequences ............................................................ 97
  2.5.4 Other Considerations ....................................................... 102
2.6 CONCLUSIONS ........................................................................................................ 103

CHAPTER 3 DEVELOPMENT OF NOVEL IN VITRO SELECTION VARIATION (FINGERMARK-SELEX) ....................................................................................... 106

3.1 INTRODUCTION ..................................................................................................... 106

3.2 APTAMERS ........................................................................................................... 107

3.2.1 Comparisons to Antibodies ........................................................................... 107

3.3 IN VITRO SELECTION .......................................................................................... 109

3.3.1 General SELEX Process .................................................................................. 109

3.3.1.1 Library Design ......................................................................................... 110

3.3.1.2 Target Molecule ...................................................................................... 110

3.3.1.3 Selection Conditions .............................................................................. 111

3.3.2 SELEX Variations ........................................................................................... 111

3.4 OBJECTIVES ....................................................................................................... 112

3.5 EXPERIMENTAL DESIGN ................................................................................... 113

3.5.1 Materials and Instrumentation ....................................................................... 115

3.5.2 Methods .......................................................................................................... 119

3.5.2.1 Substrate Preparation and Fingermark Collection ........................................ 119

3.5.2.2 In Vitro Selection Process ......................................................................... 120

3.5.2.3 Sequence Analysis for Aptamer Sequence Candidate Screening ............. 129

3.5.2.4 GC-MS Analysis for Target Identification ............................................... 132

3.6 RESULTS AND DISCUSSION ............................................................................. 134

3.6.1 In Vitro Selection Process ............................................................................... 134

3.6.2 Sequence Analysis for Aptamer Sequence Candidate Screening ................. 137

3.6.3 GC-MS Analysis for Target Identification ..................................................... 139

3.6.4 Advantages and Limitations .......................................................................... 142

3.7 CONCLUSIONS ................................................................................................... 144
## Table of Contents

### Chapter 4 Investigation into “Fingerprint” Aptamer Candidates for Fingerprint Detection

4.1 Introduction ................................................................. 146

4.2 Aptamers in Fingerprint Detection ................................. 147

4.3 Objectives ........................................................................ 151

4.4 Experimental Design ........................................................ 151

4.4.1 Materials and Instrumentation ...................................... 154

4.4.2 Methods ........................................................................... 157

4.4.2.1 Proof-of-Concept .......................................................... 157

4.4.2.2 Comparisons to Routine Fingerprint Enhancement Reagents ..... 162

4.4.2.3 Comparisons to Single- and Multi-Target Biomolecular Recognition Approaches ....................................................... 163

4.5 Results and Discussion ...................................................... 166

4.5.1 Proof-of-Concept .......................................................... 166

4.5.2 Comparisons to Routine Fingerprint Enhancement Reagents .......... 180

4.5.3 Comparisons to Single- and Multi-Target Biomolecular Recognition Approaches ....................................................... 182

4.5.3.1 Comparisons to Single-Target Aptamer-Based Reagents .......... 182

4.5.3.2 Comparisons to Single-Target Antibody-Based Reagents .......... 186

4.5.3.3 Comparisons to Multi-Target Antibody-Based Reagents .......... 190

4.5.4 Advantages and Limitations ........................................... 193

4.5.5 Conclusions ..................................................................... 196

### Chapter 5 General Discussion, Recommendations, and Conclusions

5.1 General Discussion ........................................................ 198

5.2 Recommendations .......................................................... 199

5.3 Conclusions ....................................................................... 201
# Table of Contents

APPENDIX I ROUTINE FINGERMARK ENHANCEMENT METHOD FORMULATIONS AND APPLICATIONS ........................................................... 203

APPENDIX II FINGERMARK-SELEX DONOR INFORMATION .............. 206

APPENDIX III PRIMER SEQUENCES ............................................................. 207

APPENDIX IV PHENOL CHLOROFORM AND ETHANOL PRECIPITATION .................................................................................................... 208

APPENDIX V PREDICTED SECONDARY STRUCTURES........................... 209

APPENDIX VI OBSERVATIONS ON BACKGROUND FLUORESCENCE AND NON-SPECIFIC BINDING........................................................... 210

