A novel approach to latent fingermark detection using aptamer-based reagents

by

Michael Wood

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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.

Michael Wood

DATE

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Now without further ado, boil the kettle, get a brew on, grab some of your favourite biscuits and sit back, relax and enjoy (hopefully!) this thesis. Failing this I recommend a strong Whiskey of some description.

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Abbreviations

ACE-V	Analysis, Comparison, Evaluation - Verification
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALISA	Aptamer-linked immobilised sorbent assay
AMD	Age-related macular degeneration
AMP	Adenosine monophosphate
AMPs	Antimicrobial peptides
ASPV	Apple stem pitting virus
ATP	Adenosine triphosphate
AuNPs	Gold nanoparticles
BSA	Bovine serum albumin
CE	Capillary electrophoresis
CEDIA	Cloned enzyme donor immunoassay
CE-SELEX	Capillary electrophoresis SELEX
DAB	Diaminobenzidine
DCM	Dichloromethane
DFO	1,8-diazafluoren-9-one
DMAC	Dimethylaminocinnamaldehyde
DNA	Deoxyribose nucleic acid
dsDNA	Double-stranded DNA
ECL	Electrochemiluminescence
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELONA	Enzyme-linked oligonucleotide assay
EMIT	Enzyme-multiplied immunoassay technique
FAM	5(6)-Carboxyfluorescein
FBI	Federal Bureau of Investigation
FRET	Fluorescence resonance energy transfer
FTIR	Fourier transform infrared
HCl	Hydrochloric acid
HEX	Hexachloro-6-carboxyfluorescein

HFEs	Hydrofluoroethers
HIV	Human immunodeficiency virus
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
IND	1,2-Indanedione
IND-Zn	1,2-Indanedione-zinc
ISA	Individual autoantibody profile
KCl	Potassium Chloride
K_d	Dissociation constant
LIF	Laser-induced fluorescence
MALDI-MS/P	Matrix Laser Assisted Desorption/Ionisation – Mass Spectrometry/Profiling
MCAR	Mixed cell agglutination reaction
MMD	Multi-metal deposition
MW	Molecular weight
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide
NBT	Nitro blue tetrazolium
NCFS	National Centre for Forensic Studies
NIR	Near infrared
ORO	Oil red O
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PD	Physical developer
PEG	Poly(ethylene glycol)
PSMA	Prostate-specific membrane antigen
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
QD's	Quantum dots
RCMP	Royal Canadian Mounted Police
RDT	Rapid diagnostic tests
RNA	Ribonucleic acid
RP	Ruhemann's purple
RP-Cd	Ruhemann's purple-cadmium

RP-Zn	Ruhemann's purple-zinc
RT-PCR	Reverse transcription PCR
RTX	Ruthenium tetroxide
SDS	Sodium dodecyl sulfate
SELEX	Systematic evolution of ligands by exponential enrichment
siRNA	Small interfering RNAs
SMD	Single-metal deposition
SND	Single-metal nanoparticle deposition
SPR	Surface Plasmon Resonance
ssDNA	Single-stranded deoxyribonucleic acid
TAR	Trans-activation response
TBS	Tris-buffered saline
THC	Δ^9-Tetrahydrocannabinol
THF	Tetrahydrofuran
TiO_2	Titanium dioxide
TLC	Thin layer chromatography
TMCS	Trimethylchlorosilane
TNT	Trinitrotoluene
TTBS	Tween 20 and tris-buffered saline
UK	United Kingdom
UTP	Uridine triphosphate
UV	Ultra Violet
VEGF	Vascular endothelial growth factor
VMD	Vacuum metal deposition
VSC	Video spectral comparator

Abstract

Research into latent fingermark detection and visualisation has taken many paths over the years as researchers and practitioners explore numerous methods to improve existing reagents. The majority of past research has resulted in providing small, incremental improvements to existing techniques. Currently, some researchers have opted to seek more transformational improvements in detection sensitivity, selectivity and visualisation. One such area being investigated is utilising immunology to target proteins, amino acids and drug metabolites in the latent fingermark deposit. Research to date has indicated that antibodies have great potential in providing these transformational improvements due to their ability to bind to certain fingermark components with high sensitivity and selectivity.

Following on from the antibody research, aptamers have been highlighted as the next potential immunogenic technique for several reasons, including reduced health and safety issues, lower cost, greater sensitivity and selectivity, and ease of design and versatility. Aptamers are specifically selected oligonucleotides comprised of either ribonucleic acid (RNA) or single-stranded deoxyribonucleic acid (ssDNA). Due to the selection strategies employed, aptamers can be designed to target most molecules and bind to them with detection limits in the sub-micromolar to nanomolar ranges. Although aptamers have been successfully used in a variety of highly sensitive and selective detection devices, they have not been investigated for use in the detection and visualisation of latent fingermarks prior to this project.

Initially, this project focussed on aptamers targeting amino acids as a means of visualising latent fingermarks. However, it was found that strong, non-specific interactions occurred with both the aptamer and the fluorescent tag, resulting in a lack of success with this approach.

In order to address these issues, aptamers selected to the protein lysozyme were used on fingermarks placed on both PVDF and plain white copier paper. Lysozyme was selected as it was found to be a component in human sweat, while aptamers selected to lysozyme, with binding affinities in the nanomolar range were available. It was found that the aptamer-based reagents possessed high levels of sensitivity with the clear detection of lysozyme at very low concentrations (1 ng). Latent fingermarks from various donors were able to be detected on both substrates, with primary and secondary level detail being clearly visible. Results,

however, were very inconsistent, with marks older than a couple of days being difficult to detect. This was found to be due to the degradation of lysozyme in the latent fingermark. Unfortunately, aptamers to other, possibly more suitable, fingermark components that would circumvent this problem were not available for this project. Despite the difficulties encountered, this project has, for the first time, demonstrated the potential of detecting and visualising latent fingermarks with an aptamer-based reagent. The study has laid the groundwork for future successful investigations that exploit the benefits of aptamers while overcoming the limitations identified in this project.