

Functionalised Organic Field-Effect Transistors as Biosensors for Circulating-Tumour DNA Detection

Ijeoma Patrick

A thesis presented for the degree of
Doctor of Philosophy

Department of Materials
Faculty of Engineering
Imperial College London
January 2023

Declaration of originality

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not already been submitted to satisfy degree requirements at any university or for any professional qualification. Where I have quoted from the work of others, the source is provided.

Ijeoma Patrick

14th January 2023

Supervisors:

Prof. Molly Stevens

Imperial College London

Prof. Martin Heeney

Imperial College London

Examined on 24th February 2023 by:

Dr. Sylvain Ladame

Imperial College London

Dr. Christian Nielsen

Queen Mary University of London



Department of Materials & Centre for Processable Electronics

Imperial College London

South Kensington Campus

London

SW7 2AZ

United Kingdom

The copyright of this thesis rests with the author. Unless otherwise indicated, its contents are licensed under a Creative Commons Attribution-Non Commercial 4.0 International Licence (CC BY-NC). Under this licence, you may copy and redistribute the material in any medium or format. You may also create and distribute modified versions of the work. This is on the condition that: you credit the author and do not use it, or any derivative works, for a commercial purpose. When reusing or sharing this work, ensure you make the licence terms clear to others by naming the licence and linking to the licence text. Where a work has been adapted, you should indicate that the work has been changed and describe those changes. Please seek permission from the copyright holder for uses of this work that are not included in this licence or permitted under UK Copyright Law.

Abstract

This thesis describes work towards the development of an organic field-effect transistor biosensor prototype for breast cancer circulating-tumour DNA detection. The work begins with characterisation of the underlying devices, describing the fabrication of bottom-gate bottom-contact organic field-effect transistor arrays in-house, using microfabrication processes. Commercially-bought silicon substrates were primarily adopted in combination with an automatic, high-throughput, organic semiconductor dispensing system and molecular additive formulations, to optimise the performance of the bare device array. Dispensed organic semiconductor films exhibited lower performance in devices compared to spin coated films, in terms of a lower mobility and ON/OFF ratio. However, devices formed with dispensed films exhibited low inter-device variation. Additionally, a molecular additive (2,3,5,6-Tetrafluoro-7,7,8,8-tetracyanoquinodimethane, F4-TCNQ) was shown to have the most stabilising effect on devices among the additives screened. Towards sensor fabrication, a novel biofunctionalisation strategy was developed. A semiconducting polymer was synthetically modified to generate reactive azide groups on the molecular backbone, that were subsequently used to covalently bind biorecognition elements through click chemistry. This was demonstrated to be successful in covalently-binding polyethylene glycol groups, biotin molecules and nucleic acid sequences, directly to the conjugated polymer surface, which was confirmed using a combination of surface analysis techniques. A streptavidin-biotin proof-of-concept system was employed to validate the BioFET sensing capability. Detection of streptavidin using biotin-functionalised OFETs was achieved spanning several orders of magnitude, with a high fM limit of detection. The thesis concludes with work towards the detection of a nucleic acid sequence associated with a prevalent breast cancer mutation. Through measuring shifts in the threshold voltage parameter, detection of the single-stranded DNA probe immobilisation and the subsequent sensing of 100 nM of the complementary target sequence was achieved. The level of non-specific interactions was evaluated and the selectivity was assessed using base pair mismatch sequences from the target.

Acknowledgements

I have many people to thank for their support in producing this thesis and I sincerely appreciate their contributions.

I would first like to thank my supervisor Prof. Molly Stevens for the years of guidance and kind support and for providing me the opportunity to be part of a group with many fun, talented people. I would especially like to thank my assistant supervisor Dr. Stuart G. Higgins for the continuous advice, empathy and friendship throughout my PhD. I feel lucky to have experienced working so closely with such a talented scientist, because his enthusiasm, professionalism and high standards have provided me with a great foundation. I thank Dr. Adam Creamer for the care and attention he took to training a new PhD student in many new concepts and techniques and for the mentorship and camaraderie that has enriched my experience in the project. A huge thank you to my good friends and colleagues in the Stevens group for being a brilliant lab family and for being great company during the laughs, the cries, the lunches, the parties and everything in between.

Thanks to Florent and Katarzyna in the Centre for Bio-Inspired Technology clean room for the friendly and helpful support at the start of my PhD. Thank you also to the support staff and engineers at the London Centre for Nanotechnology for the helpful assistance in the clean room. Thanks to Gwilherm for the XPS training and for politely responding to my messages at weird times of the day when the equipment was playing up. I would also like to thank the academics, students and support staff in the Centre Processable Electronics and the Doctoral Training Centre program for the continuing support during my PhD - special thanks to Cohort 10 for fostering such a caring environment in which to begin this journey in.

Grazie millie to Lorenzo for being a wonderful friend, confidante and fellow dreamer. An eternal thank you to my Dad, Mum, Chioma and Obinna for the relentless love, support and for being my personal hype team.

Contents

List of Acronyms	8
List of Figures	9
I Background	21
1 Introduction	22
References	25
2 Fundamentals of organic transistor biosensors	27
2.1 Device physics of processable electronics	27
2.1.1 Charge transport in organic semiconductors	27
2.1.2 Organic semiconducting materials	31
2.1.3 Organic field effect transistors	35
2.2 Analytical biosensors	42
2.2.1 Overview on biosensing	42
2.2.2 Performance metrics	43
2.2.3 Organic BioFET sensing	45
References	51
II Results and Discussion	56
3 Organic field-effect transistors	57
3.1 Motivation	57
3.2 Materials and Methods	59
3.2.1 In-house fabricated devices	59
3.2.2 Pre-formed OFET substrates	64
3.3 Results and Discussion	66
3.3.1 In-house fabricated devices	66
3.3.2 Devices fabricated on pre-formed substrates	69
3.4 Conclusions	82
References	84

4	Surface-functionalisation of conjugated polymers	87
4.1	Motivation	87
4.2	Materials and Methods	89
4.2.1	Azide-functionalisation of IDT-FBT	89
4.2.2	Surface biofunctionalisation	89
4.3	Results and Discussion	91
4.3.1	Azide-functionalisation of IDT-FBT	91
4.3.2	PEG immobilisation	92
4.3.3	Biotin immobilisation	96
4.3.4	Single-stranded DNA immobilisation	102
4.4	Conclusions	107
	References	108
5	Streptavidin-targeting BioFET	111
5.1	Motivation	111
5.2	Materials and Methods	112
5.3	Results and Discussion	112
5.3.1	Effect of biotin probe density on the sensor response	112
5.3.2	Evaluating the selectivity of the sensor in response to a non-specific protein	115
5.3.3	Effect of the gate voltage extraction value and device dimensions on the sensor response	116
5.3.4	Expanding the streptavidin BioFET dynamic range and the effect of PEGylation on non-specific interactions	117
5.3.5	Effect of dispensing or spin coating the organic semiconductor on the sensor response	120
5.3.6	Fitting sigmoidal models to BioFET concentration-response curves	121
5.4	Conclusions	124
	References	126
6	Circulating tumour DNA-targeting BioFET	128
6.1	Motivation	128
6.2	Materials and Methods	131
6.2.1	BioFET fabrication and operation	131
6.2.2	Fluorescence assay	131
6.3	Results and Discussion	132
6.3.1	Electronic response of devices immobilised with single-stranded DNA in comparison with bare devices	132
6.3.2	Electrical detection of DNA hybridisation	135
6.3.3	Effect of the ssDNA probe density on target strand hybridisation efficiency	138
6.3.4	Non-specific binding evaluation with sensors formed with pre-reacted ssDNA probes	143
6.3.5	Response comparison between the target and a mismatch sequence exposed to the sensor	145
6.3.6	Toehold-mediated strand displacement in solution	146
6.4	Conclusions	148

References	150
7 Summary and outlook	152
References	158

List of Acronyms

4PL four-parameter logistic

ABN 4-aminobenzonitrile

bpm base-pair mismatch

BioFET field-effect transistor-based biosensor

BSA bovine serum albumin

C₁₆IDT-BT indacenodithiophene-benzothiadiazole

CDT-BT cyclopentadithiophene-benzothiadiazole

ctDNA circulating-tumour DNA

CTC circulating tumour cell

cfDNA cell-free DNA

D-A donor-acceptor

DBCO dibenzocyclooctyne

DMSO dimethyl sulfoxide

DCB dichlorobenzene

DI deionised

dsDNA double-stranded DNA

EGOFET electrolyte-gated organic field-effect transistor biosensor

EV extracellular vesicle

EDL electrical double layer

ELISA electrochemical enzyme-linked immunosorbent assay

F4TCNQ tetrafluorotetracyanoquinodimethane

HOMO highest occupied molecular orbital

IPA Isopropyl alcohol

LOD limit of detection

LUMO lowest unoccupied molecular orbital
MOSFET metal–oxide–semiconductor field-effect transistor
MO molecular orbital
TAE Tris acetate EDTA
rpm revolution per minute
OEET organic electrochemical transistor
OFET organic semiconductor
OTMS octadecyltrimethoxysilane
OSC organic semiconductor
PBS phosphate buffered saline
PCR polymerase chain reaction
PEG polyethylene glycol
pI isoelectric point
KOH potassium hydroxide
PEDOT:PSS poly(3,4-ethylenedioxythiophene) polystyrene sulfonate
P3HT poly(3-hexylthiophene)
PBTTT poly(2,5-bis(thiophen-2-yl)thieno[3,2-b]-thiophene)
PMMA poly(methyl methacrylate)
PTFE polytetrafluoroethylene
PVDF poly(vinylidene fluoride)
PQT poly(3,3'-dialkyl-quaterthiophene)
SAM self-assembled monolayer
ssDNA single-stranded DNA
SPAAC strain-promoted azide-alkyne click
TCNQ tetracyanoquinodimethane
TMSD toehold-mediated strand displacement
ToF-SIMS Time-of-flight secondary ion mass spectrometry
QCM-D Quartz crystal microbalance with dissipation monitoring
XPS X-ray photoelectron spectroscopy

List of Figures

2.1	Electronic configurations of carbon during sp^2 hybridisation.	28
2.2	The formation of σ -bonds and π -bonds from two sp^2 hybridised carbon atoms and four hydrogen atoms to give ethene.	28
2.3	Depiction of energy-level splitting in alkenes with increasing conjugation length, with the band gaps (the lowest-energy transitions from HOMO to LUMO) shown as green arrows. Black arrows represent spin-paired electrons.	29
2.4	Classic charge-transport pathways for semi-crystalline conjugated polymers such as polythiophenes - reproduced with permission from Reference [5] Copyright © 2007 Elsevier Ltd. (a) Charge transport is fastest in the conjugation direction and the π - π stacking direction. Charge transport is slowest in the alkyl stacking direction. (b) Plane-on orientation where the alkyl stacking direction is in the plane of the substrate is unfavourable for OFETs because this is the plane of current flow. (c) Edge-on orientation where alkyl stacking direction is perpendicular to the substrate is preferable.	30
2.5	Schematic of the hopping transport in amorphous organic semiconductors. Carriers are highly localised with a large average hopping distance and large energetic σ and off-diagonal disorder Σ . This transport mode requires large activation energies.	31
2.6	Chemical structures of polymeric semiconductors discussed in this chapter.	32
2.7	Schematic illustrations of the microstructure of conjugated polymers showing a (a) crystalline, (b) semi-crystalline polymer and (c) completely amorphous morphology.	33
2.8	Schematic illustrations of conjugated polymer deposition methods showing (a) drop casting, (b) spin coating and (c) ink-jet printing.	34
2.9	Main geometries for OFETs a) top gate, top contacts, b) top gate, bottom contacts, c) bottom gate, bottom contacts and d) bottom gate, top contacts. Electronic path shown with red arrows.	35
2.10	Carrier concentration distribution and the associated output characteristics of an OFET a) in the linear regime, b) during pinch-off and c) in the saturation regime.	37

2.11	Electrical characterisation of a semiconducting polymer OFET showing (a) the transfer characteristics, with $\sqrt{I_D}$ vs V_D plotted (red points) for the linear fit extraction of the threshold voltage in the saturation regime (indicated with the slope and arrow) and (b) the output characteristics for an OFET device. Arrows indicate forwards and backwards sweeps.	39
2.12	Schematic depiction of the main biosensor components.	42
2.13	Illustration of an approach to the limit of detection calculation from the calibration curve slope and the blank sample response $B_{mean} + 3k\sigma_B$	43
2.14	Illustrations of the common of BioFET structures, the (a) OFET which encompasses a solid-state dielectric, (b) the electrolyte gated OFET (EGOFET) which features an aqueous dielectric and semiconducting polymer and c) the organic electrochemical transistor which features an aqueous dielectric and a conducting polymer (OECT).	46
2.15	Illustration of the gate electrode biofunctionalisation method employed in many EGOFETs and OECTs.	47
2.16	Illustration of the conjugated polymer biofunctionalisation methods commonly employed in organic BioFETs showing (a) physical adsorption and (b) deposition of reactive handles via PE-CVD and subsequent covalent immobilisation.	47
3.1	Process flow depicting the BGBC OFET fabrication process prior to organic semiconductor deposition. First the gate electrodes are patterned, then the dielectric is deposited and finally the source and drain electrodes are patterned on top.	60
3.2	Screenshots of the photomask computer-aided designs showing (a) the 4" wafer layout of the device layers overlaid and (b) a close up of transistor T43. The red layer is the pattern for the the source and drain mask. The turquoise layer is for the dielectric mask, the yellow layer represents the interdigitated gate and the green layer represents where the semiconductor is to be deposited.	60
3.3	Microscope images of the patterned bilayer photoresist used to define the gate electrodes showing a) the resolution marks and b) a 10 μm channel length device. The undercut is seen as the thin shadow around the pattern edges. Scale bar = 100 μm . The resolution marks refer to the designed width of the lines in the adjacent resulting pattern.	61
3.4	Photographs of the gate patterning process showing 4" diameter wafer substrates (a) after Au evaporation, (b) the metallized wafers in remover and (c) the wafer after lift-off with the resulting patterned electrodes. . . .	61

3.5	Microscope images of the gold gate electrodes showing a) resolution marks and b) a 10 μm channel length device. Scale bar = 100 μm . The resolution marks refer to the designed width of the lines in the adjacent resulting pattern.	61
3.6	Water contact angle images of the dielectric materials used for the OFETs.	62
3.7	Microscope images of the patterned bilayer resist used to define the source and drain electrodes showing (a) a device with standard electrode and b) a device with an interdigitated electrode design. The undercut can be seen as a thin shadow/border along the pattern edges. Scale bar = 100 μm . . .	63
3.8	Photographs of the source and drain patterning process on 4" wafers showing (a) the substrates after evaporation and (b) the wafer after the rinsing and drying left with the patterned electrodes.	63
3.9	Microscope images of patterned source and drain electrodes showing (a) a device with standard electrode and b) a device with interdigitated electrode design. Scale bar = 100 μm	63
3.10	(a) Photograph of the BCBC chip quadrant, (b) photograph of the chip spin coated with $\text{C}_{16}\text{IDT-BT}$ (5 mg mL^{-1} at 1000 rpm, 200 rpm s^{-1} , 30 s) and (c) microscope images of one of the transistors with source, drain and gate contacted by the probe station tips for electrical characterisation. Scale bar of (a) and (b) = 1 cm and scale bar of (c) = 100 μm	64
3.11	Chemical structure of OTMS.	65
3.12	Illustration of OFET fabrication on the pre-formed Si/SiO ₂ substrates. . .	65
3.13	Photograph of the pre-formed Si/SiO ₂ substrate after spin coating with the semiconducting polymer.	65
3.14	(a) Photograph of the BioDOT system during dispensing of the OSC ink array and (b) the resulting printed array. Scale bar = 1 mm.	66
3.15	Device characteristics of an OFET device (T39) with dimensions $W = 10$ mm, $L = 50$ μm dimensions (T39) including (a) the transfer characteristics (black) with the leakage current plotted (red) with 5 double sweep replicates and (b) the output characteristics. Arrows represent the forwards and backwards sweeps.	66
3.16	Heat map representing the threshold voltage V_T values of the the OFET array where grey boxes represent defected devices.	67
3.17	(a) Plot depicting the mean mobility and (b) the mean threshold voltage for four 100 μm channel-length devices denoted T30, T35, T45 and T50. $N = 5$ per device.	68
3.18	Chemical structure of the semiconducting polymer IDT-BT-N ₃	69
3.19	Contact angle measurements of the SiO ₂ surface during the silanization process. $N = 3$ on three areas of the sample.	70

3.20	Transfer characteristics of 5 mg mL ⁻¹ IDT-BT-N ₃ OFETs formed on Si/SiO ₂ substrates with and without OTMS treatment. 3 current-voltage sweep replicates are shown. The arrows and filled and unfilled symbols represent the forwards and backwards sweeps.	70
3.21	Plot depicting the mean (a) ON/OFF ratio, (b) mobility and (c) mean threshold voltage for 5 mg mL ⁻¹ IDT-BT-N ₃ OFET devices of the same size formed on Si/SiO ₂ with and without OTMS treatment. N = 3. Error bar represents the standard deviation.	71
3.22	Transfer characteristics of 5 mg mL ⁻¹ IDT-BT-N ₃ OFETs formed on Si/SiO ₂ substrates via spin coating. Plot depicts devices T1 - T16. Colour gradient from light to dark represents the channel-lengths 20 μm, 10 μm, 5 μm, 2.5 μm. 5 current-voltage sweep replicates are shown. Arrows represent the forwards and backwards sweeps.	73
3.23	Plot depicting the mean (a) ON/OFF ratio, (b) mobility and (c) threshold voltage for 16 devices formed on Si/SiO ₂ substrates via spin coating 5 mg mL ⁻¹ IDT-BT-N ₃ , grouped by channel-length. N = 4. Error bars represent the standard deviation.	73
3.24	Transfer characteristics of 5 mg mL ⁻¹ IDT-BT-N ₃ OFETs formed on Si/SiO ₂ substrates via dispensing. Plot depicts devices T1 - T16. Colour gradient from light to dark represents the channel-lengths 20 μm, 10 μm, 5 μm, 2.5 μm. 5 current-voltage sweep replicates are shown. Arrows represent the forwards and backwards sweeps.	74
3.25	Plot depicting the mena (a) ON/OFF ratio, (b) mobility and (c) mean threshold voltage for 16 devices formed on Si/SiO ₂ substrates via dispensing 5 mg mL ⁻¹ IDT-BT-N ₃ droplets, grouped by channel-length. N = 4. Error bar represents the standard deviation.	75
3.26	Chemical structures of the additives used in this section: ABN, TCNQ and F4TCNQ.	76
3.27	Transfer characteristics of devices incorporated with various additives into the IDT-BT-N ₃ formulation. Plot depicts 5 current-voltage sweep replicates of four devices T5 - T8 per additive formulation. Each additive was incorporated in the semiconductor at 10 wt%. Arrows represent forward and backwards sweeps.	77
3.28	Heat maps depicting (a) the mean ON/OFF ratio for devices T5, T6, T7, T8 and (b) the associated standard deviation. N = 5.	78
3.29	Heat maps depicting (a) the mean threshold voltage for devices T5, T6, T7, T8 and (b) the associated standard deviation. N = 5	79
3.30	Heat maps depicting (a) the mean mobility for devices T5, T6, T7, T8 and (b) the associated standard deviation. N = 5	79

3.31	Transfer characteristics of devices incorporated with F4TCNQ additives in the IDT-BT-N ₃ formulation from 1 - 10 wt.%. Plot depicts 5 x IV sweep replicates of four devices T5 - T8 per chip. Arrows represent forward and backwards sweeps.	80
3.32	Heat maps depicting (a) the mean ON/OFF ratio across 5 replicates for devices T5, T6, T7, T8 and (b) the associated standard deviation.	81
3.33	Heat maps depicting (a) the mean threshold voltage across 5 replicates for devices T5, T6, T7, T8 and (b) the associated standard deviation.	81
4.1	Schematic of the conjugated polymer surface functionalisation through strain-promoted azide-alkyne click chemistry reaction.	88
4.2	Chemical structure of the resulting of conjugated polymer after functionalisation with a DBCO-conjugate through the strain-promoted azide-alkyne click chemistry reaction. R represents the biorecognition element.	89
4.3	Schematic of the nucleophilic aromatic substitution reaction used to functionalise the conjugated polymer with azide groups. The fluorine groups on the backbone are substituted with the nucleophilic group bearing an azide.	90
4.4	Photographs of (a) IDT-FBT solution in THF and (b) IDT-BT-N ₃ solution in THF and (c) UV-Vis absorption spectra of IDT-FBT and IDT-BT-N ₃ in THF.	91
4.5	Proton NMR of the product IDT-BT-N ₃ (red spectrum) and the starting material IDT-FBT (black spectrum). The corresponding structures are provided for reference. Data collected with assistance from Dr. Adam Creamer.	92
4.6	Fluorine NMR of the product IDT-BT-N ₃ (red spectrum) and the starting material IDT-FBT (black spectrum). The corresponding structures are provided for reference. Data collected with assistance from Dr. Adam Creamer.	92
4.7	Schematic of the surface functionalisation of conjugated polymer thin films with PEG groups.	93
4.8	Chemical structure of the resulting of conjugated polymer surface after functionalisation with a DBCO-PEG2K conjugate through the strain-promoted azide-alkyne click chemistry reaction. n = 2000	93
4.9	Contact angle measurements of DBCO-PEG2K conjugates immobilised on the IDT-BT-N ₃ thin film surface. N = 3. The errors bars represent the standard deviations.	94