APPENDIX VII SUBSTRATE SUITABILITY .................................................. 212

APPENDIX VIII ASSESSMENT OF APTAMER CANDIDATE-DYE FILTRATION EFFECTIVENESS ............................................................................. 214

APPENDIX IX ATTO 550 NHS ESTER COMPATIBILITY .......................... 215

APPENDIX X LYSOZYME APTAMER TESTS .............................................. 216

APPENDIX XI FINGERMARK DEVELOPMENT ON POROUS SUBSTRATES WITH REVISED WORKING SOLUTION FORMULATION 218

REFERENCES .......................................................................................................... 221
List of Figures

Figure 1-1 Three main overall patterns (Reproduced from Champod et al. [8]). ........34

Figure 1-2 Illustrations of second level detail (Reproduced from Champod et al. [8]). 35

Figure 1-3 Schematic representation of factors influencing fingermark composition (Reproduced from Girod et al. [30]). ........................................................................... 40

Figure 2-1 Schematic representation of the detection of drugs and/or drug metabolites in latent fingerprints using a) gold nanoparticles and b) magnetic particles (Reproduced from Hazarika and Russell [86]). .............................................................................. 49

Figure 2-2 Fingermarks developed on polyvinylidene fluoride (PVDF) after being immunodetected with (a) anti-keratin 1/10, (b) anti-cathepsin-D, and (c) anti-dermcidin (Reproduced from Drapel et al. [71]). ................................................................................. 50

Figure 2-3 Fingermarks deposited on thermal paper first developed with ninhydrin (left side of each image), and then further developed with the immunolabeling method (anti-dermcidin, anti-albumin and antikeratin) (right side of each image) (Reproduced from van Dam et al. [90]). ............................................................................................ 51

Figure 2-4 Schematic diagram for the application of two conjugated nanoparticle systems – directly-bound antibodies (top left) and alkyl-thiol-linked antibodies (top right) – to latent fingermarks (Reproduced from Spindler et al. [70]). ......................... 52

Figure 2-5 Schematic representation of the detection of proteins within latent fingerprints using SERS imaging technique (Reproduced from Song et al. [94]) .... 54

Figure 2-6 Approach overview for optimisation and validation of multi-target biomolecular recognition fingermark enhancement reagents. ................................. 57

Figure 2-7 Schematic diagrams of how depleting fingermarks were processed when: (a) halved and (b) quartered. The gradient in colour between the two diagrams illustrates the decrease in fingermark residues within a depletion series .................. 66
List of Figures

Figure 2-8 Schematic diagrams depicting manner in which quartered fingermarks were processed to compare the eight different time intervals................................................................. 69

Figure 2-9 Schematic diagrams depicting the different processing configurations for: (a) latent fingermarks; (b) blood-contaminated fingermarks; and (c) saliva- and semen-contaminated fingermarks. (aq = aqueous working solution; BSA = aqueous working solution containing bovine serum albumin; EG = glycolic working solution; M# = multiplex solution containing # antibodies/aptamers; MeOH = ice-cold methanol fixing solution used; 5-SSA = 5-sulfosalicylic acid fixing solution used) ...................... 72

Figure 2-10 Schematic diagram showing how a split fingermark was processed. Used multiplex solution was created by exposing it to additional fingermarks pre-treated with the various routine technique sequences. For blood-contaminated fingermarks, 5-SSA fixing solution was used prior to the multiplex solution application. (aq = aqueous working solution; M# = multiplex solution containing # antibodies/aptamers) ........... 76

Figure 2-11 Natural fingermark deposited on PVDF and processed for 15 minutes (top right), 30 minutes (bottom right), 45 minutes (top left), and one hour (bottom left), visualised at 590 nm with a 650 nm bandpass filter. ................................................................. 79

Figure 2-12 Natural fingermark deposited on ziplock bag and processed for 1.25 hours (top left), 1.5 hours (bottom left), 1.75 hours (top right), and two hours (bottom right), visualised at 590 nm with a 650 nm bandpass filter. ................................................................. 80

Figure 2-13 Average CAST scores calculated for latent fingermarks developed on PVDF and ziplock bag to compare eight different processing times................................. 81

