UTS UNIVERSITY OF TECHNOLOGY SYDNEY

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.

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Rolanda Lam

February 17, 2018

Dedicated to Somebody I Loved Dearly –
You will forever be in my thoughts.

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For those who know me, I like to plan…and let's just say things did not go as planned after I accepted the offer to do a PhD at UTS. But hey, when life gives you lemons, make lemonade, right? (Bet you were all thinking I'd end with "eh", eh?)

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List of Abbreviations

5-SSA	5-sulphosalicylic acid
7EG	Heptaethylene glycol
AB	Amido Black
ACE-V	Analysis, Comparison, Evaluation – Verification
AgNCs	Silver nanoclusters
aq	Aqueous
Au	Gold
AuNPs	Gold nanoparticles
AY7	Acid Yellow 7
BSA	Bovine serum albumin
BY40	Basic Yellow 40
CA	Cyanoacrylate
$CaCl_2$	Calcium chloride
CAST	Centre for Applied Science & Technology
CE	Capillary electrophoresis
CMSC	Carleton Mass Spectrometry Centre
CO_2	Carbon dioxide
DCM	Dichloromethane
DFO	1,8-Diazafluoren-9-one
DI	Deionised
DNA	Deoxyribonucleic acid
ECF	Ethyl chloroformate
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EDTA	Ethylenediaminetetraacetic acid
EG	Ethylene glycol
EGA	Estimated gestational age
ELISA	Enzyme-linked immunosorbent assay
ESI	Electronic supplementary information
EtOH	Ethanol
FLS	Forensic light source
GC-MS	Gas chromatography-mass spectrometry

GYRO	Green-Yellow-Red-Orange
HDPE	High-density polyethylene
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPLC	High performance liquid chromatography
HTS	High throughput sequencing
IFRG	International Fingerprint Research Group
iMMD	Immunological multi-metal deposition
IND-Zn	1,2-Indanedione-zinc chloride
KCl	Potassium chloride
LADDER	Laboratory for Aptamer Discovery and Development of Emerging Research
LDPE	Low-density polyethylene
MeOH	Methanol
MES	2-(N-morpholino)ethanesulfonic acid
$MgCl_2$	Magnesium chloride
$MgCl_2 \cdot 6H_2O$	Magnesium chloride hexahydrate
MMD	Multi-metal deposition
NaCl	Sodium chloride
NaOAc	Sodium acetate
NHS	N-hydroxysuccinimide
NIN	Ninhydrin
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PD	Physical developer
PE	Polyethylene
PES	Polyethersulfone
PET	Polyethylene terephthalate
PiAnoS	Picture Annotation System
pNTP	*p*-Nitrothiophenol
PVDF	Polyvinylidene fluoride
R6G	Rhodamine 6G
RBC	Red blood cell

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 *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

1. **Lam, R.**; Hofstetter, O.; Lennard, C.; Roux, C.; Spindler, X. (2016) Evaluation of Multi-Target Immunogenic Reagents for the Detection of Latent and Body Fluid-Contaminated Fingermarks. *For. Sci. Int.* 264, 168-175.

ORAL PRESENTATIONS (Presenter = Underlined)

1. **Lam, R.** Novel Fingermark Detection Methods Using Biomolecular Recognition. University of Technology Sydney Stage 3 Seminar, July 21, 2017, Broadway, NSW, Australia.

2. Spindler, X.; **Lam, R.**; Sullivan-Davenport, K.; Dilag, J.; Hofstetter, O.; Lennard, C.; Roux, C. Possibilities and Challenges in Using Biomolecular Recognition for Latent Fingermark Detection. Australian and New Zealand Forensic Science Society 23rd International Symposium on the Forensic Sciences, September 22, 2016, Auckland, New Zealand.

3. **Lam, R.** Universal Immunogenic Reagents for the Detection of Latent Fingermarks. University of Technology Sydney Stage 2 Seminar, July 8, 2016, Broadway, NSW, Australia.

4. **Lam, R.;** Ruscito, A.; DeRosa, M.C.; Spindler, X.; Lennard, C.; Roux, C. Fingermark-SELEX: A Novel Approach to Develop DNA Aptamers for Fingermark Detection. 60th Annual Conference of the Canadian Society of Forensic Sciences, May 19, 2016, Montreal, QC, Canada.

5. Spindler, X.; **Lam, R.**; Dilag, J.; Sullivan, K.; Hofstetter, O.; Lennard, C.; Roux, C. Optimisation and Evaluation of Multi-Target Biomolecular Reagents for Latent and Bloody Fingermark Detection: Latest Developments. International Fingerprint Research Group Meeting, October 23, 2015, Patiala, India.

6. Spindler, X.; **Lam, R.**; Dilag, J.; Lennard, C.; Roux, C. Next-Generation Fingermark Reagents: Molecular Recognition, Multispectral Imaging and Mapping. 7th European Academy of Forensic Science Conference, September 8, 2015, Prague, Czech Republic.

7. **Lam, R.**; Spindler, X.; Lennard, C.; Roux, C. Optimisation of Multi-Target Immunogenic Reagents and Comparison to Routine Detection Methods for Latent and Body Fluid-Contaminated Fingermarks. 7th European Academy of Forensic Science Conference, September 8, 2015, Prague, Czech Republic.

8. **Lam, R.**; Spindler, X.; Lennard, C.; Roux, C. Optimisation of Multi-Target Immunogenic Reagents and Comparison to Routine Detection Methods for Latent and Body Fluid-Contaminated Fingermarks. 4th Doctoral School of the École des Sciences Criminelles, August 26, 2015, Les Diablerets, Switzerland.

9. **Lam, R.** Optimisation of Multi-Target Immunogenic Reagents and Comparison to Routine Detection Methods for Latent and Body Fluid-Contaminated Fingermarks. UTS-UWS Forensic Science Research Student Forum, June 30, 2015, Penrith, NSW, Australia.

POSTER PRESENTATIONS (Presenter = Underlined)

1. **Lam, R.;** Ruscito, A.; DeRosa, M.C.; Spindler, X.; Lennard, C.; Roux, C. Fingermark-SELEX: A Novel Approach to Develop DNA Aptamers for Fingermark Detection. 21st Triennial Meeting of the International Association of Forensic Sciences 2017, August 24, 2017, Toronto, ON, Canada.

2. **Lam, R.;** Ruscito, A.; DeRosa, M.C.; Roux, C. "Fingermark" Aptamers: From Random Oligonucleotide Library to Fingermark Detection Reagent. International Association for Identification's 102nd International Forensic Educational Conference, August 8, 2017, Atlanta, GA, USA.

3. **Lam, R.**; Ruscito, A.; DeRosa, M.C.; Spindler, X.; Lennard, C.; Roux, C. Fingermark-SELEX: A Novel Approach to Developing Immunogenic Reagents for Fingermark Detection. Australian and New Zealand Forensic Science Society 23rd International Symposium on the Forensic Sciences, September 22, 2016, Auckland, New Zealand.