4.10	The example of the QCM-D frequency signal change upon immobilisation of DBCO-PEG5K to the sensor coated with IDT-BT-N ₃ after rinsing with the DMSO immobilisation buffer. The time points at which the PEG solution was introduced to the sensor and when the buffer rinse was introduced are marked with red arrows.	95
4.11	The effect of PEGylation on the non-specific adsorption of a streptavidin-AlexaFluor790 conjugate. Examples of region of interests use to measure pixel intensity are highlighted in yellow. Scale bar = 2 mm.	96
4.12	Schematic of the surface functionalisation of conjugated polymer thin films with DBCO-PEG-Biotin groups.	97
4.13	Chemical structure of the resulting of conjugated polymer surface after functionalisation with a DBCO-PEG2K-Biotin conjugate through the strain-promoted azide-alkyne click chemistry reaction. n = 2000	97
4.14	Contact angle measurements of DBCO-PEG2K-Biotin conjugates immobilised on the IDT-BT-N ₃ thin film surface. N = 3. The error bars represent the standard deviations.	98
4.15	Fluorescent microscope images of streptavidin-AlexaFluor790 conjugates bound to biotinylated conjugated polymer thin films. Examples of region of interests used to measure pixel intensity are highlighted in yellow. Scale bar = 1.5 mm	98
4.16	TOF-SIMS total positive-ion images of characteristic PEG ion fragments (CH ₃ O ⁺ and C ₂ H ₅ O ⁺) on IDT-BT-N ₃ thin films functionalised with DBCO-PEG2K-Biotin. Colour scale represents the ion fragment signal intensity in arbitrary units. Data collected with assistance from Dr. Sarah Fearn.	99
4.17	TOF-SIMS total positive-ion images of characteristic biotin ion fragments (C ₂ H ₄ N ⁺ and C ₃ H ₈ N ⁺) on IDT-BT-N ₃ thin films functionalised with DBCO-PEG2K-Biotin. Colour scale represents the ion fragment signal intensity in arbitrary units. Data collected with assistance from Dr. Sarah Fearn.	100
4.18	XPS spectra of IDT-BT-N ₃ thin films (a) before functionalisation and (b) after functionalisation with DBCO-PEG4-Biotin.	101
4.19	Relative atomic compositions of IDT-BT-N ₃ thin films before and after functionalisation with biotin conjugates.	101
4.20	Schematic showing biofunctionalisation of conjugated polymer thin films with ssDNA.	102
4.21	Chemical structure of the resulting of conjugated polymer surface after functionalisation with a DBCO-ssDNA conjugate through the strain-promoted azide-alkyne click chemistry reaction.	102

4.22	Contact angle measurements of DBCO-ssDNA concentrations immobilised on the IDT-BT-N ₃ thin film surface. N = 3. Error bars represent the standard deviations.	103
4.23	Fluorescent microscope images of IDT-BT-N ₃ thin films reacted with DBCO-ssDNA-Alexa790 solutions through click chemistry. Examples of region of interests used to measure pixel intensity are highlighted in yellow. Scale bar = 1.5 mm	104
4.24	XPS C1s analysis (a) Comparison between control IDT-BT-N ₃ and ssDNA functionalised spectra, (b) Peak fits of the ssDNA functionalised sample to the raw data with background and residuals plotted.	104
4.25	XPS N1s analysis (a) Comparison between control IDT-BT-N ₃ and ssDNA functionalised spectra, (b) Peak fits of the ssDNA functionalised sample to the raw data with background and residuals plotted.	105
4.26	XPS O1s analysis (a) Comparison between control IDT-BT-N ₃ and ssDNA functionalised spectra, (b) Peak fits of the ssDNA functionalised sample to the raw data with background and residuals plotted.	106
4.27	XPS P2p analysis (a) Comparison between control IDT-BT-N ₃ and ssDNA functionalised spectra, (b) Peak fits of the ssDNA functionalised sample to the raw data with background and residuals plotted.	106
4.28	XPS Si2p analysis of ssDNA functionalised IDT-BT-N ₃ thin films.	106
5.1	Schematic representation of the fabrication of a streptavidin-targeting BioFET and the resulting assay operation.	112
5.2	(a) Transfer characteristics (back sweep) of a single streptavidin BioFET device functionalised with 1 mM biotin in the saturated regime $V_D = -40$ V with IDT-BT-N ₃ as the semiconducting polymer. Each data point I_D is the mean value of N = 5 current-voltage sweep replicates, (b) Normalised response of the streptavidin BioFET to increasing streptavidin concentrations. I_D and I_0 are taken at -35 V for each concentration. N = 30 for 6 devices of different dimensions. Error bars represent the standard deviations.	113
5.3	(a) Transfer characteristics (back sweep) of a single streptavidin BioFET device functionalised with 0.5 mM biotin in the saturation regime $V_D = -40$ V with IDT-BT-N ₃ as the semiconducting polymer. Each data point I_D is the mean value of N = 5 current-voltage sweep replicates. (b) Normalised response of the streptavidin BioFET to increasing streptavidin concentrations. I_D and I_0 are taken at -35 V for each concentration. N = 25 for 5 devices. Error bars represent the standard deviations.	114

5.4	Response comparison of a biotin-functionalised device exposed to increasing streptavidin (black) and bovine albumin serum concentrations (red). $N = 20$ for 4 devices. Error bars represent the standard deviations.	115
5.5	Depiction of the response extraction method used so far in this results chapter. (a) The I_D and I_0 values are extracted from the raw data at $V_G = -35$ V. (b) The extracted values are inputted into the response equation and to result in normalised response values that can be plotted against the dose.	116
5.6	Effect of the gate voltage extraction value V_G on the normalised current response. The optimal value for the calibration curve was chosen at $V_G = -35$ V for a balance between signal and noise.	117
5.7	(a) The response plotted per device showing populations of device dimensions $L = 20\mu\text{m}$ (purple points) and $L = 10\mu\text{m}$ (green points) and (b) the response normalised for the device dimensions with a scaling factor L/W showing a reduction in the variation.	118
5.8	Transfer characteristics (back sweep) of a streptavidin Bio-FET device ($W/L = 1000/5$) functionalised with 0.5 mM biotin in the saturation regime $V_D = -40$ V, with a 1:1 IDT-BT- N_3 as the semiconducting polymer. Mean I_D is plotted. $N = 5$	118
5.9	Response comparison of a biotin-functionalised and PEG-functionalised Bio-FET exposed to different streptavidin concentrations. I_D is taken at -35 V for each sample. $N = 20$ for 3 devices of identical dimensions. Error bars represent the standard deviations.	119
5.10	Transfer characteristics (back sweep) of a streptavidin Bio-FET device ($L/W = 5/1000$) with a printed semiconducting polymer functionalised with 0.5 mM DBCO-PEG2K-Biotin in the saturated regime $V_D = -40$ V, with 1:1 IDT-BT- N_3 :IDT-BT blend as the semiconductor. Mean I_D is plotted. $N = 5$	120
5.11	Response comparison of a BioFET fabricated with a dispensed OSC and spincoated OSC exposed to different streptavidin concentrations. I_D is taken at -35 V for each sample. $N = 15$ for three devices. Error bars represent the relative standard deviations.	121
5.12	Concentration-response curve of a streptavidin BioFET (fabricated via spin coating) with a four-parameter sigmoidal function modelled to the average response data. $N = 3$. The mean blank response level is indicated (red line) along with the error (grey area) and LOD.	122
5.13	An alternative method of LOD calculation taking into account the average noise level of the negative control experiment. The mean blank response level is indicated (red line) along with the error (grey area) and LOD. . . .	123

5.14	Concentration-response curve of a streptavidin BioFET (fabricated via dispensing) with a four-parameter sigmoidal function modelled to the average response data for 3 devices of identical dimensions. The mean blank response level is indicated (red line) along with the error (grey area) and LOD.	123
6.1	Illustration of a typical breast cancer patient journey from detection to treatment, highlighted in grey with the location where BioFET technology is proposed to enter.	129
6.2	Illustration of the liquid biopsy procedure. Tumour cells release fragments of DNA into the bloodstream as they die along with also biological materials. Such components can be isolated from blood samples and used for analysis.	130
6.3	Schematic representation of the fabrication of the ctDNA-targeting BioFET and the resulting assay operation.	131
6.4	Photograph of the OFET chip during immobilisation with ssDNA. The yellow box signifies the devices which were tested. T5 and T6 were printed with the ssDNA solution whilst T7 and T8 were left blank. Scale bar = 1 mm.	132
6.5	Transfer characteristics of four OFETs before (grey points) and after (orange points) immobilisation with 10 μ M single-stranded DNA probe sequence (T5 and T6). For the control devices that were left blank/without DNA (T7 and T8) the bare device was first tested (grey points) and then again (orange points) for comparison. Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.	133
6.6	Extracted V_T values of OFETs (T5 and T6) before (grey violin) and after (orange violin) immobilisation with 10 μ M single-stranded DNA probe sequence. For the devices that were left blank/without DNA (T7 and T8) these were first tested (grey violin) and then again (orange violin) for comparison. $N = 5$	134
6.7	Table summary of the data shown in Figure 6.6.	135
6.8	Transfer characteristics of four OFETs after ssDNA immobilisation (T5 and T6) (orange points), after the buffer reference sample (blue points) and after hybridisation with 100 nM of the target strand solution (green points). For the devices that were left blank/without probe DNA (T7 and T8) these were tested at the same time as the other devices for reference. Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.	136

6.9	Extracted V_T values of four OFETs after ssDNA immobilisation (T5 and T6) (orange points), after the buffer reference sample (blue points) and after hybridisation with 100 nM of the target strand solution (green points). For the devices that were left blank/without probe DNA (T7 and T8) these were tested at the same time as the other devices for reference. $N = 5$. . .	137
6.10	Table summary of the data shown in Figure 6.9.	138
6.11	Transfer characteristics of four OFETs before (grey points) and after immobilisation with (a) 10 μM , (b) 5 μM , (c) 2.5 μM and (d) 1.25 μM ssDNA (yellow points). Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively. .	139
6.12	Extracted V_T of OFETs before (grey) and after immobilisation with (a) 10 μM , (b) 5 μM , (c) 2.5 μM and (d) 1.25 μM ssDNA (orange). Each point represents a replicate. $N = 15$ per concentration.	140
6.13	Table summary of the data shown in Figure 6.12.	140
6.14	Transfer characteristics of OFETs after immobilisation with (a) 10 μM , (b) 5 μM , (c) 2.5 μM and (d) 1.25 μM ssDNA (orange points), also after the buffer reference sample (blue points) and after hybridisation with 100 nM of the target strand solution (green points). Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.	141
6.15	Extracted V_T of four OFETs immobilised with ssDNA (orange), after the buffer sample (blue), and after exposure to the target sample (green) for (a) 10 μM , (b) 5 μM , (c) 2.5 μM and (d) 1.25 μM ssDNA probes. $N = 15$ per concentration and status.	142
6.16	Table summary of the data shown in Figure 6.16.	142
6.17	(a) Transfer characteristics of OFETs before (grey points) and after immobilisation with 5 μM pre-reacted DBCO-ssDNA solution (orange points) and (b) the device after exposure to the buffer reference sample (blue points) and after exposure to 100 nM of the target strand solution (green points). $N = 5$ current-voltage sweep replicates for a single device. Filled and empty shapes represent the backwards and forwards sweeps respectively.	144
6.18	Extracted V_T of OFETs before (grey) and after immobilisation with pre-reacted ssDNA (orange), after the buffer reference sample (blue), and after exposure to the target sample (green). $N = 20$ per status.	144
6.19	Extracted V_T of OFETs before (grey points), after immobilisation with pre-reacted ssDNA (orange), after the buffer reference (blue), and after exposure to the 3 base-pair mismatch sequence (yellow points). $N = 20$ per assay stage.	145

6.20	Schematic depiction of the toehold-mediated strand displacement mechanism with a fluorophore-quencher system.	146
6.21	Fluorescence intensity measurements of the seven well plates (a) before and (b) after addition of target and mismatch sequences in clear-walled well plates.	147
6.22	Fluorescence intensity measurements of the seven well plates (a) before and (b) after addition of target and mismatch sequences in black-walled well plates.	147

Part I

Background

Chapter 1

Introduction

The last few years have solidified the notion that biosensors are integral to maintaining our global health. In most developed nations where life expectancy has increased significantly, these extra years are frequently impaired by long-term chronic illnesses which require constant treatment and monitoring. Traditionally, biosensing instruments have been designed for clinical applications, requiring large sample volumes, specialist equipment and complex protocols within a central laboratory. However, there is an ever growing need for patient-friendly diagnostic methods[1][2]. Specific biomarkers that can be detected with biosensors without needing hospital visits, would greatly improve the patient journey, particularly for those living in developing nations or rural areas. The rise of the field of ‘organic bioelectronics’ as coined in 2007 by Berggren and Richter-Dahlfors[3], has resulted in the creation of organic transistor-based biosensors (BioFETs), which can efficiently transduce and amplify detected biological signals into electrical signals, with promising implications for healthcare monitoring[4].

The invention of the transistor in the mid-century sparked the subsequent revolution in computing and the dawn of the electronic age[5]. Transistors have been traditionally formed on inorganic semiconductors such as silicon or germanium. However in the late 1970s, a certain class of carbon-rich molecules were demonstrated to exhibit similar electrical properties to inorganic semiconductors and not long after, the first attempts at organic field-effect transistors (OFETs) followed[6]. Intense research over the decades has improved their performance considerably, however relatively slow switching speeds and environmental instability have hindered their widespread application in consumer electronics. Nonetheless organic semiconductors, often based on conjugated polymers, are a particularly effective interface between biology and electronics[7], with applications spanning cell and tissue scaffolds[8], optoelectronic stimulation[9] and drug delivery[10]. They possess similar chemical structures to common biological components and thus show excellent biocompatibility[11][12]. The sheer variety of organic semiconductors and their ability to be designed or tailored to fulfil certain molecular design or biological functionality is central and provides a highly versatile material system with great potential.

The motivations for applying OFETs to biological sensing are manifold. Fundamentally, transistors are amplifiers, tasked with raising the strength of a weak signal thus such devices possess an inherent sensitivity. Large amounts of data can be generated and multiple parameters can be tracked simultaneously, providing rich sensing information. BioFETs operate label-free, requiring no prior tagging of the analyte of interest and typically require much shorter analysis times and lesser sample volumes compared to traditional biosensors. The key difference from inorganic devices is that OFETs are compatible with solution-processable techniques, with the potential for large-area high-throughput fabrication of devices and a considerable reduction in manufacturing costs. Also, the low-temperature deposition capability allows wider options for substrates such as glass[13], flexible plastics[14] and papers[15]. To date, organic BioFETs have been applied to the detection of cortisol[16], glucose[17], nitrates[18] and nucleic acids[19] to name a few.

Despite the broad applications of these biosensors, key bottlenecks involving factors such as the stability, sensitivity and selectivity remain. The future application of such devices is likely in single-use biosensors where long-term operational stability is not crucial, however environmental degradation should be minimised to maximise sensors lifetimes and improve reliability. The sensitivity of organic semiconductors to moisture is an ongoing limitation. Devices exposed to humid environments have exhibited degraded performance and undesirable characteristics[20]. This has inspired researchers to look for alternative device configurations, such as the incorporation of encapsulation layers[21] or spatially separating the sensing area from the organic semiconductor film[22], however this can cause a trade-off with the sensitivity. Strategies to improve the environmental stability of OFETs are still desired. Material processing methods such as incorporating dopants, molecular additives or nanoparticles into organic semiconductor formulations have also been investigated as solutions[23][24].

The issues concerning sensitivity and selectivity could be partly attributed to the lack of efficient functionalisation routes for conjugated polymers. Strategies to attach biorecognition elements to the sensor surface that do not involve laborious multi-step protocols, without significantly degrading the electronic properties and in a manner that provides sufficient sensitivity, are few. This has resulted in a growing number of reports utilising electrolyte-gated organic field-effect transistor biosensors[25] (EGOFETs) where the dielectric is replaced by an aqueous electrolyte and the gate electrode is functionalised instead. However, this sensor configuration is more complex and likely less suited for mass production processes. Ultimately, maximising the performance of these sensors is considerable engineering challenge. It will require advancement in multiple areas simultaneously, optimising the microstructure of the organic semiconductor layer, the interface of immobilised biorecognition elements and the device architecture and fabrication.

The aim of the research presented in this thesis was to develop of a highly sensitive BioFET prototype, fabricated with a unique biofunctionalisation strategy, for the targeting of nucleic acid sequences associated with a prevalent breast cancer biomarker. A brief overview of the chapters from this point are provided below:

Thesis overview

Chapter 2

Theoretical concepts provided on charge transport in conjugated polymers; device physics of organic field-effect transistors; common conjugated polymers applied in BioFETs; important concepts in analytical biosensing.

Chapter 3

Fabrication and characterisation of OFET arrays; work towards the development of OFETs in-house using microfabrication techniques; optimisation of OFET arrays formed on commercially-bought substrates utilising an automatic dispensing system; molecular additives for organic semiconductor formulations investigated.

Chapter 4

Demonstration of a versatile surface biofunctionalisation method to covalently-bind biomolecules to conjugated polymer surfaces; synthetic modifications to organic semiconductor made to generate reactive groups and utilise click chemistry; combination of surface analysis employed techniques for characterisation.

Chapter 5

Streptavidin-biotin system applied for initial validation of the BioFET platform; biotin capture probes immobilised on the sensor surface are used to target streptavidin; sensing response is analysed; non-specific interactions are assessed.

Chapter 6

Proof-of-concept system for ctDNA targeting of a prevalent breast cancer biomarker; single-stranded DNA capture probes are used to detect complementary target sequences; sensing response is analysed; non-specific interactions are assessed.

Chapter 7

Concluding comments; critical commentary on the outlook of BioFET sensing is provided.

References

- [1] P. Li, G.-H. Lee, S. Y. Kim, S. Y. Kwon, H.-R. Kim, S. Park, *ACS Nano* **2021**, *15*, 1960–2004.
- [2] S. Mondal, N. Zehra, A. Choudhury, P. K. Iyer, *ACS Applied Bio Materials* **2021**, *4*, 47–70.
- [3] M. Berggren, A. Richter-Dahlfors, *Advanced Materials* **2007**, *19*, 3201–3213.
- [4] J. Wang, D. Ye, Q. Meng, C.-a. Di, D. Zhu, *Advanced Materials Technologies* **2020**, *5*, 2000218.
- [5] I. Ross, *Proceedings of the IEEE* **1998**, *86*, Conference Name: Proceedings of the IEEE, 7–28.
- [6] H. Koezuka, A. Tsumura, T. Ando, *Synthetic Metals*, Proceedings of the International Conference of Science and Technology of Synthetic Metals **1987**, *18*, 699–704.
- [7] S. G. Higgins, A. Lo Fiego, I. Patrick, A. Creamer, M. M. Stevens, *Advanced Materials Technologies* **2020**, *5*, 2000384.
- [8] K. Fidanovski, D. Mawad, *Advanced Healthcare Materials* **2019**, *8*, 1900053.
- [9] M. Han, E. Yildiz, H. N. Kaleli, S. Karaz, G. O. Eren, I. B. Dogru-Yuksel, E. Senses, A. Şahin, S. Nizamoglu, *Advanced Healthcare Materials* **2022**, *11*, 2102160.
- [10] H.-C. Tian, J.-Q. Liu, X.-Y. Kang, Q. He, B. Yang, X. Chen, C.-S. Yang, *Sensors and Actuators A: Physical* **2015**, *228*, 28–39.
- [11] G. Lanzani, *Nature Materials* **2014**, *13*, 775–776.
- [12] J. Tropp, J. Rivnay, *Journal of Materials Chemistry C* **2021**, *9*, 13543–13556.
- [13] D. K. Hwang, R. R. Dasari, M. Fenoll, V. Alain-Rizzo, A. Dindar, J. W. Shim, N. Deb, C. Fuentes-Hernandez, S. Barlow, D. G. Bucknall, P. Audebert, S. R. Marder, B. Kippelen, *Advanced Materials* **2012**, *24*, 4445–4450.
- [14] S. G. Higgins, B. V. O. Muir, J. Wade, J. Chen, B. Striedinger, H. Gold, B. Stadlober, M. Caironi, J.-S. Kim, J. H. G. Steinke, A. J. Campbell, *Advanced Electronic Materials* **2015**, *1*, DOI 10.1002/aelm.201570013.