Figure 2-14 Frequency of CAST scores for latent fingermarks developed on PVDF and ziplock bag to assess four shorter processing times......................................................... 81

Figure 2-15 Results for latent fingermarks on non-porous substrates (a-c) and semi-porous substrates (d) organised by average CAST scores and working solutions. In Figure 2-15(d), glossy magazine was not processed with M4(BSA), while glossy cardboard was not processed with either M6 working solution. (aq = aqueous working solution; BSA = aqueous working solution containing bovine serum albumin; EG =
glycolic working solution; M# = multiplex solution containing # antibodies/aptamers)

Figure 2-16 Blood-contaminated fingermarks on (a) garbage bag and (b) beverage can, both fixed with 5-SSA solution and processed with glycolic working solution with four components (top left), glycolic working solution with eight components (bottom left), aqueous working solution with four components (top right), and aqueous working solution with eight components (bottom right). Both developed fingermarks were visualised at 590 nm with a 650 nm bandpass filter.

Figure 2-17 Quartered blood-contaminated fingermark developed with a multiplex solution of six components on a light grey shopping bag with: (a) MeOH fixing/aqueous working solutions; (b) MeOH fixing/glycolic working solutions; (c) 5-SSA fixing/aqueous working solutions; and (d) 5-SSA fixing/glycolic working solutions. All quarter marks were visualised under an excitation of 590 nm with a 650 nm bandpass filter.

Figure 2-18 Average CAST score results comparing blood-contaminated fingermarks fixed with two different fixing solutions, but all processed with a multiplex solution of six components. (aq = aqueous working solution; EG = glycolic working solution; M# = multiplex solution containing # antibodies/aptamers; MeOH = ice-cold methanol fixing solution used; 5-SSA = 5-sulfosalicylic acid fixing solution used)

Figure 2-19 Average CAST score results comparing blood-contaminated fingermarks fixed with two different fixing solutions and multiplex working solutions containing different number of components. (aq = aqueous working solution; EG = glycolic working solution; M# = multiplex solution containing # antibodies/aptamers; MeOH = ice-cold methanol fixing solution used; 5-SSA = 5-sulfosalicylic acid fixing solution used)

Figure 2-20 Comparison of aqueous (left halves) and glycolic (right halves) working solutions for fingermarks contaminated with (a) saliva on garbage bag; (b) semen on beverage can; and (c) saliva on glossy magazine. Saliva-contaminated fingermarks were visualised at 590 nm with a 650 nm bandpass filter, while the semen-contaminated fingermark was visualised at 530 nm with a 610 nm bandpass filter.
Figure 2-21 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the comparison between aqueous and glycolic working solutions for saliva- and semen-contaminated fingermarks (blue and red columns, respectively). Cling film was not used for saliva-contaminated fingermarks. All fingermarks with no development were removed from this analysis, resulting in no usable data for saliva-contaminated fingermarks on glossy magazine. A positive value favours the glycolic working solution. ...............................................................................................................90

Figure 2-22 Comparison of average CAST scores for corresponding latent fingermark halves. Non-porous substrates were only processed with the multiplex, CA, and CA → R6G, while semi-porous substrates were processed with the multiplex, CA, CA → IND-Zn, and CA → IND-Zn → PD. ...............................................................................................................91

Figure 2-23 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the comparison between the multiplex solution with routine sequences for latent fingermarks. Assessments were performed under each technique’s optimal viewing conditions (Table 2-2). Negative values correspond to a decrease in enhancement of the multiplex solution when compared to the routine technique sequence. The multiplex was compared to CA and CA → R6G on non-porous substrates, and CA, CA → IND-Zn, and CA → IND-Zn → PD on semi-porous substrates. ...............................................................................................................93

Figure 2-24 Comparison of average CAST scores for blood-contaminated fingermark halves processed with the multiplex solution and routine technique sequences............94

Figure 2-25 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the comparison between the multiplex solution with blood reagent sequences. Assessments were performed under each technique’s optimal viewing conditions (Table 2-2). Negative values correspond to a decrease in enhancement of the multiplex solution when compared to the routine technique sequence. ..................95

Figure 2-26 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the comparison of CA → dyestain → multiplex with CA → multiplex (blue) and CA → dyestain (red). Negative values favour CA → dyestain → multiplex. ......................................................................................................................97
Figure 2-27 Average enhancement scores (for a comparative scale between -2 and +2) resulting from comparisons of routine (a) latent and (b) blood reagent sequences with and without the multiplex solution as a pre-treatment. Assessments were performed under each technique’s optimal viewing conditions (Table 2-2). Negative values correspond to a decrease in enhancement of the sequences with the multiplex solutions when compared to the routine technique sequences alone. ................................. 99

Figure 2-28 Representative images of split fingermarks visualised under their respective viewing conditions depicting results for the multiplex solution as a pre-treatment: (a) latent fingermark on a ziplock bag treated with multiplex → CA (left) and CA only (right); and (b) blood-contaminated fingermark on a beverage can treated with CA → multiplex → AY7 (left) and CA → AY7 (right). Each half illustrated was visualised and recorded under optimal viewing conditions for the last enhancement technique implemented (Table 2-2). ................................................................. 100

Figure 2-29 Average enhancement scores (for a comparative scale between -2 and +2) resulting from comparisons of routine (a) latent and (b) blood reagent sequences with and without the multiplex solution as a post-treatment. Assessments were performed under each technique’s optimal viewing conditions (Table 2-2). Negative values correspond to a decrease in enhancement of the sequences with the multiplex solutions when compared to the routine technique sequences alone. ................................. 101

Figure 2-30 Representative images of fingermark halves visualised under their respective viewing conditions depicting results for the multiplex solution as a post-treatment: (a) latent fingermark half on a shopping bag treated with CA only (left) and then CA → multiplex (right); and (b) blood-contaminated fingermark half on a plastic bottle treated with CA → AY7 (left) and then CA → AY7 → multiplex (right). Each half illustrated was visualised and recorded under optimal viewing conditions for the last enhancement technique implemented (Table 2-2). ................................................ 102

Figure 3-1 Schematic diagram of the SELEX process (Reproduced from Stoltenburg et al. [129]). ......................................................................................................................... 109

Figure 3-2 Overview of fingermark-SELEX process, with the addition of GC-MS and sequencing analyses. ........................................................................................................ 115
Figure 3-3 Polymerase chain reaction temperature program used..........................126

Figure 3-4 Example PAGE gels from Round 11 visualised under (a) UV and (b) fluorescence. The bottom band outlined in red was cut out for subsequent desalting and DNA quantification.................................................................127

Figure 3-5 Example UV-vis absorbance spectrum from Round 9 with a 50x dilution of DNA sample................................................................................................................128

Figure 3-6 Graph showing percentage of DNA bound in each negative and positive selection round of fingermark-SELEX. Selection was monitored by the fluorophore attached to the DNA in all rounds except Round 1. The concentration of DNA was lowered in Rounds 2 and 3 to increase stringency............................................134

Figure 3-7 Sequencing data distribution for select SELEX rounds. (# = selection round; N = negative selection round; P = positive selection round)..........................138

Figure 3-8 Squalene peak (highlighted in red box) was detected in Round 6 (top), but not in subsequent Rounds 7 (middle) and 8 (bottom)..................................................140

Figure 3-9 Round 9 samples (fingermarks on garbage bag plastic) using DCM on non-polar column (top), ECF on non-polar column (middle), and ECF on polar column (bottom)..........................................................................................141

Figure 3-10 Round 10 samples (fingermarks on copy paper) using ECF and run on a polar column (top) and non-polar column (bottom). ..................................................142

Figure 4-1 Charged fingermarks deposited on PVDF, aged for 24 hours, and then developed with aptamer-based reagent, each with a different lysozyme aptamer sequence (Reproduced from Wood et al. [195])........................................148

Figure 4-2 Principle of nanoplasmonic imaging of latent fingermarks with and without cocaine present (Reproduced from Li et al.[196]). With respects to detection, a more acceptable term for “addicts” would be “users”. .................................................149
Figure 4-3 (a) Latent fingermarks deposited on marble and visualised with the aid of (b) FAM-labelled, (c) quantum dot-functionalised, and (d) UCNP-functionalised lysozyme-binding aptamer (Reproduced from Wang et al. [197]). ............................... 150