- [15] M. Y. Lee, J. Hong, E. K. Lee, H. Yu, H. Kim, J. U. Lee, W. Lee, J. H. Oh, *Advanced Functional Materials* **2016**, *26*, 1445–1453.
- [16] H.-J. Jang, T. Lee, J. Song, L. Russell, H. Li, J. Dailey, P. C. Searson, H. E. Katz, *ACS Applied Materials & Interfaces* **2018**, *10*, 16233–16237.
- [17] D. Elkington, M. Wasson, W. Belcher, P. C. Dastoor, X. Zhou, *Applied Physics Letters* **2015**, *106*, 263301.
- [18] T. Minami, Y. Sasaki, T. Minamiki, S.-i. Wakida, R. Kurita, O. Niwa, S. Tokito, *Biosensors and Bioelectronics* **2016**, *81*, 87–91.
- [19] C. Napoli, S. Lai, A. Giannetti, S. Tombelli, F. Baldini, M. Barbaro, A. Bonfiglio, *Sensors (Basel Switzerland)* **2018**, *18*, DOI 10.3390/s18040990.
- [20] H. Sirringhaus, *Advanced Materials* **2009**, *21*, 3859–3873.
- [21] E. K. Lee, M. Y. Lee, C. H. Park, H. R. Lee, J. H. Oh, *Advanced Materials* **2017**, *29*, 1703638.
- [22] J. Song, J. Dailey, H. Li, H.-J. Jang, P. Zhang, J. T.-H. Wang, A. D. Everett, H. E. Katz, *Advanced Functional Materials* **2017**, *27*, 1606506.
- [23] M. Nikolka, I. Nasrallah, B. Rose, M. K. Ravva, K. Broch, A. Sadhanala, D. Harkin, J. Charmet, M. Hurhangee, A. Brown, S. Illig, P. Too, J. Jongman, I. McCulloch, J.-L. Bredas, H. Sirringhaus, *Nature Materials* **2016**, *16*, 356.
- [24] R. A. Picca, K. Manoli, A. Luciano, M. C. Sportelli, G. Palazzo, L. Torsi, N. Cioffi, *Sensors and Actuators B: Chemical* **2018**, *274*, 210–217.
- [25] D. Wang, V. Noël, B. Piro, *Electronics* **2016**, *5*, 9.

Chapter 2

Fundamentals of organic transistor biosensors

This chapter provides an overview of the relevant theory behind organic semiconductors, their devices and the analytical biosensors formed from them. The first section outlines the origin of semiconducting behaviour in conjugated polymers and the models used to describe charge transport behaviour in these systems. The effects of disorder on charge transport is discussed along with typical design criteria considered to promote efficient transport. Next, popular conjugated polymers used in OFETs are reviewed along with important structure-property concepts. Then, organic semiconductor processing methods are evaluated before detailing the key OFET operating principles. The second section discusses essential concepts in analytical biosensing, including definitions of key performance metrics. Finally, an overview on the current state-of-the-art of organic BioFET sensing is provided.

2.1 Device physics of processable electronics

2.1.1 Charge transport in organic semiconductors

Hybridisation and molecular orbital theory

Molecules are labelled organic when one or more atoms of carbon (C) are covalently linked to atoms of other elements, commonly hydrogen, oxygen or nitrogen. The most common carbon isotope contains 6 protons, 6 neutrons, 6 electrons and has a ground state electronic configuration of $1s^2 2s^2 2p^2$. Carbon bond formation depends on the manner in which the atomic orbitals involved undergo mixing to form hybrids; these are termed sp^3 , sp^2 and sp hybridisation. The basis of conjugated polymers is based on the phenomenon that carbon can undergo sp^2 hybridisation. In sp^2 hybridisation, carbon promotes an electron from the $2s$ to $2p$ state resulting in the excited state configuration $1s^2 2s^1 2p^3$.

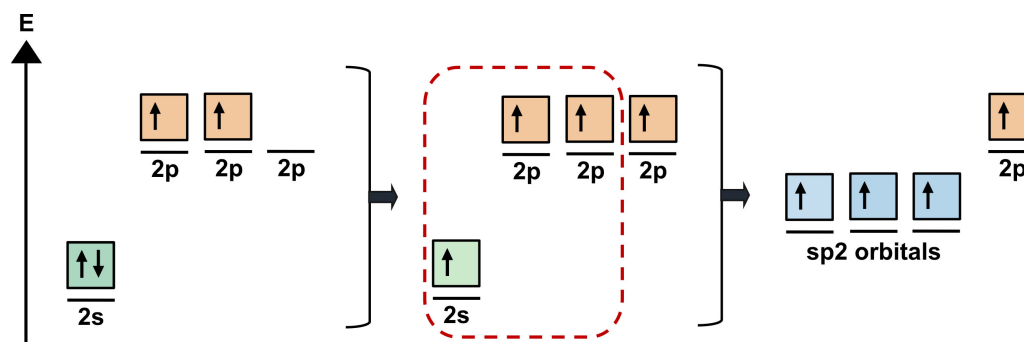


Figure 2.1: Electronic configurations of carbon during sp^2 hybridisation.

Now, it is possible for the s orbital and two of the p orbitals, $2p_x$ and $2p_y$, to overlap or *hybridise* and form three equivalent sp^2 orbitals distributed in the x-y plane with an angle of 120° between them; the remaining unhybridised p orbital, $2p_z$, lies perpendicular to that plane[1]. If two sp^2 hybridised molecules are brought together, the sp^2 orbitals will give rise to three σ -bonds, which are strong covalent bonds. Two σ -bonds are formed with hydrogen and one with the adjacent molecule's carbon. The electron in the $2p_z$ orbital of one carbon atom, will be paired with the electron in the $2p_z$ orbital of the other, forming a weak π -bond. The overlapping $2p_z$ orbitals is called a π -orbital and the electrons here will have a spatial probability density above and below the molecular axis. When looking at the electronic processes in organic molecules, the energy of molecular orbitals (MOs) should be considered. MOs are approximated as a linear combination of atomic orbitals, which is the wavefunction of an electron in an atom with an associated phase. During the C-C bond formation the $2p_z$ orbitals can either overlap in-phase (constructive interference) or out-of-phase (destructive interference) resulting in π -bonding and π^* -antibonding MOs respectively. Due to this interference and the Pauli exclusion principle, regions of filled π -bonding and unfilled π^* -antibonding orbitals will form, resulting in a pathway for electrons to move through, from the the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).

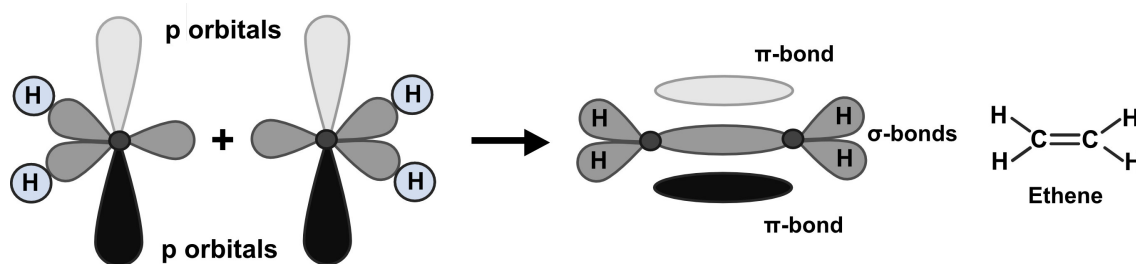


Figure 2.2: The formation of σ -bonds and π -bonds from two sp^2 hybridised carbon atoms and four hydrogen atoms to give ethene.

π -conjugated molecules

Introducing many more carbon atoms into this system will produce a chain of σ -bonds and π -bonds between neighbouring carbon atoms. These bonds provide organic semiconductors with the characteristic properties of (i) mechanical robustness from the strong σ -bond backbone and (ii) delocalised π -bonds which allow the π -electrons to travel freely across the carbon chain and carry a current. In a conjugated system, which contains alternating double and single bonds between the carbon atoms and thus alternating lengths, there is a change to the periodicity of the carbon backbone which distorts the electronic band structures. This phenomenon is known as Peierls distortion and effect causes the filled states to go to a lower energy and the empty states to a higher energy. An energy gap between the HOMO and the LUMO levels arises called the band gap, which is the origin of the semiconducting properties of these materials. Further increasing the conjugation length of these molecules into a polymeric system broadens the energy states into quasi-continuous ‘bands’, analogous to a valence band and conduction band in an inorganic semiconductor.

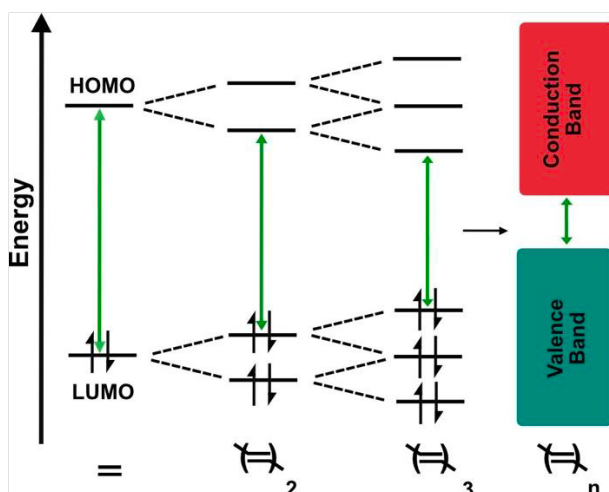


Figure 2.3: Depiction of energy-level splitting in alkenes with increasing conjugation length, with the band gaps (the lowest-energy transitions from HOMO to LUMO) shown as green arrows. Black arrows represent spin-paired electrons.

Charge transport in conjugated polymers

Conjugated polymers are fundamentally large networks of polymer backbones held together by weak van der Waals forces and the ability of the polymer chains to flex and bend leads to disruptions in the conjugation length. The conjugated backbone can be pictured as broken into segments, within which the delocalisation of π -orbitals occurs, rather than across the whole molecule. This variation of conjugation lengths also gives rise to the different band gap energies in various materials. Considering the microstructure of these materials as a disordered web of conduction pathways, the consequence is that charge

carriers are localised on conjugated segments and generally move through the material via a ‘hopping’ mechanism, a temperature-assisted tunnelling process from site to site[2]. Thus, for amorphous conjugated polymers where hopping occurs from one highly localised state to the next, charge transport is very affected by levels of disorder. There are various hopping models proposed related to the different microstructures of organic semiconductors[3]. An idealised schematic of conduction pathways for a popular class of conjugated polymers, semi-crystalline polythiophenes, is depicted below in Figure 2.4 showing highly anisotropic conduction pathways. In these systems, charge transport is fastest in the conjugation direction, however when chains pack, the supramolecular ordering is such that there is partial overlap between neighbouring π -orbitals, which assists interchain transport. In the third and slowest direction, the insulating alkyl chains attached to the backbone hinder charge transport. This high anisotropy has been demonstrated by comparing the electrical characteristics of OFETs and diodes. For these semi-crystalline materials in OFET devices, edge-on orientation is preferred, where the slow alkyl stacking direction is perpendicular to the plane of charge transport[4].

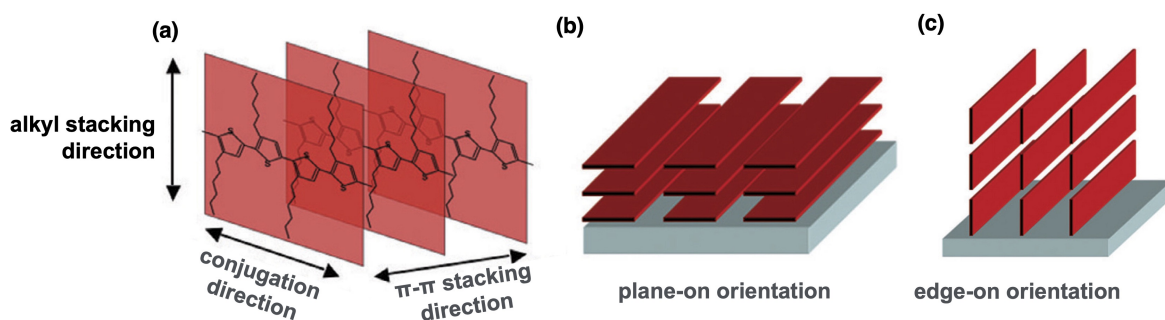


Figure 2.4: Classic charge-transport pathways for semi-crystalline conjugated polymers such as polythiophenes - reproduced with permission from Reference [5] Copyright © 2007 Elsevier Ltd. (a) Charge transport is fastest in the conjugation direction and the π - π stacking direction. Charge transport is slowest in the alkyl stacking direction. (b) Plane-on orientation where the alkyl stacking direction is in the plane of the substrate is unfavourable for OFETs because this is the plane of current flow. (c) Edge-on orientation where alkyl stacking direction is perpendicular to the substrate is preferable.

Bässler’s Gaussian Disorder model is typically adopted to explain the behaviour of amorphous organic semiconductors[6]. The model proposes that in a disordered conjugated system, the density of states can be approximated by a Gaussian distribution with a standard deviation. There are two main types of disorder: energetic and static, the former includes variations in hopping site energies and charge trapping while the latter includes variations in the morphological structure such as intermolecular distances and orientations. Both factors contribute to a broadening of the density of states, which is a property of most amorphous polymeric semiconductors[7]. Thus, this transport mode is restricted to low mobilities (typically $< 0.1 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$) and large activation energies.

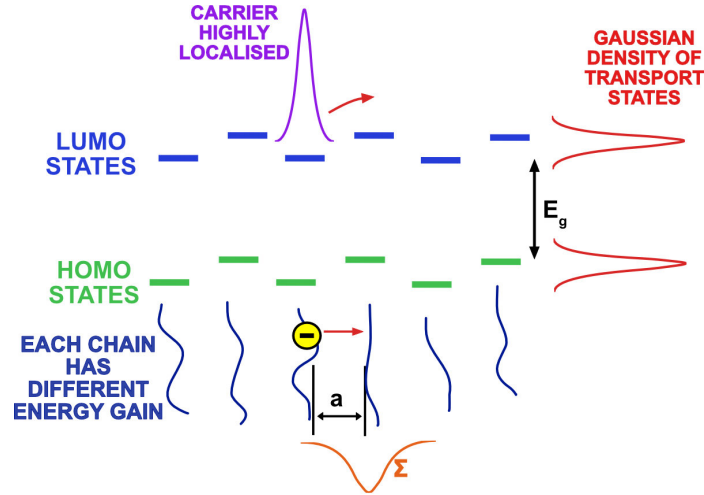


Figure 2.5: Schematic of the hopping transport in amorphous organic semiconductors. Carriers are highly localised with a large average hopping distance and large energetic σ and off-diagonal disorder Σ . This transport mode requires large activation energies.

2.1.2 Organic semiconducting materials

Types of polymeric semiconductors

Common conjugated polymers employed in OFETs are summarised here. Most polymeric semiconductors are comprised of a mix of ordered (a.k.a crystalline) and disordered (a.k.a amorphous) regions due to variations in macromolecular packing and folding. For the past few decades, the focus regarding polymeric semiconductors in OFETs was on semicrystalline thiophene-based polymers such as poly(3-hexylthiophene) (P3HT), poly(3,3'-dialkyl-quaterthiophene) (PQT) and poly(2,5-bis(thiophen-2-yl)thieno[3,2-b]-thiophene) (PBTTT). These materials tend to self-assemble into crystallites when cast into thin films. The supramolecular ordering is such that the π -orbitals belonging to adjacent polymer chains are stacked cofacially, adopting edge-on, lamellar structures which aides charge transport. P3HT was the first polymeric semiconductor to surpass the $0.1 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ mobility threshold[8], and combined with wide commercial availability, this material has been heavily investigated. Design guidelines based on thiophene-based polymers were used by McCulloch *et al.* to synthesise poly[2,5-bis(3-tetradecylthiophen-2-yl)thieno[3,2-b]thiophene] (PBTTT), a highly crystalline material due to the side-chain interdigitation of the alkyl side-chains, with a mobility of $1.1 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ [9].

More recently, this design criteria has been challenged by the rise of donor-acceptor (D-A) copolymers. These are largely amorphous structures with no long range order as verified in X-ray diffraction measurements[10]. Yet these materials often show higher mobilities compared to polymeric semiconductors with more crystalline structures, directly challenging the well-accepted ideal charge transport scenario. D-A copolymers consist of electron rich (donor) coupled to electron poor (acceptor) monomers result-

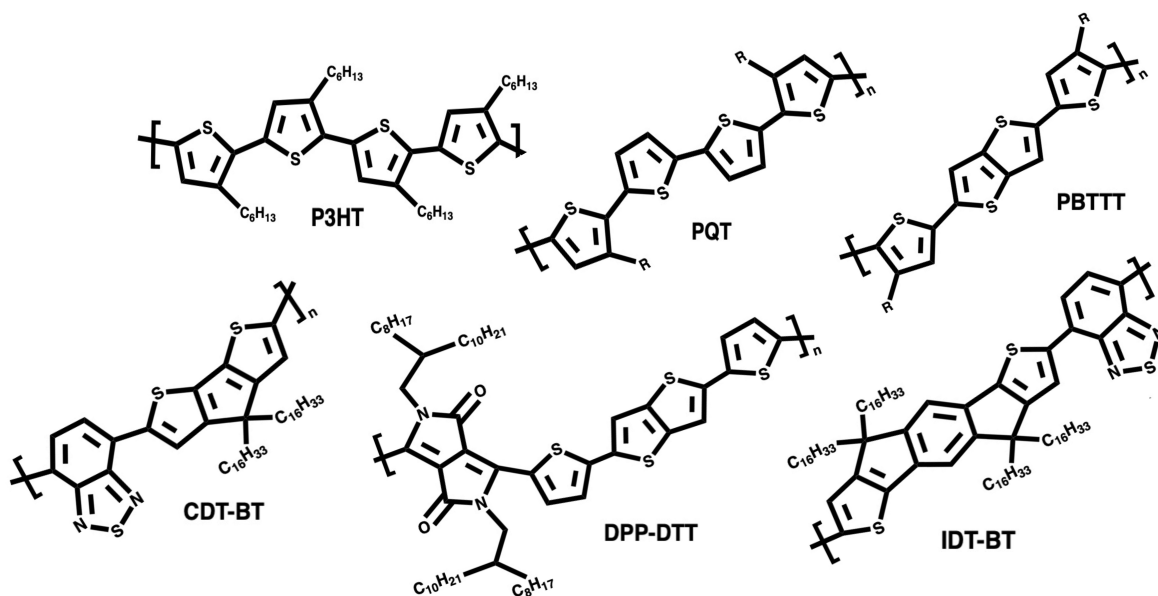


Figure 2.6: Chemical structures of polymeric semiconductors discussed in this chapter.

ing in low band gap molecules. One of the first D-A copolymers employed in OFETs was cyclopentadithiophene–benzothiadiazole (CDT-BT), an amorphous material with a mobility of $0.17 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ [11]. Classed as high-mobility, the D-A copolymer indacenodithiophene–benzothiadiazole (IDT-BT) has attracted much attention. With respect to molecular packing, X-ray absorption spectroscopy measurements revealed IDT-BT to have strong preference for face-on orientation[12], different from the previously established ideal for OFETs of edge-orientation. However, unexpectedly, high mobilities of $1.2 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ [12] and $3.6 \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ [13] have been achieved. The origin of the high mobility of D-A copolymers with respect to their microstructures is still somewhat a mystery, but a few recent studies have shed some light on this.

Structure-property relationships

A feature central to the properties of D-A copolymers is the highly planar backbone conformation. IDT-BT in particular, assumes a rigid-rod conformation with minimal steric hindrance between the donor and acceptor units. Molecular simulations have shown a wavy, but mostly torsion free backbone resulting in a structure with low energetic disorder and close chain packing with a small π – π stacking distance[10]. Further simulations in semiconducting polymers have shown that when polymer chains are rigid and long enough, charge carriers can travel efficiently on a single backbone chain before the slower, inefficient interchain hopping takes place[14]. This is thought to be the mechanism underway in IDT-BT, with the highly planar, rigid backbone facilitating efficient charge transport along the polymer chain with sporadic interchain hopping. Therefore suggesting that for D-A copolymers, in contrast to polythiophenes, the important design criteria involves

torsion restriction or conformation locking through covalent or non-covalent bonds to increase the backbone planarity[15].

As mentioned, in the case of thiophene-based polymers, close supramolecular packing is key for efficient charge transport and side-chain engineering is a common method to achieve this. Tuning the length, shape and position of alkyl side chains can facilitate closer $\pi - \pi$ stacking packing. In addition, functional groups can be incorporated to strengthen the intermolecular packing through hydrogen bonding.

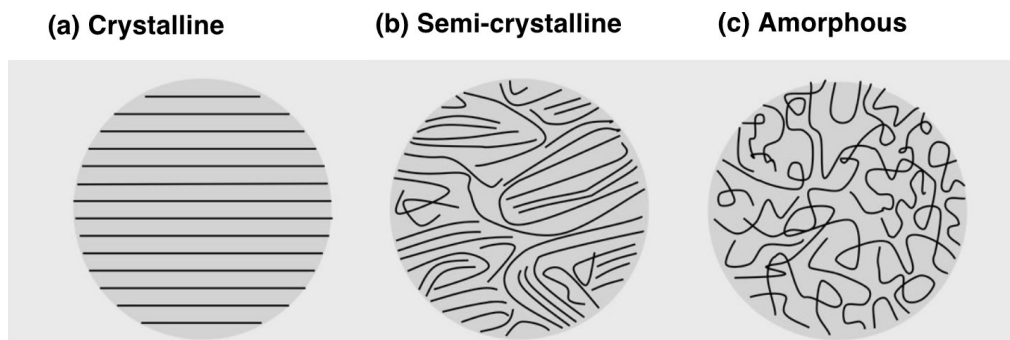


Figure 2.7: Schematic illustrations of the microstructure of conjugated polymers showing a (a) crystalline, (b) semi-crystalline polymer and (c) completely amorphous morphology.