Figure 4-4 Overview of pilot study involving “fingermark” aptamer candidates from fingermark-SELEX process. ................................................................. 153

Figure 4-5 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the comparison between working solutions containing the corresponding short (i.e., 1, 2, 3, 4) and long (i.e., 5, 6, 7, 8, respectively) aptamer candidate sequences. Positive values correspond to an increase in enhancement with the working solution containing the longer aptamer candidate sequence when compared to that with its corresponding shorter aptamer candidate sequence. ........ 168

Figure 4-6 Development obtained on PVDF with working solutions containing corresponding short (left) and long (right) aptamer candidate sequences under: (a) white light and (b) luminescence conditions (530 nm with 590 nm bandpass filter). 169

Figure 4-7 Three-day-old (top row) and 12-day-old (bottom row) fingermarks developed and observed under luminescence conditions (530 nm with 590 nm bandpass filter) on (from left to right) Australian garbage bag, Canadian garbage bag, Australian copy paper, and Canadian copy paper. .................................................. 170

Figure 4-8 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the direct comparison of pairs of longer aptamer candidate sequences. Positive values correspond to an increase in enhancement with the aptamer candidate sequence listed second in its pairing when compared to the aptamer candidate sequence listed first. ................................................................. 171

Figure 4-9 Examples of reverse development on Australian garbage bag (left) and ziplock bag (right) observed under luminescence conditions (530 nm with 590 nm bandpass filter). ................................................................. 172

Figure 4-10 Chemical spot test results for fresh working solution (Sequence 5) by Method 1 (top to bottom): 0.001 M, 0.01 M, and 0.1 M for (left to right): (a) L-serine, glycine, L-ornithine, L-alanine, L-threonine, L-histidine, L-valine, L-leucine, L-
isoleucine, L-lysine, and L-phenylalanine with water control; and (b) stearic acid, oleic acid, squalene, palmitic acid*, and cholesterol* with dichloromethane and blank substrate controls. *Denotes chemical solutions prepared to 0.05 M instead of 0.1 M.

Figure 4-11 Distribution of CAST scores for larger donor population organised by aptamer candidate sequences per substrate. ................................................................. 174

Figure 4-12 Natural fingermarks from the larger donor population study illustrating various types of development: (a) 2-week-old fingerprint aluminium foil with continuous ridge detail; (b) 3-day-old fingerprint on plastic grocery bag with reverse development; and (c) 2-week-old fingerprint on aluminium foil with spotty development indicating pore locations. All fingermarks were visualised at 530 nm with 590 nm bandpass filter. ....................................................................................... 176

Figure 4-13 Three-day-old natural fingermarks developed with Method 1 (left halves) and: (a) Method 2 on Australian garbage bag; (b) Method 3 on Australian garbage bag; and (c) Method 4 on aluminium foil. All fingermarks were visualised at 530 nm with 590 nm bandpass filter. ............................................................................................... 178

Figure 4-14 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the direct comparison of Method 1 to three other working-rinse solution combinations. Positive values correspond to an increase in enhancement with Method 1 when compared to the others. ........................................................................................................ 178

Figure 4-15 Comparison of CAST score frequency distribution between CA → R6G and working solutions containing “fingermark” aptamer candidates (“FM”) per substrate. ....................................................................................................................... 180

Figure 4-16 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the direct comparison of working solutions containing “fingermark” aptamer candidates to CA → R6G. Negative values correspond to a decrease in enhancement with the working solutions when compared to the routine sequence. .. 181
Figure 4-17 Distribution of CAST score frequency as percentages for each working solution per substrate, due to additional samples processed with “fingermark” aptamer candidate-containing reagent (“FM”) and lysozyme aptamer-based reagent. 182

Figure 4-18 One-week-old natural fingermark half deposited by a male donor on aluminium foil, developed with the working solution containing Sequence #5, and visualised at 530 nm with a 590 nm bandpass filter. 183

Figure 4-19 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the direct comparison of working solutions containing “fingermark” aptamer candidates to those containing aptamers against cathepsin, IgE, or lysozyme. Positive values correspond to an increase in enhancement of the novel reagent when compared to the single-target aptamers. 184

Figure 4-20 One-week-old fingermark on aluminium foil with both halves imaged: (a) under optimal viewing conditions (e.g., exposure time adjusted) for both halves; and (b) at the same exposure time. (left half = working solution with “fingermark” aptamer candidate; right half = working solution with single-target aptamer). 185

Figure 4-21 Distribution of CAST score frequency as percentages for each working solution per substrate since “fingermark” aptamer candidate-containing reagent (“FM”) processed all corresponding halves to the three single-target antibodies. 187

Figure 4-22 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the direct comparison of working solutions containing “fingermark” aptamer candidates to those containing anti- cathepsin D, anti-L-amino acid, or anti-serotonin. Positive values correspond to an increase in enhancement of the novel reagent when compared to the single-target antibodies. 189

Figure 4-23 Normal development with working solution containing tris buffer (left) compared to reverse development with working solution containing PBS with non-fat milk (right). Fingermark visualised at 530 nm with 590 nm bandpass filter. 189

Figure 4-24 Distribution of CAST score frequency as percentages for each working solution per substrate since “fingermark” aptamer candidate-containing reagent (“FM”)
processed all corresponding halves to the two multiplex solutions. (M4 and “M8” = multiplex solutions of four and seven antibodies, respectively) ................................. 190

Figure 4-25 Average enhancement scores (for a comparative scale between -2 and +2) resulting from the direct comparison of working solutions containing “fingermark” aptamer candidates to a multiplex solution of four (blue columns) and of seven (red columns) antibodies. Positive values correspond to an increase in enhancement of the novel reagent when compared to the multiplex solutions. .......................................... 192

Figure 4-26 One-week-old fingermarks developed on: (a) Australian garbage bag with working solution containing “fingermark” aptamer candidate (left half) and multiplex of four antibodies (right half); and (b) grocery bag with working solution containing “fingermark” aptamer candidate (left half) and multiplex of seven antibodies (right half). Fingermarks visualised at 530 nm with 590 nm bandpass filter. ....................... 192

Figure X-1 Two-day-old sebaceous fingermark on PVDF developed with working solution containing Aptamer 1 (left) and Aptamer 2 (right) viewed under (a) white light and (b) 505 nm with 555 nm bandpass filter. ................................................................. 217

Figure XI-1 Three-day-old sebaceous fingermarks deposited on (a) Australian and (b) Canadian copy paper and subjected to the working solution for 15 (left halves) and 20 (right halves) seconds. Fingermarks were visualised at 530 nm with a 590 nm bandpass filter. .............................................................................. 219
List of Tables

Table 1-1 Main chemical constituents of the glandular secretions (Adapted from Champod et al. [8]; Lee et al. [27]; Knowles [28]). ...................................................... 38

Table 1-2 Summary of relative abundance (serine ratio) of amino acids in fingerprint deposits (Reproduced from Ramotowski [27]). ............................................................ 41

Table 2-1 List of antibodies, aptamers, and luminescent dyes included in the multiplex solutions. All aptamers were DNA aptamers except vitamin B12. All luminescent dyes were commercially bought except isoindole-1, which was previously synthesised by Spindler (see ESI of [100]). ..................................................................................... 63

Table 2-2 Visualisation conditions utilised............................................................................. 66

Table 2-3 The CAST scale utilised to evaluate the halved fingermarks individually. Representative image examples of fingermark halves are included. ......................... 67

Table 2-4 The UC scale utilised to evaluate corresponding halved fingermarks. Representative image examples illustrate comparisons between the assessed technique (left) and the other technique (right). ............................................................................ 68

Table 2-5 Summary of variables for optimisation study.......................................................... 71

Table 2-6 Routine fingermark detection sequences selected for non-porous and semi-porous substrates. Only latent fingermarks deposited on semi-porous substrates were sequentially processed with three techniques. .............................................................. 74

Table 2-7 Routine sequences used to assess the effects of routine techniques on the multiplex solutions....................................................................................................... 75