Molecular weight remains an important parameter for charge transport. Very low molecular weight polymers result in poorly connected crystal domains. Slow charge transport occurs because the hops of charge carriers between these domains are very inefficient. Conversely, high molecular weight polymers form domains with ample chains connecting them thus increasing the carrier mobility. This positive correlation between molecular weight and mobility has been demonstrated for D-A copolymers such as IDT-BT[13].

Intriguingly, a recent study by Centra *et al.* used transmission electron microscopy to image the microstructure of IDT-BT film areas comparable to electronic devices and discovered an unexpected degree of short- and medium-range order in this material routinely classed as amorphous[16]. They reported locally aligned regions of polymer chains throughout the entire of the sample with frequent overlaps of the domains that appear to display preferential angles and described similarities to liquid-crystalline behaviour. This study highlights that with innovative characteristic methods, the goal towards understanding the morphology of these complex, heterogeneous structures becomes less elusive.

Conjugated polymer deposition methods

In their native state, most conjugated polymers are highly insoluble due to the rigidity of the backbone and lack of conformational freedom in solution. However, the central advantage of utilising such materials is their ability to be synthetically modified to achieve certain functionalities such as solubility. Solubilising groups typically involve long or branched alkyl chains to sterically hinder aggregation and are typically introduced dur-

ing monomer synthesis[17]. However there is a trade-off, as long and bulky side-chains can inhibit molecular ordering and lead to low charge carrier mobility in devices. The benefit of solution processing organic semiconductors is that deposition can occur at ambient temperatures and pressures, which expands the list of suitable device substrates to include flexible plastics, textiles and paper[18]. It also reduces the significant infrastructure costs associated with ultra-high vacuum systems. Common solution deposition methods used to form conjugated polymer thin films include drop-casting, spin coating and inkjet printing among several others[19]. Each method and its associated advantages and disadvantages which will be discussed briefly here.

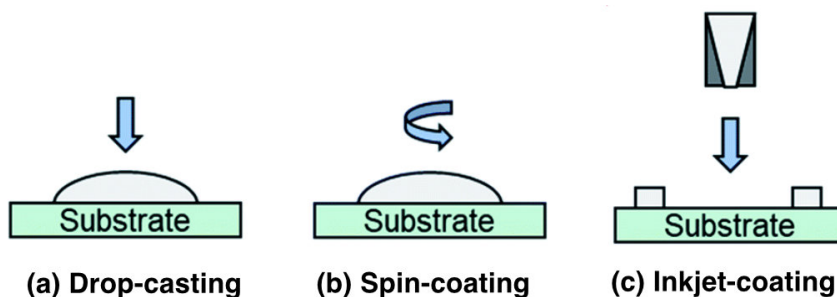


Figure 2.8: Schematic illustrations of conjugated polymer deposition methods showing (a) drop casting, (b) spin coating and (c) ink-jet printing.

The drop casting method is the simplest. The organic semiconductor solution is dropped onto the substrate and left for the solvent to evaporate under room temperature conditions (or on a hotplate), leaving behind a thin film. The advantage is this method creates no wastage, but the film coverage is non-uniform and thicknesses are hard to control. Spin coating is the one of the most widely used thin-film deposition tools in academia due to its simple operation and accessibility. A small volume of semiconductor solution is deposited onto the centre of the substrate which is then spun at high speeds (≈ 1000 rpm or higher). The acceleration causes the solution to spread over the substrate and eventually off its edge, leaving behind a thin film. The thickness and the quality of the film depends on the nature of the semiconductor solution, the spin parameters and the underlying substrate. The main disadvantage of this technique is the wastefulness, at least 90% of the material used is flung off the sides and unable to be recovered. For this reason, and because it's inherently a batch process, renders this method less suitable for mass production.

Due to the ability to print at high resolutions and relatively fast speeds, inkjet printing has emerged as an effective organic semiconductor patterning method with low material consumption. It operates via a jet of ink in the form of small droplets, which are ejected via a piezoelectric mechanism onto a substrate. Inherently an additive process, this method minimises wastage providing a large economical benefit. The ink-substrate interaction influences the properties of the final film, with the viscosity of the semiconducting ink and the wettability of the underlying substrate, becoming key. These factors can alter the

drying behaviour of a droplet, which also determines the morphology and structure of the deposited ink spot; the so-called ‘coffee rings’ are a common artefact of this deposition[20]. However, this method is compatible with large-area deposition and therefore has mass production potential. Overall, though solution-based deposition methods have been a key focus in academia and industry to fabricate thin films for devices, the lack of controllability and reproducibility remain obstacles in the way of commercialisation.

2.1.3 Organic field effect transistors

Device architectures

An OFET comprises several layers of components assembled on a substrate (i) an organic semiconductor thin film, (ii) an insulating layer called the dielectric and (iii) three conducting electrodes namely the source, drain and gate. These layers can be combined in a choice of four main geometries: bottom gate, bottom contact (BGBC), bottom gate, top contact (BGTC), top gate, bottom contact (TGBC), and top gate, top contact (TGTC).

The choice of geometry is associated with certain trade-offs. The BGBC architecture, where the organic semiconductor is deposited in the last step, allows for quick screening of new materials and processing methods. It also reduces the opportunity for the semiconductor to become damaged during subsequent fabrication steps which can degrade the device performance. However, this configuration leaves the semiconductor exposed to the ambient conditions, which can speed up degradation due to the thin film absorbing oxygen and water in the atmosphere.

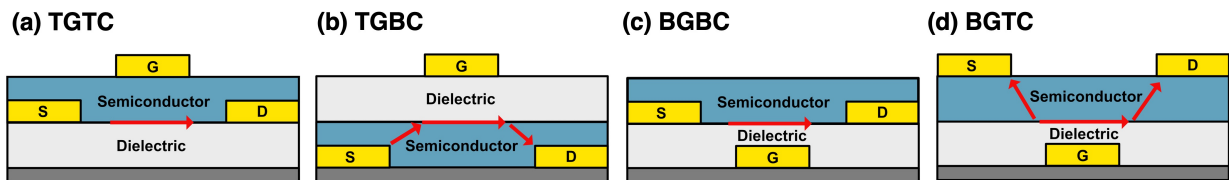


Figure 2.9: Main geometries for OFETs a) top gate, top contacts, b) top gate, bottom contacts, c) bottom gate, bottom contacts and d) bottom gate, top contacts. Electronic path shown with red arrows.

Structures with a top gate electrode, such as TGBC and TGTC, can hinder the environmental degradation of the semiconductor and the dielectric can also act as an encapsulation layer. However, careful consideration of materials, solvents and fabrication steps must be taken to ensure the underlying semiconductor remains intact.

The four structures can also be divided into classifications of ‘co-planar’ (BGBC and TGTC) and ‘staggered’ (BGTC and TGBC). Co-planar refers to the source and drain electrodes and the conducting channel being located on the same plane. Staggered refers to the conducting channel being offset from the plane of the source and drain electrodes. Co-planar structures are typically associated with larger contact resistance compared to

the staggered structures, due to the smaller contact area of the source electrode with the charge accumulation layer in the semiconductor[21]. For this reason, most of the highest performing OFETs report this staggered geometry[22].

OFET operating principles

OFETs are operated by the application of two potentials, one at the gate electrode V_G and the other at the drain electrode V_D while the source is typically grounded. This discussion will concern p-type organic semiconductors where the majority charge carriers are holes rather than n-type where the majority charge carriers are electrons. For n-type the same operating principle applies but simply with reversed polarities.

OFETs primarily operate in accumulation mode, therefore in the ideal scenario when no bias is applied $V_G = 0$, no charge accumulation occurs and the device is in an ‘OFF’ state. When a negative gate bias is applied $V_G < 0$, the electric field will polarise the dielectric, resulting in an accumulation of holes at the semiconductor-dielectric interface and the device is in an “ON” state. This layer of accumulated charge forms the conducting channel, the critical region where charge transport occurs, and is located within the few molecular layers of the semiconductor[23].

Once this accumulation of free holes has occurred, a second bias can be applied between the source and the drain electrodes, termed the drain voltage V_D . Free holes can then be injected and extracted from the source and drain electrodes generating a drain current denoted I_D . The charge density in the transistor channel and resulting current are modulated by the magnitudes of the applied fields V_G and V_D , hence the term ‘field-effect’. In the ideal case when $V_D > 0$ the charges should only travel from source to drain. However, in reality there may be a current flowing between the source and the gate I_G known as the gate leakage current. Therefore at $V_G = 0$ the device may not be fully turned ‘OFF’.

Often in devices, a smaller negative V_G is first required to fill charge traps at the semiconductor-dielectric interface before the free charge carriers can accumulate in the channel. The gate voltage at which all traps are filled is known as the threshold voltage V_T , a measure of what gate bias is required to turn the device ‘ON’. The amount of V_D bias applied affects the resistance across channel which affects how the charges are distributed; this results in three operating regimes as illustrated in Figure 2.10.

If $V_D = 0$, there are no changes to the accumulation layer forming the conducting channel. When $0 < V_D \ll V_G - V_T$, charges will be injected from source to drain and be subjected to a constant resistance across the channel. The resulting current will vary linearly with V_D meaning the device is in the ‘linear regime’. Figure 2.10(a) depicts the uniform mobile carrier density across the channel in the linear regime with the associated output characteristics (a measurement the drain current I_D as a function of the drain voltage V_D at a fixed gate voltage V_G). Further increasing V_D to the point where

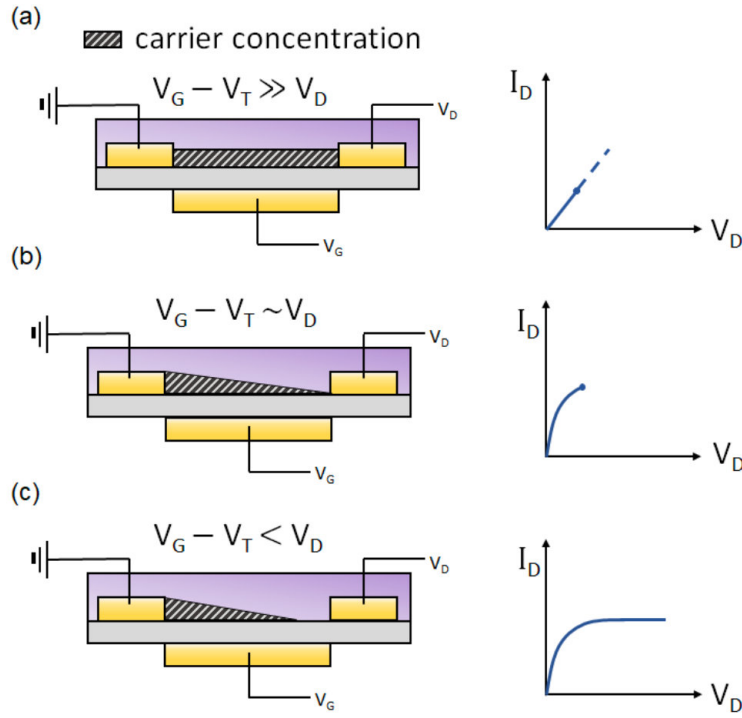


Figure 2.10: Carrier concentration distribution and the associated output characteristics of an OFET a) in the linear regime, b) during pinch-off and c) in the saturation regime.

$V_G - V_T \approx V_D$ there will be an area at the drain electrode that becomes depleted of charge carriers and the channel becomes ‘pinched-off’ as shown in Figure 2.10(b). Increasing V_D further to the point where $V_G - V_T < V_D$, a depletion region forms next to the drain electrode which leads to a current-limiting resistance and the conductivity across the channel ceases to be linear; this is known as the saturation regime as shown in Figure 2.10(c).

OFET parameter extraction

Important figures of merit for these devices can be extracted by measuring the current flowing through the channel. Shockley’s gradual channel approximation is a model developed to describe the current-voltage behaviour in inorganic metal-oxide semiconductor field-effect transistor (MOSFET) devices[24], quite a different system to OFETs. The ‘non-ideal’ behaviours that OFETs typically exhibit cause the traditional Shockley model to become less applicable for accurate parameter extraction[25]. Despite this, applying the model to OFET current-voltage data provides useful parameters to monitor to guide research and optimisation. However, future academic and industrial research would benefit from new models by which the extracted parameters truly reflect device properties.

The model makes the assumption that the electric field between the source and gate electrodes is much larger than the electric field between the source and drain electrodes. Typically, the channel length between the source and drain (on the order of micrometres)

is much larger than the thickness of the dielectric and semiconducting layer (on the order of nanometres) which causes this model to break down in devices with very short channels. Consequently, the approximation assumes that the electric field created by the gate voltage dictates the charge distribution within the channel which can be approximated as one-dimensional[26]

The accumulated mobile charge carriers in the channel can be described by:

$$Q_{mob} = C_i(V_G - V_T - V(x)) \quad (2.1)$$

where C_i is the specific capacitance of the dielectric, V_T is the potential required to fill traps in the material before accumulated charge carriers become mobile. $V(x)$ is the potential resulting from the applied V_D with x representing the distance across the channel from the source to the drain electrode. The mobile charges in one-dimension create the current I_D which gives:

$$I_D = \mu Q W E_x \quad E_x = \frac{dV}{dx} \quad (2.2)$$

Substituting Equation 2.1 into Equation 2.2 becomes:

$$I_D dx = \mu W C_i (V_G - V_T - V(x)) dV \quad (2.3)$$

where μ is the charge carrier mobility and W is the width of the channel. Integrating Equation 2.3 along the channel from $x = 0$ to the channel length $x = L$, and from $V = 0$ to $V = V_D$ gives:

$$\int_0^L I_D dx = \mu W C_i \int_0^{V_D} (V_G - V_T - V(x)) dV \quad (2.4)$$

which results in the following:

$$I_{D,lin} = \mu \frac{W}{L} C_i [(V_G - V_T) V_D - \frac{1}{2} V_D^2] \quad (2.5)$$

This equation is valid in the linear regime, however as mentioned, when $V_D = V_G - V_T$ the pinch-off at the saturation regime arises. Substituting this into Equation 2.5 yields the equation for the drain current in the saturation regime.

$$I_{D,sat} = \mu \frac{W}{2L} C_i (V_G - V_T)^2 \quad (2.6)$$

From the derivatives of 2.5 and 2.6 with respect to V_G , the mobility μ in the linear and saturation regimes can be expressed as:

$$\mu_{lin} = \frac{L}{W C_i V_D} \frac{\partial I_D}{\partial V_G} \quad (2.7)$$

$$\mu_{sat} = \frac{2L}{WC_i} \left(\frac{\partial I_D}{\partial V_G} \right)^2 \quad (2.8)$$

In an ideal device μ_{lin} and μ_{sat} are equal, however in practice the contact resistance lowers the effective V_D resulting in a significantly lower μ_{lin} . Applying the gradual-channel approximation model for I_D to experimental current-voltage data, the important performance parameters can be extracted. As stated, the output characteristics describes the measurement when V_D is varied at a constant V_G and I_D is measured. The other main measurement, the transfer characteristics, describes when V_G is varied at a constant V_D and I_D is measured. Key parameters to extract from the experimental data include the absolute drain current I_D , the current ON/OFF ratio $I_{ON/OFF}$, the threshold voltage V_T , the saturation mobility μ_{sat} and sometimes the subthreshold slope SS . Typically in organic electronics, high performance transistors are characterised by a large gain $I_{ON/OFF}$ and a high subthreshold slope SS , which indicates clear switching behaviour. Additionally, a V_T as close to zero as possible, indicates low operating voltages required to power the device and a high μ_{sat} indicates efficient charge transport within the device.

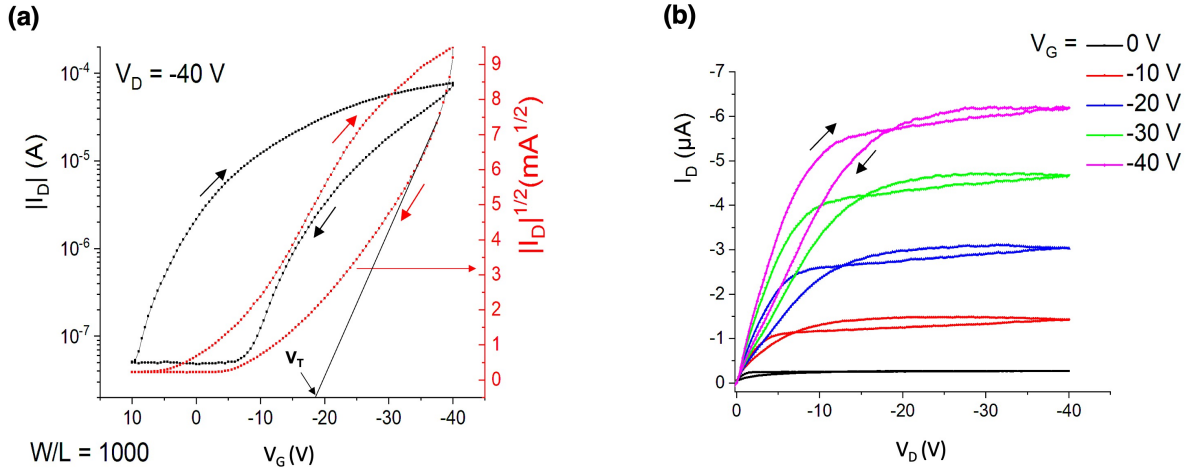


Figure 2.11: Electrical characterisation of a semiconducting polymer OFET showing (a) the transfer characteristics, with $\sqrt{I_D}$ vs V_D plotted (red points) for the linear fit extraction of the threshold voltage in the saturation regime (indicated with the slope and arrow) and (b) the output characteristics for an OFET device. Arrows indicate forwards and backwards sweeps.

OFET non-idealities and optimisation strategies

Each layer of the OFET architecture has properties that can cause deviation from ‘ideal’ device behaviour and will be discussed briefly here. Starting with the electrodes, these are typically metal contacts but also can be conducting polymers[27]. Contact resistance in devices increases the barrier to charge carrier injection from the electrodes into the conducting channel. This also becomes more prominent as the channel length decreases,

however the gradual-channel approximation model for the OFET current-voltage relationship assumes this to be negligible, leading to inaccuracy in extracted parameters[26]. The injection barrier height of metal electrodes can be minimised by choosing a metal with a work function well matched to the HOMO (or LUMO) of the organic semiconductor. Additionally, electron-poor (or rich) self-assembled-monolayers can be used to modify electrodes by increasing (or decreasing) the work function[28].

The dielectric layer is crucial in the OFET structure because the bulk and interfacial properties both influence device performance. Equation 2.1 relates the charge accumulation in the channel directly to the operative voltage and the dielectric capacitance. Many semiconducting polymer-based OFETs still require high operating voltages which is a big obstacle for commercialisation, thus increasing the capacitance of the dielectric layer is pivotal to reducing power consumption. The following equation provides two options for increasing the dielectric specific capacitance:

$$C_i = \epsilon_0 \frac{k}{d} \quad (2.9)$$

where ϵ_0 is the permittivity of free space, k is the dielectric constant and d is the thickness. Reducing the thickness of the dielectric layer or choosing a material with a higher dielectric constant can increase the capacitance. However, too-thin dielectrics can increase the possibility of pinholes, which can cause the devices to short and break-down. Thin dielectrics can also lead to higher gate leakage current, so generally increasing the dielectric constant is preferred.

One of the most common dielectrics employed in OFETs is silicon dioxide (SiO_2). However, it is difficult to make very thin, defect-free layers of this material and with $k \approx 4$, this material is typically unable to generate the capacitance required to facilitate low-voltage operation. Additionally, substrate cleaning steps can lead to hydroxyl groups on the dielectric surface in the form of silanols. These surface groups can increase charge trapping in the device and cause undesirable device characteristics. Charge carriers pass through the semiconducting layer at the semiconductor-dielectric interface, therefore the interface characteristics and the semiconductor molecular packing are central to achieving a high performance. Surface treatments in the form of thin insulating layers and silane self-assembled monolayers can be used to passivate these groups prior to semiconductor deposition[29].

For fully solution-processed devices, polymer dielectrics can also be considered; common choices include the perfluoropolymer CYTOP ($k \approx 2$) polystyrene (PS, $k \approx 2.6$) and poly(methyl methacrylate)(PMMA, $k \approx 3.5$). However, to achieve robust films, layers of a few hundred nanometres must be deposited, which significantly reduces the capacitance which these materials can achieve. Thus recently, high- k fluoropolymers such as poly(vinylidene fluoride) (PVDF) copolymers have been considered as gate dielectric ma-

terials. These materials possess very high dielectric constants ($k \approx 10 - 40$) and a lack of functional groups known to trap charges[30]. However, PVDF copolymers are known to demonstrate ferroelectric behaviour because of the large dipole moment of the carbon-fluorine (C-F) under high electric fields. A trade-off arises because this ferroelectric property can cause undesirable hysteresis, therefore the incorporation of a thinner low- k material at the interface to suppress this can be considered[31].