Table 2-8 Incorporation of the multiplex solution at different positions within the routine sequences. Only samples of latent fingermarks on semi-porous substrates included the steps in grey. ................................................................. 78
Table 3-1 Summary of aptamer advantages over antibodies (Adapted from O’Sullivan et al. [130]; Jayasena [131]). ...................................................................................................................... 108

Table 3-2 Formulation for PCR master mix .................................................................................................................. 125

Table 3-3 Primer pairing for selected DNA pools for sequencing. (# = selection round; P = positive selection round; N = negative selection round) ................................................. 130

Table 4-1 “Fingermark” aptamer candidate sequences tested. *Predicted secondary structure strength based on manufacturer’s website. Secondary structures predicted by RNAstructure software [203] can be found in Appendix V. ................................................. 159

Table 4-2 Fingermark constituents used for chemical spot tests. (*0.5 M instead of 0.1 M [36]) ........................................................................................................................................... 160

Table 4-3 Various working solution and rinsing solution combinations compared. .......................................................... 161

Table 4-4 Summary of variables used to compare working solutions containing “fingermark” aptamer candidates to previous UTS biomolecular recognition methods. .......................................................................................................................... 164

Table VI-1 Background fluorescence visualised at 530 nm with 590 nm bandpass filter for substrates processed with, from top left (clockwise): Sequences #1 to #8. The middle piece is untreated substrate. .................................................................. 211

Table VII-1 Observations of processed natural and sebaceous fingermarks under luminescence conditions .......................................................................................................................... 213

Table X-1 Lysozyme aptamer sequences used in Wood’s PhD research [101]........ 216
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-SSA</td>
<td>5-sulphosalicylic acid</td>
</tr>
<tr>
<td>7EG</td>
<td>Heptaethylene glycol</td>
</tr>
<tr>
<td>AB</td>
<td>Amido Black</td>
</tr>
<tr>
<td>ACE-V</td>
<td>Analysis, Comparison, Evaluation – Verification</td>
</tr>
<tr>
<td>AgNCs</td>
<td>Silver nanoclusters</td>
</tr>
<tr>
<td>aq</td>
<td>Aqueous</td>
</tr>
<tr>
<td>Au</td>
<td>Gold</td>
</tr>
<tr>
<td>AuNPs</td>
<td>Gold nanoparticles</td>
</tr>
<tr>
<td>AY7</td>
<td>Acid Yellow 7</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>BY40</td>
<td>Basic Yellow 40</td>
</tr>
<tr>
<td>CA</td>
<td>Cyanoacrylate</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>CAST</td>
<td>Centre for Applied Science &amp; Technology</td>
</tr>
<tr>
<td>CE</td>
<td>Capillary electrophoresis</td>
</tr>
<tr>
<td>CMSC</td>
<td>Carleton Mass Spectrometry Centre</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DFO</td>
<td>1,8-Diazafluoren-9-one</td>
</tr>
<tr>
<td>DI</td>
<td>Deionised</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECF</td>
<td>Ethyl chloroformate</td>
</tr>
<tr>
<td>EDC</td>
<td>1-ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EG</td>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>EGA</td>
<td>Estimated gestational age</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ESI</td>
<td>Electronic supplementary information</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>FLS</td>
<td>Forensic light source</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>GYRO</td>
<td>Green-Yellow-Red-Orange</td>
</tr>
<tr>
<td>HDPE</td>
<td>High-density polyethylene</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HTS</td>
<td>High throughput sequencing</td>
</tr>
<tr>
<td>IFRG</td>
<td>International Fingerprint Research Group</td>
</tr>
<tr>
<td>iMMD</td>
<td>Immunological multi-metal deposition</td>
</tr>
<tr>
<td>IND-Zn</td>
<td>1,2-Indanedione-zinc chloride</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>LADDER</td>
<td>Laboratory for Aptamer Discovery and Development of Emerging Research</td>
</tr>
<tr>
<td>LDPE</td>
<td>Low-density polyethylene</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
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<tr>
<td>MES</td>
<td>2-(N-morpholino)ethanesulfonic acid</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>MgCl$_2$·6H$_2$O</td>
<td>Magnesium chloride hexahydrate</td>
</tr>
<tr>
<td>MMD</td>
<td>Multi-metal deposition</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NaOAc</td>
<td>Sodium acetate</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
</tr>
<tr>
<td>NIN</td>
<td>Ninhydrin</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PD</td>
<td>Physical developer</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>PES</td>
<td>Polyethersulfone</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene terephthalate</td>
</tr>
<tr>
<td>PiAnoS</td>
<td>Picture Annotation System</td>
</tr>
<tr>
<td>pNTP</td>
<td>$\rho$-Nitrothiophenol</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>R6G</td>
<td>Rhodamine 6G</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
</tbody>
</table>
RNA  Ribonucleic acid
RT-PCR Reverse transcription polymerase chain reaction
SELEX Systematic Evolution of Ligands by EXponential enrichment
SERS Surface-enhanced Raman scattering
SiO$_2$ Silicon dioxide
ssDNA Single-stranded deoxyribonucleic acid
SWGFAST Scientific Working Group on Friction Ridge Analysis, Study and Technology
TBS Tris-buffered saline
TEMED Tetramethylethylenediamine
THF Tetrahydrofuran
TNT Trinitrotoluene
TTBS Tris-buffered saline with Tween® 20
UC University of Canberra
UCNPs Upconversion nanoparticles
UTS University of Technology Sydney
UV-vis Ultraviolet-visible
VMD Vacuum metal deposition
Abstract

Over the past decade, there has been a resurgence of interest to design fingermark enhancement reagents capable of biomolecular recognition; such reagents would offer high selectivity and sensitivity, two areas where some believe improvement is desired with current fingermark detection methods. In addition to these, a high degree of adaptability for visualisation can be achieved with biomolecular recognition probes, such as antibodies and aptamers, allowing for the selection of the most appropriate visualisation wavelength for a particular luminescent probe or substrate without the need for sophisticated instrumentation or imaging systems. However, the major hurdle to overcome is the balance between sensitivity and selectivity. Single-target biomolecular recognition may be highly selective, purported to have better detection limits than chemical reactions or stains, and can provide information about identity and/or activity, but often results in incomplete ridge pattern development because only a fraction of the fingermark residue is being specifically targeted.

Consequently, the development and evaluation of multi-target biomolecular reagents for fingermark enhancement was investigated, with the focus on endogenous eccrine secretions. A variety of parameters (i.e., processing time, fixing and working solution conditions) were optimised on a wide range of non-porous and semi-porous substrates representative of casework materials to assess the suitability of the biomolecular reagents for potential operational use. The relative performance of biomolecular reagents was compared to that of routine methods applied to latent and body fluid-contaminated fingermarks. The incorporation of these novel reagents into routine technique sequences was also investigated. The experimental results indicated that the multi-target biomolecular reagents were not a suitable alternative to routine detection methods, did not provide any significant enhancement when included in routine sequences; however, they may still have potential for a niche application yet to be identified.

While a larger fraction of the fingermark was being targeted by multi-target reagents, the resulting development seemed to be influenced by inter-donor variability; it was unknown which combination of biomolecular recognition probes would be the most
“universal”. The focus of this research shifted to aptamers due to their many advantageous features over antibodies, one being their versatile \textit{in vitro} selection process called Systematic Evolution of Ligands by EXponential enrichment or SELEX. Up to sixteen fingermark donors deposited variously aged natural fingermarks onto two realistic substrates (i.e., pooled target approach), which were then subjected to a novel SELEX variation termed fingermark-SELEX. Select DNA aptamer candidates, developed specifically against genuine fingermark residues, were subsequently incorporated into a fingermark enhancement reagent. The proof-of-concept work demonstrated this novel reagent’s ability to successfully develop friction ridge detail on non-porous substrates. Its relative performance was superior to that of single-target and multi-target biomolecular reagents previously designed within the same research group. This study has further opened up the possibilities of incorporating biomolecular recognition into fingermark detection methods by recognising and tapping into the potential of SELEX and resulting aptamer candidates in this forensic discipline.
Publications and Presentations

PEER-REVIEWED PUBLICATIONS


ORAL PRESENTATIONS (Presenter = Underlined)


**POSTER PRESENTATIONS** (Presenter = Underlined)