There are also optimisation strategies concerning the organic semiconductor microstructure to improve charge transport. Molecular additives can be blended with the organic semiconductor inks during processing, to enhance charge carrier mobilities and improve device stability. Luo *et al.* reported the incorporation of ionic additive tetramethylammonium iodide (NMe₄I) into the conjugated polymer DPP-DTT resulting in an improved mobility by a factor of 24 compared to films without the additive[32]. Scattering measurements and AFM suggested that the ionic additive had the effect of reducing torsion of the alkyl side chains, facilitating a more ordered molecular packing and $\pi - \pi$ interactions. The molecules tetracyanoquinodimethane (TCNQ), tetrafluorotetracyanoquinodimethane (F4TCNQ) and 4-aminobenzonitrile (ABN) were selected for in a study as reported by Nikolka *et al.* for incorporation within conjugated polymer formulations [33][34]. Small percentages of these molecules were incorporated in IDT-BT thin films resulting in devices that demonstrated improved environmental stability and reduced contact resistance. The additives are thought to bind to the water and oxygen molecules absorbed within the microstructure of the thin film and prevent them from interacting unfavourably with the polymer. However, molecules such as F4TCNQ with a LUMO deeper than the HOMO of the host polymer IDT-BT, can lead to charge transfer doping and an increase in OFF current which may be undesirable. Nikolka *et al* also demonstrated that by reducing the annealing time from 1 h to under 2 min, the residual solvent in the film also behaves like an additive with improved environmental stability however this effect is limited by the solvent volatility. Solid additives with a strong hydrogen bonding group are preferred for long-term applications[35].

The macrostructure of the organic semiconductor can also affect the device performance. Patterning of the semiconductor through photolithography and dry etching[36] or inkjet printing[37] can be utilised to eliminate parasitic leakage and reduce crosstalk among neighbouring devices[38]. Very high feature resolution can be achieved with photolithography however dry-etching risks damage to semiconducting and dielectric layer. Incorporating an inter-layer to protect the semiconductor during patterning has been reported as a solution to this[38].

The barriers that OFETs face in regards to operational and environment instability are clear. Nevertheless, in the context of biosensors, achieving OFETs with very high mobilities and on/off ratios while desirable, is not the ultimate objective. It is the aim,

however, that devices are sufficiently well-performing to exhibit stable signals such that they can be relied upon to detect and quantify concentrations of biomolecules.

2.2 Analytical biosensors

2.2.1 Overview on biosensing

A biosensor is an analytical device which undergoes a response when exposed to an analyte and converts this response into a signal that can be measured. Typically the main components of a biosensor include: (i) the analyte i.e the substance of interest that needs detection, (ii) the biorecognition element i.e. the molecule that specifically recognises the analyte and (iii) the transducer i.e. the element that converts one form of energy into another, which in this context is the OFET. The 1962 development of the glucose sensor by Clark and Lyons which operated using enzyme electrodes is widely cited as the first ever biosensor [39]. Since then, the field has expanded to include a wide range of biosensor systems with biorecognition elements in the form of capturing proteins, antibodies, nucleic acids and even complex tissues[40].

Biosensors are broadly separated into two categories *label-based* and *label-free*. Label-based biosensors rely on the covalent attachment of labels to the analyte, such as fluorescent markers, quantum dots or radioactive groups, to provide the transduction. The final sensor signal, proportional to the number of labels, provides the number of bound target molecules. This method includes the famously sensitive electrochemical enzyme-linked immunosorbent assay (ELISA), however labelling strategies can alter the natural state of the target molecules and may demand laborious, lengthy protocols. Label-free based biosensors, which do not require any labelling steps, are inherently simpler. Techniques include micro-cantilevers, surface plasmon resonance and quartz crystal microbalance measurements[41]. However these methods have drawbacks with regards to reliability, miniaturisation, sensitivity and equipment costs, limiting their suitability for widespread use in health applications.

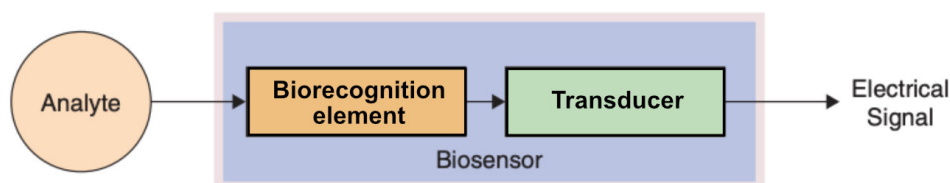


Figure 2.12: Schematic depiction of the main biosensor components.

2.2.2 Performance metrics

This section will discuss important figures of merit used across many biosensing systems to describe the detection behaviour. The International Union of Pure and Applied Chemistry guidelines are often used for analytical biosensor validations, however many variations in the definitions of performance indicators and calculations of figures of merits exist. Nevertheless, key concepts referenced in this work are described below.

Biosensor calibration

Typically, a biosensor is calibrated by measuring the response R across a range of concentrations of analyte solutions. This is used to determine the dynamic range of the sensor, which describes the concentration window over which the device can reliably report changes in signal response. A calibration curve can be plotted based on the obtained responses and from this, test samples of unknown concentrations can be interpolated based on the response. Ideally 5 or more solutions covering two orders of magnitude should be measured and where possible, in physiological buffer conditions. Ultimately, clinical biomarkers will determine the required dynamic range from the limits of the interested biomarkers. A blank solution (no analyte) is usually tested for the background signal R_0 so that the normalised response $(R - R_0)/R_0$ can be plotted against the analyte concentrations.

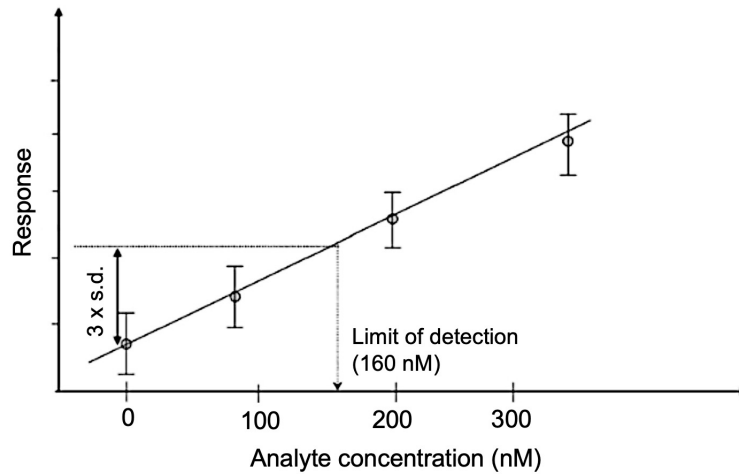


Figure 2.13: Illustration of an approach to the limit of detection calculation from the calibration curve slope and the blank sample response $B_{mean} + 3k\sigma_B$.

Sensitivity and limit of detection

The sensitivity of a biosensor indicates the ability to discriminate small differences in the concentration of target analyte and can be determined by the slope of the calibration curve. Typically for BioFETs, the limit of detection (LOD) is reported. The LOD

describes the concentration at which the response is deemed to be sufficiently above the blank response. A common estimate of the LOD evaluates this value as the lowest target concentration that can provide a detectable response, as observed visually based on the calibration curve. Another approach to calculating the LOD is to repeatedly measure a blank sample, calculate the mean B_{mean} and standard deviation σ_B of this response, then find the intersection at the value $B_{mean} + 3k\sigma_B$ with the calibration slope to give the LOD[42]. For biosensors to be clinically relevant, this could mean achieving a LOD as low as pg mL^{-1} or even fg mL^{-1} .

Selectivity

The capability of a biosensor to deliver signals that are free from interfering molecules and provide “true results” is of great importance. A common definition of selectivity is the extent to which a biosensor can determine particular analyte(s) in a complex mixture without interference from other components in the mixture[43]. Across biosensing fields, the terms selectivity and specificity are often used interchangeably bringing some confusion over their definitions, however specificity can be described as the upper limit of selectivity[44]. To determine the selectivity of the biosensor, the response against non-target/interfering analytes should be measured under the same experimental conditions and the calibration curves between the interfering and target analytes can be compared.

Repeatability and reproducibility

Repeatability is the ability of a biosensor to generate a consistent response, to a statistically-defined acceptable level, in duplicate assays under the same experimental conditions. For example, the variation in response of a single biosensor to the target analyte under the same conditions. Reproducibility is the ability of a biosensor to generate a consistent response, to a statistically-defined acceptable level, in duplicate assays under different experimental conditions. For example, the variation in response of several nominally identical biosensors to the target analyte.

Stability

The stability of a biosensor can relate to different aspects depending on the context. For BioFET applications, the stability can refer to the ability of the response to remain consistent with time, while all other parameters remain constant. i.e. is a measure of the degree to which a device response might degrade over time.

The ideal biosensor

To summarise, the ideal biosensor could be described as a device with (i) a steep calibration curve slope such that small changes in analyte concentration result in large changes in

the response i.e. high sensitivity, (ii) a clinically relevant LOD for the target analyte, (ii) a dynamic range covering the clinically relevant span for the target, (iii) high selectivity for the target against interfering analytes (iv) responses that are repeatable and reproducible and (v) responses that are robust against environmental factors and consistent with time. Additionally, for commercial applications, the response times would be short, it would be low-cost to fabricate and easy to use. BioFETs are proposed as potentially effective platforms to move towards these ideals.

2.2.3 Organic BioFET sensing

A discussion of the state-of-the art of BioFET sensing requires descriptions of the main categories of devices commonly employed. This will be provided here before reviewing the analytical performances currently being achieved.

Organic BioFET variations

BioFET is an umbrella term which includes several categories of transistor-based biosensors. OFET-based biosensors are derived from the structures described in section 2.1.3, with a solid state dielectric layer. When biorecognition elements are endowed to an interface in the device, the trapping of target analytes leads to physical or chemical changes in the semiconducting layer, which leads to changes in the current flow through the device that can be recorded as the sensor response.

Organic electrochemical transistors (OECTs) and electrolyte-gated OFETs (EGOFETs) are BioFET structures in which the gate electrode is separated from the conjugated polymer by a liquid electrolyte which serves as an aqueous dielectric. The active layer in OECTs is typically a conducting polymer such as poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS). Its conductivity is modulated by an electrochemical doping/de-doping process at the polymer-electrolyte interface, involving the migration of mobile ions into/out of the semiconducting layer, in response to a gate bias[45]. The high volumetric capacitance of the electrolyte in these configurations allows these devices to be powered at very low voltages (~ 1 V), allowing the possibility of real-time sensing without the risk of hydrolysis during operation.

EGOFETs utilise semiconducting polymers in the active layer; the gate bias triggers the formation of electrical double layers (EDLs) at both the gate-electrolyte and electrolyte-semiconductor interfaces, without any ion penetration. The EDLs are just a few nanometres thick and thus possess a very high capacitance, causing high charge carrier densities to be accumulated in the conducting channel at very low voltages (~ 1 V)[42]. Trapping of the target analyte to biorecognition elements at an interface in this device, can cause changes in the surface potential and/or capacitances, which influence the channel current thus enabling sensing. These configurations have the advantage of

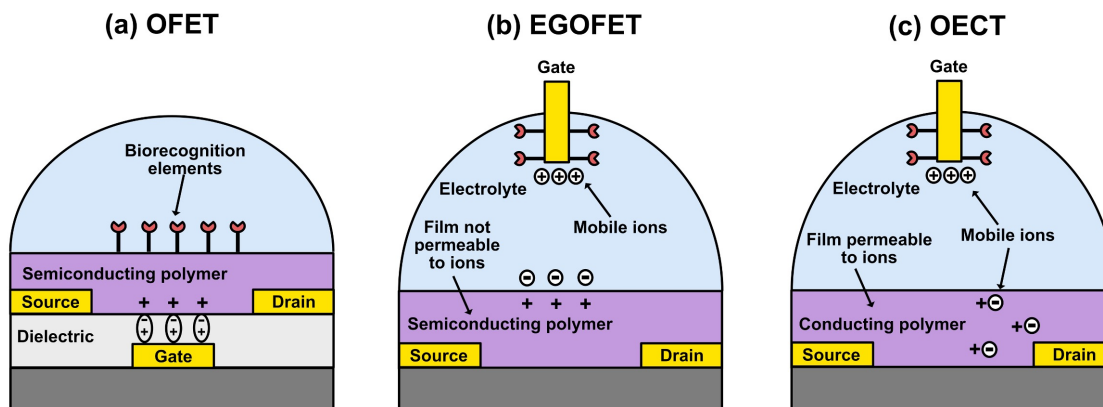


Figure 2.14: Illustrations of the common of BioFET structures, the (a) OFET which encompasses a solid-state dielectric, (b) the electrolyte gated OFET (EGOFET) which features an aqueous dielectric and semiconducting polymer and c) the organic electrochemical transistor which features an aqueous dielectric and a conducting polymer (OECT).

low voltage operation but they come with certain trade-offs. With respect to OECTs, successive penetration of ions into the active layer, can lead to quicker degradation of the conducting polymer. Additionally, the reliance of these devices on ion migration for the transduction, means they typically exhibit slower response times than their OFET-based counterparts. The requirement for a gate electrode immersed at a well-defined distance into a aqueous dielectric, complicates the form factor considerably potentially impacting manufacturability.

Biofunctionalisation strategies

To fabricate BioFETs, choices must be made about (i) the biorecognition elements to incorporate, (ii) on which interface to endow them and (iii) the method of immobilisation. Selective probe elements such as enzymes, nucleic acid sequences, antibodies or capturing proteins are typically utilised as receptors. Different immobilisation strategies exist depending on the BioFET configuration. In devices with aqueous dielectrics, it is common to find the gate electrode functionalised with bioreceptors as illustrated in Figure 2.14(b) and (c).

Gold is a common electrode material, therefore thiol-gold interactions are often exploited and have been used to functionalise gate electrodes with antibodies[46][47], DNA sequences[48][49], SAMs[50][51] and enzymes[52]. Utilising the gate in this method ensures no changes to the conducting channel properties during immobilisation. However, with this method, the binding event is spatially separated from the conducting channel, hindering the direct coupling of this event to the signal response. Additionally, the positioning of a functionalised gate electrode at a defined distance within the electrolyte solution can be hard to control, leading to low reproducibility.

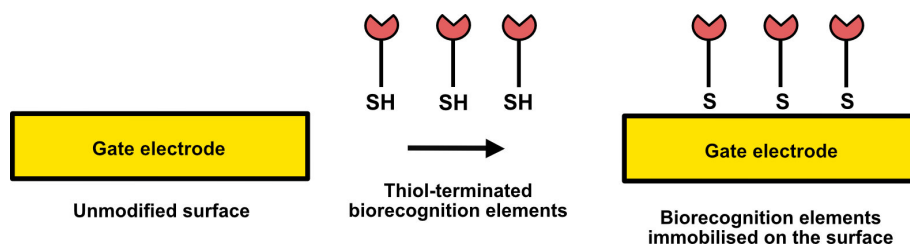


Figure 2.15: Illustration of the gate electrode biofunctionalisation method employed in many EGOFETs and OECTs.

The other main method is to functionalise the organic semiconductor surface. There are various strategies reported to achieve this with their associated advantages and disadvantages. Physical adsorption of bioreceptors on the organic semiconductors is often used, where weak Van der Waals interactions drive assembly on the surface as depicted in Figure 2.16. Quick, easy and requiring no modifications that affect the conducting channel, this method has been used in BioFETs to immobilise antibodies specific to a cancer biomarker[53], a sepsis biomarker[54] a pathogen biomarker[55] and DNA sequences[56]. However, there is a lack of control over the amount and orientation of adsorbed receptor and questions remain over the long-term stability of this immobilisation method.

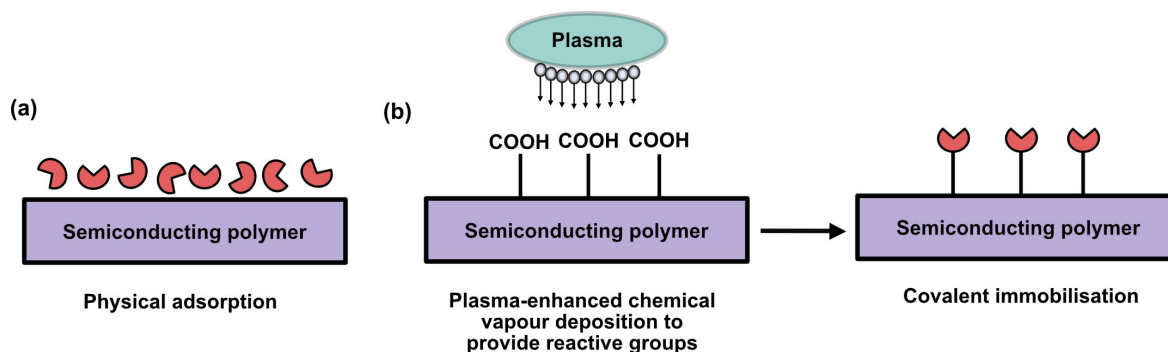


Figure 2.16: Illustration of the conjugated polymer biofunctionalisation methods commonly employed in organic BioFETs showing (a) physical adsorption and (b) deposition of reactive handles via PE-CVD and subsequent covalent immobilisation.

For a more stable binding to the surface, covalent immobilisation is the preferred route, however generating functional groups to facilitate this can be complex. Synthetically grafting the biorecognition element directly to the the polymer backbone where they are also present within the bulk, is generally not preferred. A study reported 25% of the side chains of the semiconducting polymer poly(9,9-dioctylfluorene-alt-benzothiadiazole) (F8BT) functionalised with biotin, and the devices exhibited poor performance compared to the unmodified polymer. This suggests biotin groups sterically interact with each other, reducing the backbone planarity and hindering charge transport[57]. For this reason, techniques which leave the conjugated polymer bulk unchanged to preserve charge transport and modify only the surface, are favoured.

Plasma-enhanced vapour chemical deposition (PE-CVD) allows the deposition of

nanometre-thick coatings rich in functional groups, such as carboxylic acid groups (-COOH) or amino groups (-NH₂). This method has been utilised in BioFETs to deposit functional groups for the subsequent functionalisation of peptide nucleic acid (PNA) sequences[58], capturing proteins[59], biotinylated phospholipids[60] and enzymes. However, PE-CVD requires specialist, high-vacuum facilities which hinder low-cost fabrication goals[61]. Additionally, the short deposition times required to avoid damaging the organic semiconductor during the process can result in uneven deposited coatings. However, once the conjugated polymer bears the necessary reactive groups, either the biomolecule can be bound directly or intermediary steps can be introduced such as the use of affinity coupling.

The streptavidin-biotin interaction is a particularly popular coupling, where biotinylated molecules can be bound to avidin-modified surfaces and vice versa. This method can provide more control over density and orientation than physical adsorption, however either the biotin/avidin molecules and the conjugated polymer would need to bear the necessary functional groups to bond thus likely involves more experimental steps. Self-assembled monolayers (SAMs) are also a reported intermediary, where the SAMs exhibit two different functional groups on either side. One end binds to the surface of the BioFET sensor and the other anchors the biomolecules[60]. Rarer functionalisation strategies have included decorating the organic semiconductor surface with gold nanoparticles to provide functionalisation sites for covalent attachment of thiolated DNA aptamers specific to thrombin[62].

An alternative to covalent immobilisation is through the integration of a functional biological inter-layer. In this configuration, the probe receptor layer is sandwiched between the dielectric and the OSC thin film, requiring no modification to the bulk semiconductor. However, the analyte must permeate through the OSC film to the binding sites beneath, which could negatively affect charge transport and/or degrade the active layer[63].

Regardless of the chosen method, the biofunctionalisation protocol will directly affect the quality of the probe layer. Subjection to organic solvents, extreme temperatures, non-physiological pHs or high salt concentrations can potentially destroy the native conformation of the biological probes and limit their ability to selectively bind to their targets.

BioFET detection

This section will provide a review of the state-of-the-art of BioFET performance capability. As this project focuses on BioFET with a solid dielectrics, these structures will be the focus of the discussion. BioFETs can operate in slightly different ways depending on the device configuration, the type of analyte and its charge. Generally, the target analyte binds with the biorecognition element, and this charged, bound product has the effect of modulating the effective gate voltage that the device experiences, leading to a measurable I_D or V_T shift.

Focusing on protein targeting BioFETs initially, antibody-antigen binding events are central to many biosensors. Minamiki *et al.* used an extended-gate electrode structure in their device for the detection of biotinylated antibodies. A LOD of 8 nM was achieved and shown to be highly selective in competition interfering proteins[64]. The response manifested in a negative shift of V_T which was attributed to increasing positively-charged antibody concentrations reducing carrier concentrations in the channel. In a top-gated configuration, Martínez-Domingo *et al.* chose to target a model antigen and achieved a LOD of $5.71 \mu\text{g mL}^{-1}$. The isoelectric point of the antigen (pI) ranges from 6.6–8.5, causing it to be negatively charged at the pH of their working buffer (pH 9), thus the response was displayed as an increase current as the antigen concentration increases[65]. Macchia *et al.* chose to target the C-reactive protein (CRP) with an EGOFET featuring a gate-electrode functionalised with a SAM of densely packed capturing anti-CRP proteins. A significantly low LOD of just 13 proteins was achieved in human saliva, with the highly compact SAM causing an amplification effect[66].

The first demonstration of nucleic acid detection with BioFETs was reported by Khan *et al.* using covalently-bound peptide-nucleic acid sequences as the biorecognition element[58]. Upon DNA injection to the sensor, a decrease in I_D was observed. DNA sequences are negatively-charged due to the phosphate ions in the structure, therefore the sensing mechanism proposed was that the bound DNA acts as a negatively-charged top gate that attracts charge carriers away from the channel region. Lai *et al.* report demonstrated DNA hybridization detection in a BioFET with an extended-gate structure with a LOD of 100 pM[48]. A marked reduction/positive shift in V_T and an increase in I_D was observed both after single-stranded DNA probe immobilisation and complementary target hybridization. This was assigned to the increased negative charges in the sensing area due to the bound target DNA molecules, causing increased accumulation in the p-type organic semiconductor conducting channel. A similar configuration was used by Napoli *et al.* using hairpin shaped oligonucleotides probes[67]. Complementary ssDNA sequences were injected in the measurement environment and a current increase proportional to the concentration of target molecules in solution was obtained with a eventual LOD of 100 pM. It is clear when reviewing BioFET reports that the proposed sensing mechanisms can differ significantly depending on the system. Thus careful understanding of individual systems is required to interpret the response data and more fundamental studies of these transduction mechanisms would greatly benefit academic decisions.

BioFETs to-date have demonstrated high sensitivity, fast response times and small sample volumes required for the assays, which have very important implications in medical diagnostics. However, there are bottlenecks that limit the clinical applicability of these devices, one of which is the effect of Debye screening in high ionic strength solutions. Physiologically relevant solutions that can be comparable to blood or serum are required to have salt concentrations of $\sim 150 \text{ mM}$. Charged target molecules in solution will be

screened by counterions in solution, e.g. negative species such as streptavidin or DNA will be surrounded by a cage of positively charged ions driven through electrostatic interactions. At a certain length, called the Debye length λ_D , the number of net positive counterions will equal the number of negative charges on the streptavidin or DNA and this scales inversely with the ionic strength of the solution. This can be expressed as:

$$\lambda_D = \frac{\epsilon k T}{q \sqrt{C}} \quad (2.10)$$

where C is the ionic strength, ϵ is the dielectric constant, T is the temperature of the sample solution and k is the Boltzmann's constant. It describes a screening effect such that the electrostatic potential arising from charges on the streptavidin or DNA will decay exponentially towards zero with distance. Reports have demonstrated that when a binding event occurs further than λ_D , it goes undetected[68]; in real-world samples this can be sub-nanometre, often smaller than the size of the capture probes themselves. Therefore, the choice of probe can have a large effect on sensing efficiency, with small probes like aptamers[69] bringing the binding event within λ_D rather large probes, such as antibodies. Debye screening issues also mean that often studies use diluted plasma, serum or PBS as the measurement buffer however this can cause instability of the biomolecules in solution and reduced affinity for the binding event. The Debye length, however, is not a hard cut-off. The model makes several assumptions that are inapplicable to most real-world systems so often there are exceptions, including reports of sensing beyond the Debye length in high ionic strength solutions[70][71].

To summarise, great progress has been made in organic BioFET sensing, but for employment in realistic applications, much more must be achieved. Continued exploration of high performing organic semiconductor materials, effective designs of device configurations and extensive study of intrinsic mechanisms, would boost the development of this field considerably.

References

- [1] A. Köhler, H. Bässler, *Electronic processes in organic semiconductors : an introduction*, Wiley-VCH Verlag GmbH & Co., Weinheim, Germany, **2015**.
- [2] N. Tessler, Y. Preezant, N. Rappaport, Y. Roichman, *Advanced Materials* **2009**, *21*, 2741–2761.
- [3] C. Liu, K. Huang, W.-T. Park, M. Li, T. Yang, X. Liu, L. Liang, T. Minari, Y.-Y. Noh, *Materials Horizons* **2017**, *4*, 608–618.
- [4] Y. Yao, H. Dong, W. Hu, *Advanced Materials* **2016**, *28*, 4513–4523.
- [5] A. Salleo, *Materials Today* **2007**, *10*, 38–45.
- [6] H. Bässler, *physica status solidi (b)* **1993**, *175*, 15–56.
- [7] R. Noriega, J. Rivnay, K. Vandewal, F. P. V. Koch, N. Stingelin, P. Smith, M. F. Toney, A. Salleo, *Nature Materials* **2013**, *12*, Number: 11 Publisher: Nature Publishing Group, 1038–1044.
- [8] Z. Bao, A. Dodabalapur, A. J. Lovinger, *Applied Physics Letters* **1996**, *69*, Publisher: American Institute of Physics, 4108–4110.
- [9] I. McCulloch, M. Heeney, C. Bailey, K. Genevicius, I. MacDonald, M. Shkunov, D. Sparrowe, S. Tierney, R. Wagner, W. Zhang, M. L. Chabinyc, R. J. Kline, M. D. McGehee, M. F. Toney, *Nature Materials* **2006**, *5*, Number: 4 Publisher: Nature Publishing Group, 328–333.
- [10] D. Venkateshvaran, M. Nikolka, A. Sadhanala, V. Lemaire, M. Zelazny, M. Kepa, M. Hurhangee, A. J. Kronemeijer, V. Pecunia, I. Nasrallah, I. Romanov, K. Broch, I. McCulloch, D. Emin, Y. Olivier, J. Cornil, D. Beljonne, H. Sirringhaus, *Nature* **2014**, *515*, 384–388.
- [11] M. Zhang, H. N. Tsao, W. Pisula, C. Yang, A. K. Mishra, K. Müllen, *Journal of the American Chemical Society* **2007**, *129*, Publisher: American Chemical Society, 3472–3473.

- [12] X. Zhang, H. Bronstein, A. J. Kronemeijer, J. Smith, Y. Kim, R. J. Kline, L. J. Richter, T. D. Anthopoulos, H. Sirringhaus, K. Song, M. Heeney, W. Zhang, I. McCulloch, D. M. DeLongchamp, *Nature Communications* **2013**, *4*, Number: 1 Publisher: Nature Publishing Group, 2238.
- [13] W. Zhang, J. Smith, S. E. Watkins, R. Gysel, M. McGehee, A. Salleo, J. Kirkpatrick, S. Ashraf, T. Anthopoulos, M. Heeney, I. McCulloch, *Journal of the American Chemical Society* **2010**, *132*, Publisher: American Chemical Society, 11437–11439.
- [14] P. Carbone, A. Troisi, *The Journal of Physical Chemistry Letters* **2014**, *5*, Publisher: American Chemical Society, 2637–2641.
- [15] M. Kim, S. U. Ryu, S. A. Park, K. Choi, T. Kim, D. Chung, T. Park, *Advanced Functional Materials* **2020**, *30*, 1904545.
- [16] C. Cendra, L. Balhorn, W. Zhang, K. O'Hara, K. Bruening, C. J. Tassone, H.-G. Steinrück, M. Liang, M. F. Toney, I. McCulloch, M. L. Chabinyc, A. Salleo, C. J. Takacs, *ACS Macro Letters* **2021**, *10*, 1306–1314.
- [17] J. Mei, Z. Bao, *Chemistry of Materials* **2014**, *26*, Publisher: American Chemical Society, 604–615.
- [18] V. Raghuwanshi, D. Bharti, A. K. Mahato, I. Varun, S. P. Tiwari, *ACS applied materials & interfaces* **2019**, *11*, 8357–8364.
- [19] Y. Yunus, N. A. Mahadzir, M. N. Mohamed Ansari, T. H. Tg Abd Aziz, A. Mohd Afdzaluddin, H. Anwar, M. Wang, A. G. Ismail, *Polymers* **2022**, *14*, 1112.
- [20] R. D. Deegan, O. Bakajin, T. F. Dupont, G. Huber, S. R. Nagel, T. A. Witten, *Nature* **1997**, *389*, Number: 6653 Publisher: Nature Publishing Group, 827–829.
- [21] C. N. R. Rao, A. Müller, A. K. Cheetham, *Nanomaterials Chemistry: Recent Developments and New Directions*, Google-Books-ID: aydHhdmjGycC, John Wiley & Sons, **2007**, 424 pp.
- [22] D. Choi, T. K. An, Y. J. Kim, D. S. Chung, S. H. Kim, C. E. Park, *Organic Electronics* **2014**, *15*, 1299–1305.
- [23] C.-a. Di, Y. Liu, G. Yu, D. Zhu, *Accounts of Chemical Research* **2009**, *42*, Publisher: American Chemical Society, 1573–1583.
- [24] H. Klauk, *Organic Electronics II: More Materials and Applications*, Google-Books-ID: qjsBuDZSDY0C, John Wiley & Sons, **2012**, 443 pp.
- [25] H.-I. Un, J.-Y. Wang, J. Pei, *Advanced Science* **2019**, *6*, 1900375.
- [26] Z. A. Lamport, H. F. Haneef, S. Anand, M. Waldrup, O. D. Jurchescu, *Journal of Applied Physics* **2018**, *124*, Publisher: American Institute of Physics, 071101.

-
- [27] S. Wang, Z. Wang, Y. Huang, Y. Hu, L. Yuan, S. Guo, L. Zheng, M. Chen, C. Yang, Y. Zheng, J. Qi, L. Yu, H. Li, W. Wang, D. Ji, X. Chen, J. Li, L. Li, W. Hu, *ACS Applied Materials & Interfaces* **2021**, *13*, Publisher: American Chemical Society, 17852–17860.
- [28] B. H. Hamadani, D. A. Corley, J. W. Ciszek, J. M. Tour, D. Natelson, *Nano Letters* **2006**, *6*, Publisher: American Chemical Society, 1303–1306.
- [29] Y. Lei, B. Wu, W.-K. E. Chan, F. Zhu, B. S. Ong, *Journal of Materials Chemistry C* **2015**, *3*, Publisher: The Royal Society of Chemistry, 12267–12272.
- [30] A. T. Lill, A. F. Eftaiha, J. Huang, H. Yang, M. Seifrid, M. Wang, G. C. Bazan, T.-Q. Nguyen, *ACS Applied Materials & Interfaces* **2019**, *11*, Publisher: American Chemical Society, 15821–15828.
- [31] A. Rahmanudin, D. J. Tate, R. Marcial-Hernandez, N. Bull, S. K. Garlapati, A. Zamhuri, R. U. Khan, S. Faraji, S. R. Gollu, K. C. Persaud, M. L. Turner, *Advanced Electronic Materials* **2020**, *6*, 1901127.
- [32] H. Luo, C. Yu, Z. Liu, G. Zhang, H. Geng, Y. Yi, K. Broch, Y. Hu, A. Sadhanala, L. Jiang, P. Qi, Z. Cai, H. Sirringhaus, D. Zhang, *Science Advances* **2016**, *2*, Publisher: American Association for the Advancement of Science, e1600076.
- [33] M. Nikolka, I. Nasrallah, B. Rose, M. K. Ravva, K. Broch, A. Sadhanala, D. Harkin, J. Charmet, M. Hurhangee, A. Brown, S. Illig, P. Too, J. Jongman, I. McCulloch, J.-L. Bredas, H. Sirringhaus, *Nature Materials* **2016**, *16*, 356.
- [34] M. Nikolka, G. Schweicher, J. Armitage, I. Nasrallah, C. Jellett, Z. Guo, M. Hurhangee, A. Sadhanala, I. McCulloch, C. B. Nielsen, H. Sirringhaus, *Advanced Materials* **2018**, *30*, 1–8.
- [35] Y. Chen, W. Deng, X. Zhang, M. Wang, J. Jie, *Journal of Physics D: Applied Physics* **2022**, *55*, 053001.
- [36] L. Han, J. Li, S. Ogier, Z. Liu, L. Deng, Y. Cao, T. Shan, D. Sharkey, L. Feng, A. Guo, X. Li, J. Zhang, X. Guo, *Advanced Electronic Materials* **2022**, *8*, 2200014.
- [37] Y.-J. Kwon, Y. D. Park, W. H. Lee, *Materials* **2016**, *9*, 650.
- [38] W. Tang, J. Zhao, L. Feng, P. Yu, W. Zhang, X. Guo, *IEEE Electron Device Letters* **2015**, *36*, Conference Name: IEEE Electron Device Letters, 59–61.
- [39] P. Bergveld, *Sensors and Actuators A: Physical* **1996**, *56*, 65–73.
- [40] S. Mohanty, E. Kougiannos, *IEEE Potentials* **2006**, *25*, Conference Name: IEEE Potentials, 35–40.
- [41] R. L. Caygill, G. E. Blair, P. A. Millner, *Analytica Chimica Acta* **2010**, *681*, 8–15.

- [42] L. Torsi, M. Magliulo, K. Manoli, G. Palazzo, *Chemical Society Reviews* **2013**, *42*, 8612–8628.
- [43] J. Vessman, R. I. Stefan, J. F. v. Staden, K. Danzer, W. Lindner, D. T. Burns, A. Fajgelj, H. Müller, *Pure and Applied Chemistry* **2001**, *73*, Publisher: De Gruyter, 1381–1386.
- [44] K. Danzer, *Fresenius' Journal of Analytical Chemistry* **2001**, *369*, 397–402.
- [45] J. Rivnay, S. Inal, A. Salleo, R. M. Owens, M. Berggren, G. G. Malliaras, *Nature Reviews Materials* **2018**, *3*, Number: 2 Publisher: Nature Publishing Group, 1–14.
- [46] M. Berto, E. Vecchi, L. Baiamonte, C. Condò, M. Sensi, M. Di Lauro, M. Sola, A. De Stradis, F. Biscarini, A. Minafra, C. A. Bortolotti, *Sensors and Actuators B: Chemical* **2019**, *281*, 150–156.
- [47] S. Demuru, J. Kim, M. El Chazli, S. Bruce, M. Dupertuis, P.-A. Binz, M. Saubade, C. Lafaye, D. Briand, *ACS Sensors* **2022**, *7*, Publisher: American Chemical Society, 2721–2731.
- [48] S. Lai, M. Demelas, G. Casula, P. Cosseddu, M. Barbaro, A. Bonfiglio, *Advanced Materials* **2013**, *25*, 103–107.
- [49] S. P. White, K. D. Dorfman, C. D. Frisbie, *Analytical Chemistry* **2015**, *87*, Publisher: American Chemical Society, 1861–1866.
- [50] K. Guo, S. Wustoni, A. Koklu, E. Díaz-Galicia, M. Moser, A. Hama, A. A. Alqah-tani, A. N. Ahmad, F. S. Alhamlan, M. Shuaib, A. Pain, I. McCulloch, S. T. Arold, R. Grünberg, S. Inal, *Nature Biomedical Engineering* **2021**, *5*, Number: 7 Publisher: Nature Publishing Group, 666–677.
- [51] S. Casalini, F. Leonardi, T. Cramer, F. Biscarini, *Organic Electronics* **2013**, *14*, 156–163.
- [52] T. Minami, Y. Sasaki, T. Minamiki, S.-i. Wakida, R. Kurita, O. Niwa, S. Tokito, *Biosensors and Bioelectronics* **2016**, *81*, 87–91.
- [53] M. Magliulo, D. De Tullio, I. Vikholm-Lundin, W. M. Albers, T. Munter, K. Manoli, G. Palazzo, L. Torsi, *Analytical and Bioanalytical Chemistry* **2016**, *408*, 3943–3952.
- [54] P. Seshadri, K. Manoli, N. Schneiderhan-Marra, U. Anthes, P. Wierzchowicz, K. Bonrad, C. Di Franco, L. Torsi, *Biosensors and Bioelectronics* **2018**, *104*, 113–119.
- [55] H. Runfang, Y. Yangfan, L. Leilei, J. Jianlong, Z. Qiang, D. Lifeng, S. Shengbo, L. Qiang, *Journal of Biotechnology* **2022**, *359*, 75–81.
- [56] Q. Zhang, V. Subramanian in 2007 IEEE International Electron Devices Meeting, 2007 IEEE International Electron Devices Meeting, ISSN: 2156-017X, **2007**, pp. 229–232.

-
- [57] Z.-S. Kim, S. C. Lim, S. H. Kim, Y. S. Yang, D.-H. Hwang, *Sensors* **2012**, *12*, 11238–11248.
- [58] H. U. Khan, M. E. Roberts, O. Johnson, R. Förch, W. Knoll, Z. Bao, *Advanced Materials* **2010**, *22*, 4452–4456.
- [59] H. U. Khan, J. Jang, J.-J. Kim, W. Knoll, *Journal of the American Chemical Society* **2011**, *133*, 2170–2176.
- [60] M. Magliulo, A. Mallardi, M. Y. Mulla, S. Cotrone, B. R. Pistillo, P. Favia, I. Vikholm-Lundin, G. Palazzo, L. Torsi, *Advanced Materials* **2013**, *25*, 2090–2094.
- [61] H. Shen, Y. Zou, Y. Zang, D. Huang, W. Jin, C.-a. Di, D. Zhu, *Materials Horizons* **2018**, *5*, Publisher: Royal Society of Chemistry, 240–247.
- [62] M. L. Hammock, O. Knopfmacher, B. D. Naab, J. B.-H. Tok, Z. Bao, *ACS Nano* **2013**, *7*, Publisher: American Chemical Society, 3970–3980.
- [63] M. D. Angione, S. Cotrone, M. Magliulo, A. Mallardi, D. Altamura, C. Giannini, N. Cioffi, L. Sabbatini, E. Fratini, P. Baglioni, G. Scamarcio, G. Palazzo, L. Torsi, *Proceedings of the National Academy of Sciences* **2012**, *109*, 6429.
- [64] T. Minamiki, T. Minami, R. Kurita, O. Niwa, S.-i. Wakida, K. Fukuda, D. Kumaki, S. Tokito, *Applied Physics Letters* **2014**, *104*, 243703.
- [65] C. Martínez-Domingo, S. Conti, A. de la Escosura-Muñiz, L. Terés, A. Merkoçi, E. Ramon, *Organic Electronics* **2020**, *84*, 105794.
- [66] E. Macchia, K. Manoli, B. Holzer, C. Di Franco, R. A. Picca, N. Cioffi, G. Scamarcio, G. Palazzo, L. Torsi, *Analytical and Bioanalytical Chemistry* **2019**, *411*, 4899–4908.
- [67] C. Napoli, S. Lai, A. Giannetti, S. Tombelli, F. Baldini, M. Barbaro, A. Bonfiglio, *Sensors (Basel Switzerland)* **2018**, *18*, DOI 10.3390/s18040990.
- [68] L. Kergoat, B. Piro, M. Berggren, M.-C. Pham, A. Yassar, G. Horowitz, *Organic Electronics* **2012**, *13*, 1–6.
- [69] I. M. Bhattacharyya, G. Shalev, *ACS Sensors* **2020**, *5*, Publisher: American Chemical Society, 154–161.
- [70] G. Palazzo, D. De Tullio, M. Magliulo, A. Mallardi, F. Intranuovo, M. Y. Mulla, P. Favia, I. Vikholm-Lundin, L. Torsi, *Advanced Materials* **2014**, *27*, 911–916.
- [71] J. Song, J. Dailey, H. Li, H.-J. Jang, P. Zhang, J. T.-H. Wang, A. D. Everett, H. E. Katz, *Advanced Functional Materials* **2017**, *27*, 1606506.

Part II

Results and Discussion

Chapter 3

Organic field-effect transistors

This results chapter begins with work concerning the microfabrication of BGBC OFETs in-house. However, this progress was halted during the 2020 Covid-19 pandemic where access to external clean rooms were restricted for long time periods. The chapter continues with the optimisation and characterisation of OFETs formed on commercially-bought substrates. The exploration of self-assembled monolayer substrate treatments and semi-conducting inks incorporated with molecular additives, is presented. Additionally, the utilisation of an unconventional organic semiconductor dispensing system for OFET array fabrication is demonstrated.

3.1 Motivation

OFETs have been developed in the world of organic electronic technology, with their potential in low-cost, large-area device fabrication driving intense research. Their compatibility with solution deposition techniques and flexible, transparent substrates, have made OFETs emerge as promising technology for diverse applications, including for driving display switches, radio-frequency identification tags and sensors[1][2]. As OFET are intrinsic amplifiers, small changes in the gate voltage V_G result will result in large changes in source-drain current I_D , which explains the particular focus on biosensing. The OFET acts as a transducer, by translating a binding event into a modulation of current that can be measured, an effect which has been taken advantage of in many BioFET configurations[3].

This project focuses on sensors using conventional solid-state dielectrics, as opposed to aqueous electrolytes, which possess a much lower capacitance (nF cm^{-2} rather than $\mu\text{F cm}^{-2}$). While generally less sensitive due to this large difference in charge accumulation ability, the fabrication steps and mechanism for sensing are less complex. Ultimately, the sensitivity of a biosensor should be in the clinically relevant range of the target analyte, which BioFETs utilising solid state dielectrics have already proven to achieve[4]. The strategy of spatially separating the regions where the analyte binding event and the

OFET-based amplification occurs through extended-gate configurations have been utilised to improve the sensor's overall lifetime by preserving the organic semiconductor[5][6], however these configurations are less compact with more complex form factors. Additionally, there is the hypothesis that sensitivity could be limited by this indirect coupling of binding events on a separated gate, with the active layer where charge accumulation occurs.

In this work, a configuration is chosen where the binding event takes place directly above the conducting channel in a direct coupling. The notion is that the effect of bound, charged analytes to probes grafted on top of the organic semiconductor could be modelled as a dual-gate OFET system, which typically comprises, a bottom gate electrode, dielectric, organic semiconductor, source and drain electrodes and finally the top dielectric and top gate[7]. In this configuration, the device still operates according to the conventional OFET mechanisms, however a top-gate bias (which could be approximated as bound charged analytes) could deplete/enhance the charge carrier accumulation driven by a bottom-gate bias.

The performance metrics that have typically guided the development of OFETs for use in integrated circuits, such as high mobilities and fast switching speeds, are not necessarily the criteria required for BioFETs. Nevertheless, the idea is that the electrical performance of the bare device will have an effect on the detection performance of the resulting biosensor. For example, a material with a sufficiently high mobility and a dielectric with an adequate specific capacitance, are required for stable electrical outputs with a low signal-to-noise ratio. Additionally, low hysteresis and minimal bias stress effects are desirable for accurate measurements of transfer curve shifts in response to a target analytes[8]. However, the exact relationship between OFET performance metrics and biosensor performance metrics such as sensitivity is still unclear.

The issues surrounding the environmental and operational stability of OFETs remain one of the main bottlenecks across many applications[9]. Unintentional doping from impurities and/or from moisture in ambient conditions can lead to non-ideal electrical characteristics, such as high off currents I_{OFF} , hysteresis and performance degradation under continuous gate bias. However, in recent years much progress has been made in tackling these instabilities, significantly enhancing device lifetimes and suitability for sensing applications[10][11][12].

Printing processes that could facilitate the mass production of OFETs are strongly sought-after for applications in low-cost, disposable electronics[13]. This has promising implications for single-use point-of-care biosensors and the ability to rationally design and synthetically modify organic semiconductors is central to this. Polymeric semiconductors are typically designed to be solution-processable through side-chain engineering of flexible, solubilizing side chains[14]. This allows these materials to be blended into inks, which in turn can be blended together with other materials to tune device characteristics[15][16]. This synthetic versatility also allows for the organic semiconductor to be functionalised

directly with biological receptors for sensing applications, which is central to this work.

The initial choice to fabricate the OFETs in-house was to have control over the design, materials and processing to maximise the electrical performance. As discussed in section 2.1.3, the choice of dielectric directly affects charge accumulation in the conducting channel, so a high- k fluoropolymer was employed for the insulating bulk. For the device architecture, a BGBC structure was utilised, where the organic semiconductor is deposited in the last step. This is to minimise the damage to the active layer during the fabrication process and to allow complete access to the thin film surface for the subsequent biofunctionalisation and BioFET assays. For the device fabrication on commercially bought Si/SiO₂ chips, silane treatments were carried on the substrates to optimise the interface properties. An unconventional automatic dispensing system for preparing organic semiconductor thin films was employed to reduce material waste and add more fabrication control with an automatic high-throughput process. Molecular additives were explored for blends with semiconductor inks in an attempt to maximise the environmental and operational stability of the underlying array, with the assumption that this will lead to increased stability in future biosensing measurements.

3.2 Materials and Methods

3.2.1 In-house fabricated devices

A simplified process flow for the fabrication of BGBC OFETs is illustrated in Figure 3.1. The first step was to pattern the gate electrodes using photolithography. The photomasks used throughout this process were designed jointly with Dr. Stuart G. Higgins in the group of Prof. Molly Stevens using an electronics design tool (Layout Editor, juspertor GmbH). A screen capture of the 4" wafer design is presented in Figure 3.2(a) and includes 4 quadrants with 25 transistors in 5x5 arrays. T1 - T50 with an interdigitated (ID) design, T50 - T75 with a standard electrode design, and T75 - T100 with certain designs for contact resistance measurements. The channel widths (W) are 10 mm and the channel lengths (L) from rows 1-5 are 5, 10, 20, 50 and 100 μm respectively. The self-aligned architecture design employed here, where the gate is used to define the source and drain contacts, avoids significant overlap of electrodes which can lead to overlap capacitance which does not contribute to the transconductance[17].

Patterning the gate electrodes

A ‘lift-off’ method used to pattern the gate electrodes. This creates a controlled undercut profile with edges in contact with the substrate which has shown to result in improved device performance[18]. 4" borofloat glass wafers (Pi-Kem) were first subjected to O₂ plasma cleaning (10 mins, 300 W, 0.4 - 0.6 mbar). The lift-off resist (LOR1A, MicroChem)

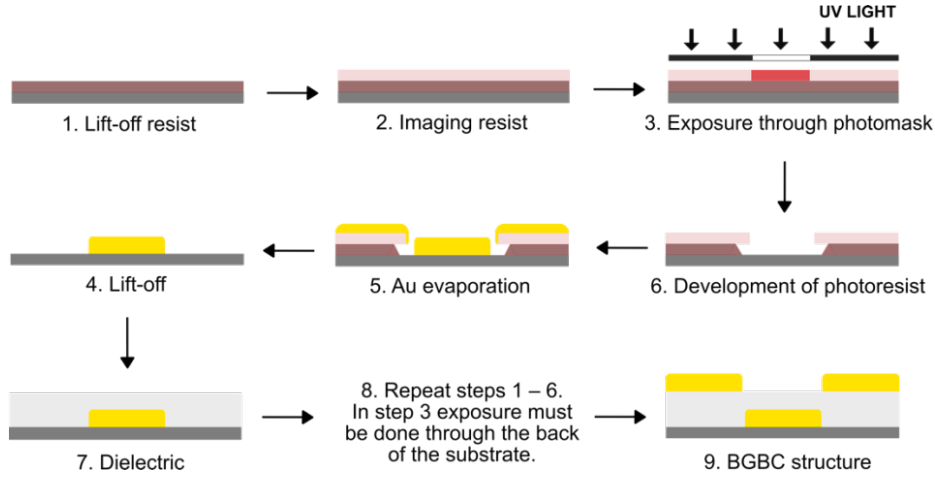


Figure 3.1: Process flow depicting the BGBC OFET fabrication process prior to organic semiconductor deposition. First the gate electrodes are patterned, then the dielectric is deposited and finally the source and drain electrodes are patterned on top.

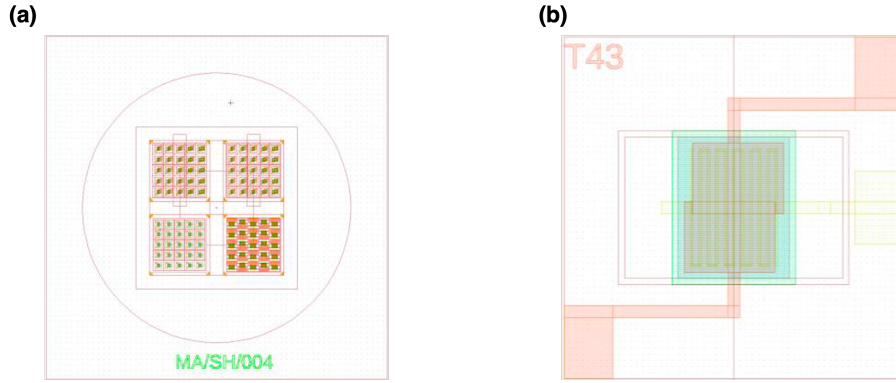


Figure 3.2: Screenshots of the photomask computer-aided designs showing (a) the 4'' wafer layout of the device layers overlaid and (b) a close up of transistor T43. The red layer is the pattern for the the source and drain mask. The turquoise layer is for the dielectric mask, the yellow layer represents the interdigitated gate and the green layer represents where the semiconductor is to be deposited.

was spin coated (2000 rpm, 1000 rpm s^{-1} , 45 s) to produce a $\sim 150 \text{ nm}$ layer and baked on a hotplate (5 min at 190°C). A positive photoresist (S1813, Microposit) was spin coated (4000 rpm , 1000 rpm s^{-1} , 45 s) to produce a $\sim 1.3 \mu\text{m}$ layer and baked on a hotplate (60 s at 115°C). The substrate was then exposed to UV light through the gate photomask (365 nm, 15 s, 340 W) before being submerged (15 s) in developer (MF319). After, the substrates were rinsed with deionised (DI) water and dried with N_2 gas to result in the patterned photoresist (Figure 3.3). In the next step, a 5 nm chrome adhesion layer was evaporated onto the substrate (10^{-7} mbar , 0.2 nm s^{-1}) and subsequently 50 – 100 nm of gold (Au) on top (Figure 3.4(a)). The substrates were then submerged in remover (Microposit 1165) for the lift-off (Figure 3.4(b)). The underlying photoresist was dissolved and the wafers were then rinsed with remover, DI water, IPA and dried with N_2 leaving the resulting patterned gates (Figure 3.4(c) and Figure 3.5).

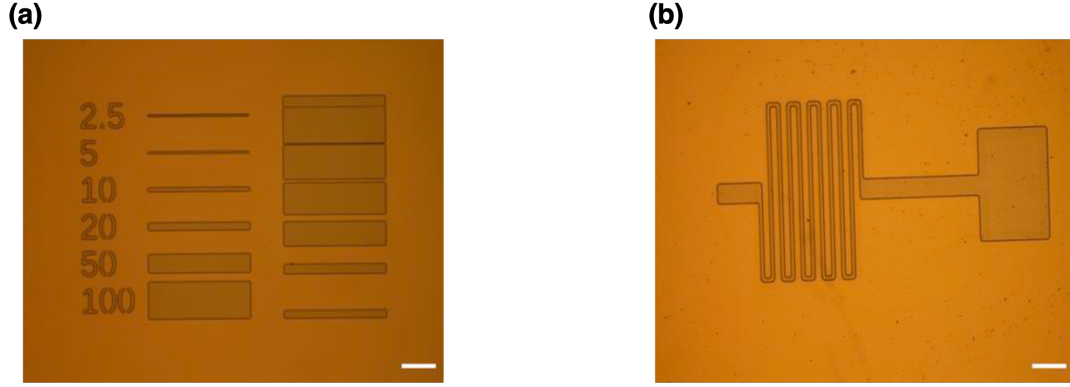


Figure 3.3: Microscope images of the patterned bilayer photoresist used to define the gate electrodes showing a) the resolution marks and b) a $10\ \mu\text{m}$ channel length device. The undercut is seen as the thin shadow around the pattern edges. Scale bar = $100\ \mu\text{m}$. The resolution marks refer to the designed width of the lines in the adjacent resulting pattern.

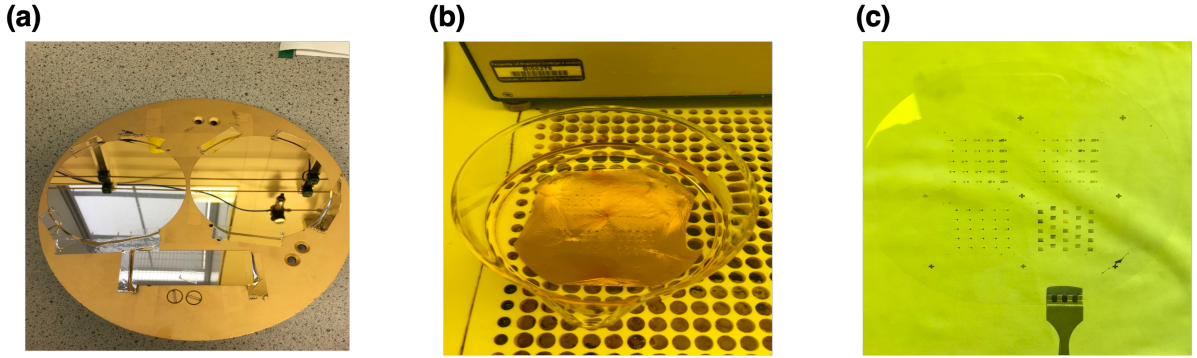


Figure 3.4: Photographs of the gate patterning process showing 4" diameter wafer substrates (a) after Au evaporation, (b) the metallized wafers in remover and (c) the wafer after lift-off with the resulting patterned electrodes.

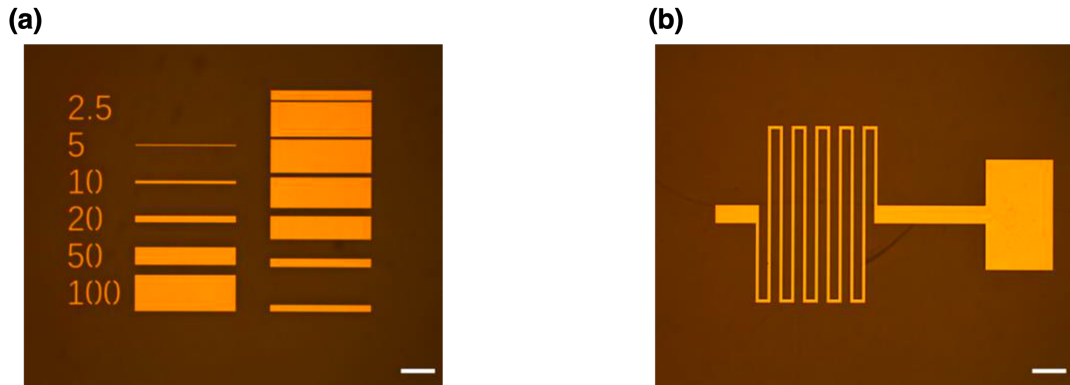


Figure 3.5: Microscope images of the gold gate electrodes showing a) resolution marks and b) a $10\ \mu\text{m}$ channel length device. Scale bar = $100\ \mu\text{m}$. The resolution marks refer to the designed width of the lines in the adjacent resulting pattern.

Depositing the dielectric layer

For the dielectric layer, a combination of a low- k and a high- k insulator was used; a ferroelectric fluoropolymer P(VDF-HFP) (3M) film for the thicker insulating bulk and a thinner PVP film (Sigma Aldrich) for the interface. The former was utilised to lower the device operating voltages by increasing the dielectric specific capacitance[19] whilst the latter was used to passivate the highly polar surface of the high- k insulator that can increase carrier trapping and result in undesirable hysteresis[8]. The P(VDF-HFP) material came with thermally activated crosslinkers within the formulation. For the PVP layer, the thermal crosslinker poly(melamine-co-formaldehyde) methylated (PMF) (Sigma Aldrich) was used. P(VDF-HFP) solutions (80 mg mL^{-1}) were obtained by dissolving in 2-butanone and leaving to stir overnight. PVP solutions (40 mg mL^{-1}) were obtained by dissolving in PGMEA, adding 10 wt.% PMF and leaving to stir overnight. P(VDF-HFP) solution was spin coated (4000 rpm, 500 rpm s^{-1} , 45 s) to produce a $\sim 1 \text{ }\mu\text{m}$ layer and then left on a hotplate to cure (6 hr at $180 \text{ }^{\circ}\text{C}$). PVP was spin coated next and left to cure (3 hr at $200 \text{ }^{\circ}\text{C}$). The contact angles for each layer and the bilayer are presented in Figure 3.6. The neat P(VDF-HFP) is highly hydrophobic and after successful deposition of the highly hydrophilic PVP to create the bilayer, it is rendered less hydrophobic..

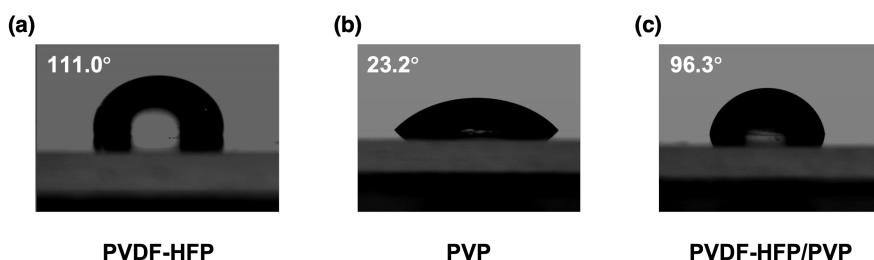


Figure 3.6: Water contact angle images of the dielectric materials used for the OFETs.

Patterning the source and drain electrodes

A lift-off resist (LOR10B, MicroChem) was spin coated (4000 rpm , 1000 rpm s^{-1} , 45 s) to produce a $\sim 90 \text{ nm}$ layer and baked on a hotplate (10 min at $190 \text{ }^{\circ}\text{C}$). A positive photoresist (S1818, Microposit) was spin coated (4000 rpm , 1000 rpm s^{-1} , 45 s) to produce a $\sim 1.9 \text{ }\mu\text{m}$ layer and softbaked on a hotplate (60 s at $115 \text{ }^{\circ}\text{C}$). A backside exposure method was used in combination with the source and drain photomasks (365 nm, 10 s, 340 W) before being submerged in developer (MF319) for approximately 4 mins. The substrates were then rinsed with DI water and dried with N_2 gas leaving the patterned photoresist bilayer (Figure 3.7). A 5 nm chrome adhesion layer was then evaporated onto the substrate (10^{-7} mbar) and then 50 – 100 nm of gold (Figure 3.8). The substrates were submerged in remover (Microposit 1165) for the lift-off, then rinsed with remover, DI, IPA, then dried with N_2 leaving the resulting patterned source and drain electrodes (Figure 3.9).

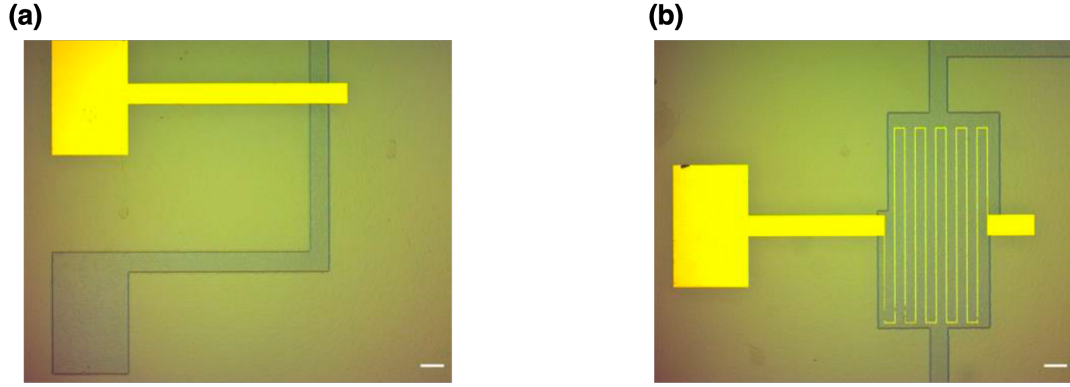


Figure 3.7: Microscope images of the patterned bilayer resist used to define the source and drain electrodes showing (a) a device with standard electrode and b) a device with an interdigitated electrode design. The undercut can be seen as a thin shadow/border along the pattern edges. Scale bar = $100\ \mu\text{m}$.

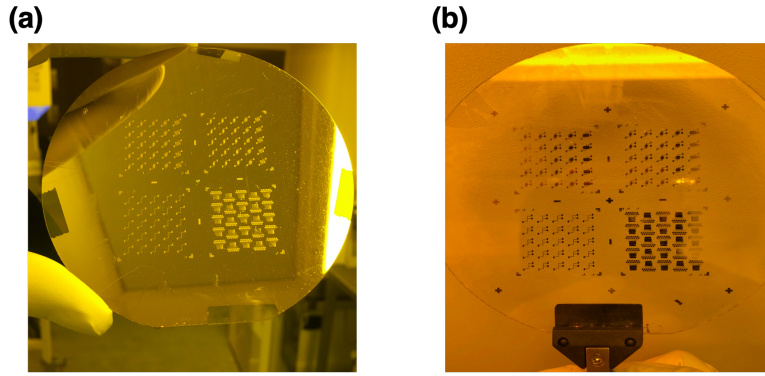


Figure 3.8: Photographs of the source and drain patterning process on 4" wafers showing (a) the substrates after evaporation and (b) the wafer after the rinsing and drying left with the patterned electrodes.

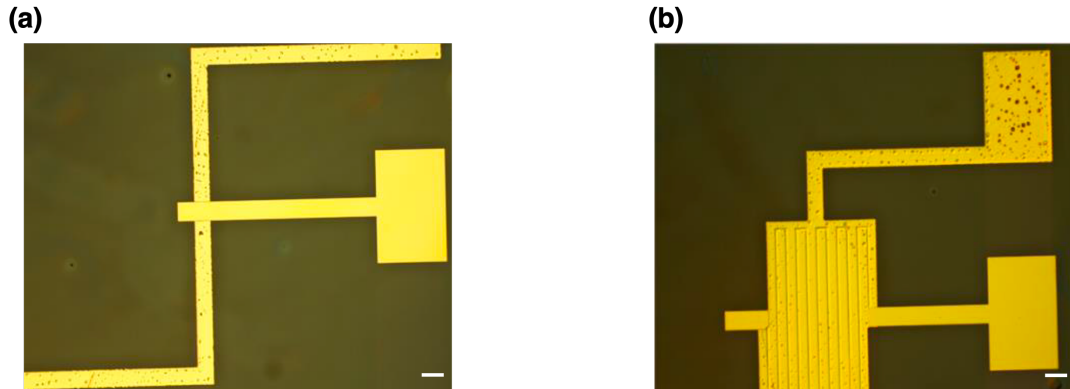


Figure 3.9: Microscope images of patterned source and drain electrodes showing (a) a device with standard electrode and b) a device with interdigitated electrode design. Scale bar = $100\ \mu\text{m}$.

Depositing the organic semiconductor

After dicing the patterned 4" wafer into separate chips and spin coating (1000 rpm, 200 rpm s⁻¹, 30 s) a thin film of the semiconducting polymer C₁₆IDT-BT from dichlorobenzene (DCB) solutions (5 mg mL⁻¹), the OFET array fabrication was completed (Figure 3.10).

OFET electrical characterisation

The electrical measurements, specifically the transfer and output curves, were conducted in ambient conditions with a semiconductor parameter analyser (Keysight B2902A) and a probe station set up. The chips are placed on an electrically isolated chuck through which a bias can be applied. Sharp metal tips are attached to probe arms which are connected to magnetic micro-manipulators on a platter with x, y and z controls. For the in-house devices, the 3 probe tips were used to contact the three electrodes as shown in Figure 3.10(c). For the commercially-obtained devices, the gate bias was sent through the bottom of the doped silicon substrate and 2 probe tips were used to contact the source and drain. The output characteristics were measured sweeping V_D from ranges of 0 V to -40 V, keeping V_G constant at of -40 V. The transfer characteristics were measured by sweeping the V_G , typically from ranges of +10 V to 40 V at a constant V_D of -40 V or of -20 V.

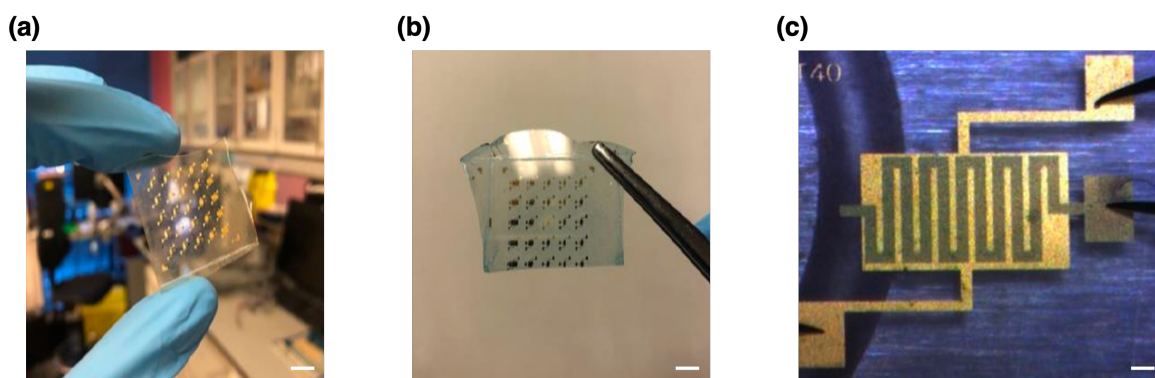


Figure 3.10: (a) Photograph of the BCBC chip quadrant, (b) photograph of the chip spin coated with C₁₆IDT-BT (5 mg mL⁻¹ at 1000 rpm, 200 rpm s⁻¹, 30 s) and (c) microscope images of one of the transistors with source, drain and gate contacted by the probe station tips for electrical characterisation. Scale bar of (a) and (b) = 1 cm and scale bar of (c) = 100 μ m.

3.2.2 Pre-formed OFET substrates

Preformed BGBC Si/SiO₂ chips (Gen. 4, Fraunhofer IPMS) were the substrates used for all electrical measurements in this chapter unless stated otherwise. A heavily-doped silicon substrate acted as the bottom gate electrode and a thermally grown silicon oxide SiO₂ (90 or 230 nm thick) served as the dielectric. Gold, interdigitated electrodes on top

form the source and drain contacts with channel widths of 10 mm and a range of channel lengths: 2.5, 5, 10, and 20 μm . The substrates received from the supplier were first cleaned with acetone, IPA and O_2 plasma (5 min at 100 W). All substrates were subjected to a surface treatment by submerging overnight in a 1.5 % octadecyltrimethoxysilane (OTMS) solution in toluene or ethanol. The substrates were then sonicated with fresh solvent and cured ($150\text{ }^\circ\text{C}$), ready for the semiconductor deposition as illustrated in Figure 3.12.

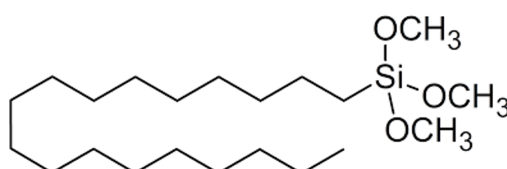


Figure 3.11: Chemical structure of OTMS.



Figure 3.12: Illustration of OFET fabrication on the pre-formed Si/SiO₂ substrates.

Spin coated OSC on pre-formed substrates

Substrates were spin coated (2000 rpm, 500 rpm s⁻¹, 45 s) with 150 μL of the semiconducting ink as shown in Figure 3.13. Chips were then placed on a hotplate ($100\text{ }^\circ\text{C}$) to evaporate any residual solvent.

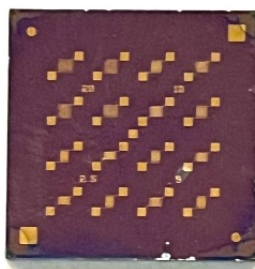


Figure 3.13: Photograph of the pre-formed Si/SiO₂ substrate after spin coating with the semiconducting polymer.

Printed OSC on pre-formed substrates

The automatic aspirate-dispense system (BioDOT AD1520) in Figure 3.14(a) bearing a 110 μm diameter ceramic tip was used to apply 20 x 0.003 μL droplets per device area with a 110 μs open time set. The resulting OFET array is shown in Figure 3.14(b).

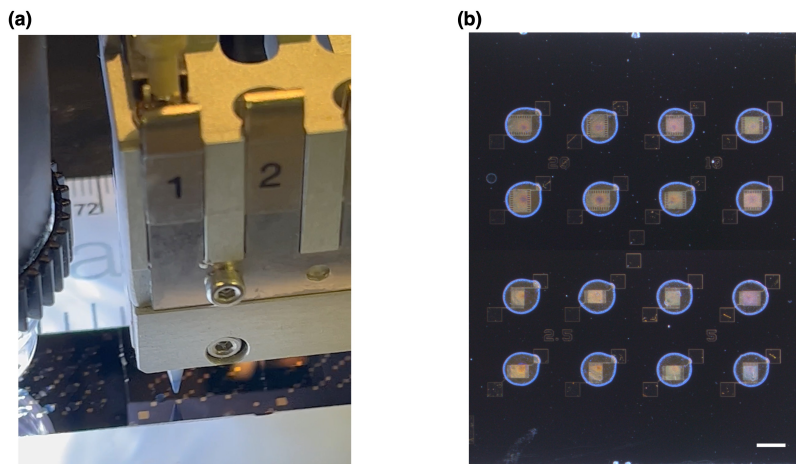


Figure 3.14: (a) Photograph of the BioDOT system during dispensing of the OSC ink array and (b) the resulting printed array. Scale bar = 1 mm.

3.3 Results and Discussion

3.3.1 In-house fabricated devices

Representative transfer and output characteristics for an OFET fabricated entirely in-house, with a P(VDF-HFP)/PVP bilayer as the dielectric and C₁₆IDT-BT as the organic semiconductor, are presented in Figure 3.15. The hole mobility μ , threshold voltage V_T and ON/OFF ratio values found for this device T39 were $4.4 \times 10^{-3} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, +1.6 V and 119 respectively. Commenting first on the mobility μ , this value is a few orders of magnitude below a few reported C₁₆IDT-BT OFETs[20][10][11]. However, these devices are typically fabricated in a top-gate top-contacts configuration which is known to have increased charge injection efficiency due to the staggered structure.

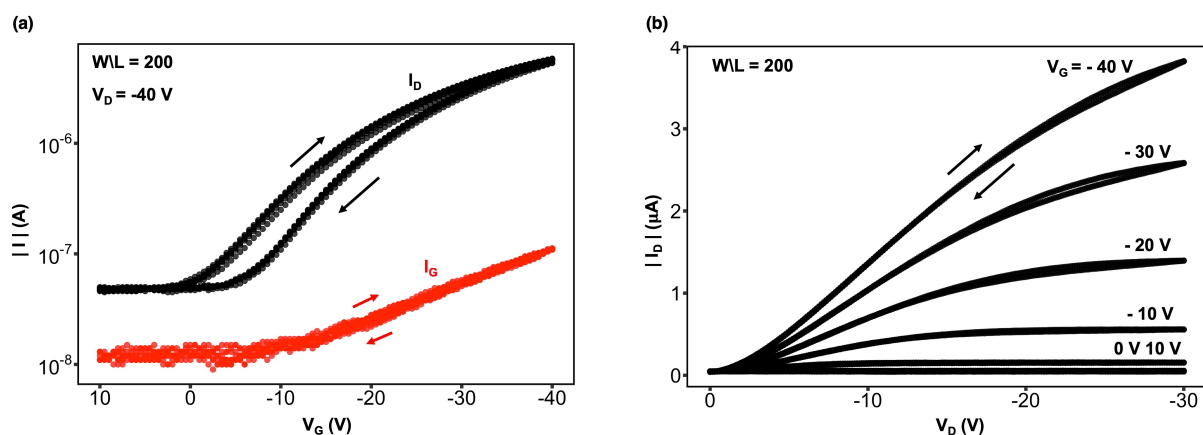


Figure 3.15: Device characteristics of an OFET device (T39) with dimensions $W = 10 \text{ mm}$, $L = 50 \text{ }\mu\text{m}$ dimensions (T39) including (a) the transfer characteristics (black) with the leakage current plotted (red) with 5 double sweep replicates and (b) the output characteristics. Arrows represent the forwards and backwards sweeps.

Nevertheless, a hole mobility of $10^{-3} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ is a reasonable value for amorphous materials such as conjugated polymers, for which achieving high purity and uniform molecular weights through synthesis can be challenging[21]. The threshold voltage value being close to zero is promising, as it indicates minimal trapping and that large gate voltages are not required to switch the device on. The ON/OFF ratio for OFET devices is typically in the range of 10^6 [22], so the value measured here is a few order of magnitude less. The leakage current I_G shown in Figure 3.15 (red curve) will likely contribute to the low ON/OFF ratio, by increasing the OFF state current I_{OFF} . The origin of this large I_G could indicate defects in the dielectric layer that degrade the insulating properties, despite the thickness of layer. Additionally, for this device the semiconductor was unpatterned and in direct electrical contact with the gate, which would also contribute to I_G .

Nevertheless, the operational stability of the device is promising, with negligible difference between all of the current-voltage sweeps as indicated by the low variation of V_T with a standard deviation $\sigma_{V_T} = \pm 1.55 \text{ V}$. Additionally, the hysteresis is relatively low in both plots, another indicator of minimal charge trapping behaviour. This suggests the thin interfacial PVP layer is acting to reduce the strong surface polarisation of the fluoropolymer.

However, one of the challenges of in-house fabrication was low yield. On the chip from which the device characteristics in Figure 3.15 were obtained, a yield of 36% functioning devices were obtained. This is represented in Figure 3.16 where the greyed-out boxes represent defected devices. A reason for this could be the dielectric cross-linking process, during which mechanical stress in the material would lead to cracks on areas of the film, which can lead to electrical shorts.

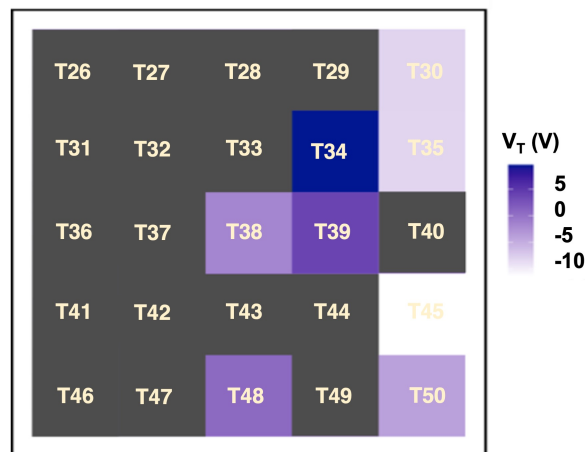


Figure 3.16: Heat map representing the threshold voltage V_T values of the the OFET array where grey boxes represent defected devices.

The $100 \mu\text{m}$ size had the most functioning devices, and the mean mobility and mean threshold voltage for these devices were extracted and plotted in Figure 3.17. Average

values of $\mu = 3.9 \times 10^{-3} \pm 6.92 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, $V_T = -8.4 \text{ V} \pm 3.1$ and 57 ± 14.2 were calculated for the mobility, threshold voltage and ON/OFF ratio respectively.

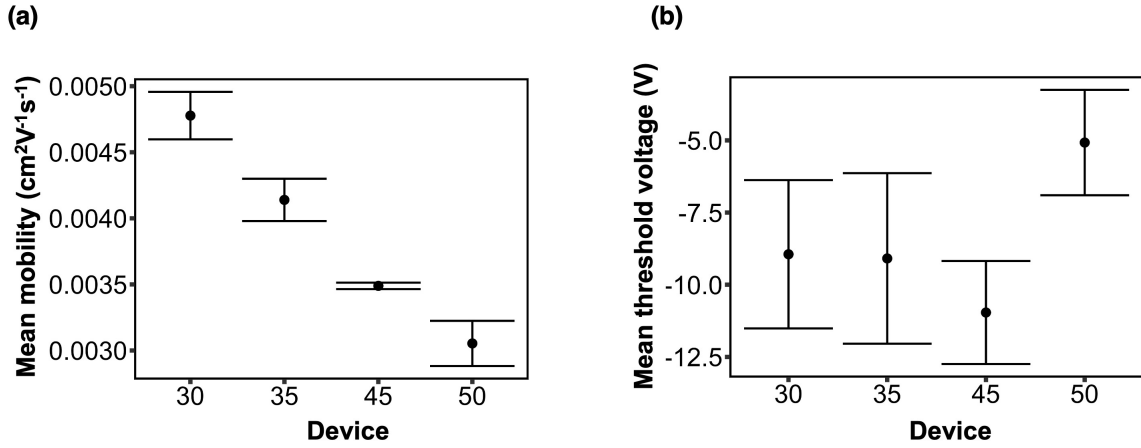


Figure 3.17: (a) Plot depicting the mean mobility and (b) the mean threshold voltage for four 100 μm channel-length devices denoted T30, T35, T45 and T50. N= 5 per device.

For most of these in-house fabricated devices, a low voltage operation was not achieved despite the efforts to employ a high- k dielectric. A reason for this could be an overestimation of the dielectric constant of this fluoropolymer formulation. A full characterisation of the dielectric properties of this formulation using impedance spectroscopy would be required to troubleshoot this. With this overestimation, employing a rather thick $\sim 1 \mu\text{m}$ layer would significantly reduce charge accumulation as the dielectric thickness is inversely proportional to the capacitance. The dielectric formulation would need to be optimised to ensure a minimum layer thickness, however there is typically a trade-off with increasing an likelihood of pinholes/defects.

Also to improve device yields, the dielectric layer processing methods should be optimised. Solution-processed layers deposited one after another can suffer from interfacial mixing and increased surface roughness. Consequently, when surface roughness increases above certain threshold, a steep drop in mobility has been reported[23][24]. Exploring options for orthogonal solvents is one strategy to reduce surface interfacial roughness[25].

The original intention was to continue optimising this microfabrication protocol, however the 2020 Covid-19 pandemic prevented access to clean room facilities for long time periods. The switch to pre-patterned substrates that can be bought from an external supplier was beneficial to continue progress with the project, however there is a trade off. There is little control over the device architectures and materials, such as the range channel lengths and the low- k SiO_2 dielectric which restricts the charge accumulation ability.

3.3.2 Devices fabricated on pre-formed substrates

The devices on these substrates may be referred to with the notation T1-T16 in the following results sections. T1 - T4 represents 20 μm channel lengths, T5 - T8 represents 10 μm channel lengths, T9 - T12 represents 5 μm channel lengths and T13 - T16 represents 2.5 μm channel lengths. The semiconducting polymer IDT-BT-N₃ is employed in the OFETs in this section.

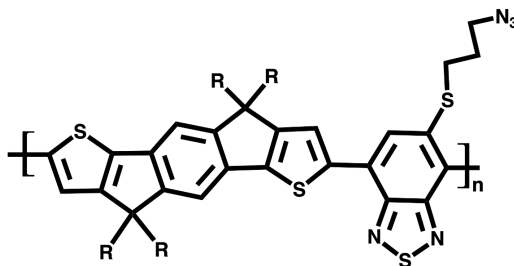


Figure 3.18: Chemical structure of the semiconducting polymer IDT-BT-N₃

Silane treatment on pre-formed substrates

As described in section 3.2.2, the substrate cleaning protocol involves O₂ plasma treating. This can result in dangling hydroxyl groups on the surface that are well known to trap charges and degrade the device performance through effects such as hysteresis[26]. Depositing alkylsilanes that can form strong covalent bonds with SiO₂ can passivate these bonds stop them interacting unfavourably with the charge carriers. Success of this surface treatment can be verified with contact angle measurements as shown in Figure 3.19. After removing the protective photoresist through sonication with acetone and IPA, the SiO₂ surface is hydrophilic with a contact angle of $44.7 \pm 3.8^\circ$.

After being subject to the plasma treatment, the contact angle is significantly reduced to essentially 0°, an effect of the polar silanol groups on the surface. Then after the overnight OTMS treatment, the contact angle increased to $92.7 \pm 7.9^\circ$, indicating a hydrophobic surface with lowered surface energy as a result of the newly deposited alkyl chains. There are many different protocols relating to silane deposition each which can affect the properties of the deposited SAM[27].

Simple solution immersion was the chosen method here due to the simplicity and ease of the setup, however it can suffer from low reproducibility. Ambient moisture getting into the system can cause the organosilane to polymerise, hindering monolayer formation.[28]. The use of anhydrous toluene rather than ethanol can facilitate extracting moisture from the oxide surface. The concentration of silane solution, the immersion time and temperature will all impact the SAM formation[29]. Also, insufficient rinsing after

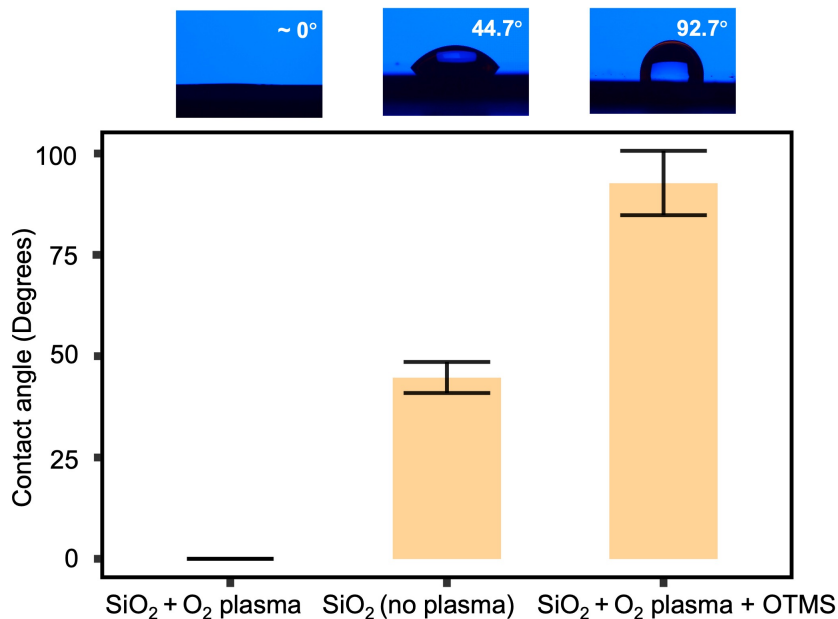


Figure 3.19: Contact angle measurements of the SiO₂ surface during the silanization process. $N = 3$ on three areas of the sample.

deposition can cause aggregation on the surface, hampering monolayer formation. Thus a sonication step was carried out in fresh solvent to wash away any unbound silane.

Figure 3.20 shows the transfer characteristics of two substrates spin coated with the same organic semiconductor ink formulation, however one was subjected to OTMS treatment prior to deposition. It is immediately clear that the OTMS treated devices exhibit superior device performance in terms of increased drain current I_D and a reduction in hysteresis. This indicates a reduced degree of charge trapping, suggesting less dangling hydroxyl groups on the surface.

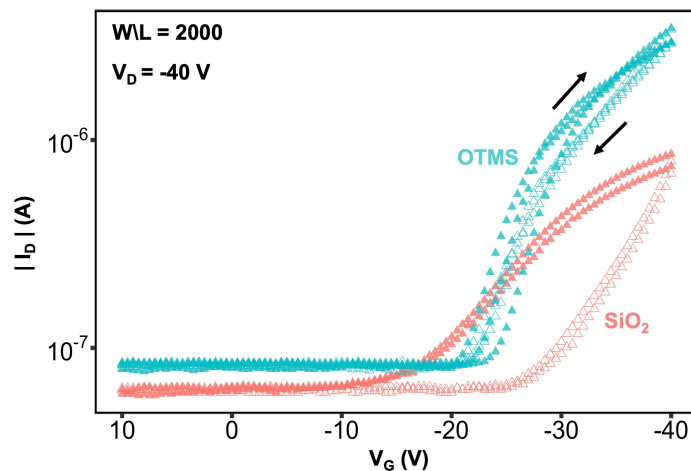


Figure 3.20: Transfer characteristics of 5 mg mL⁻¹ IDT-BT-N₃ OFETs formed on Si/SiO₂ substrates with and without OTMS treatment. 3 current-voltage sweep replicates are shown. The arrows and filled and unfilled symbols represent the forwards and backwards sweeps.

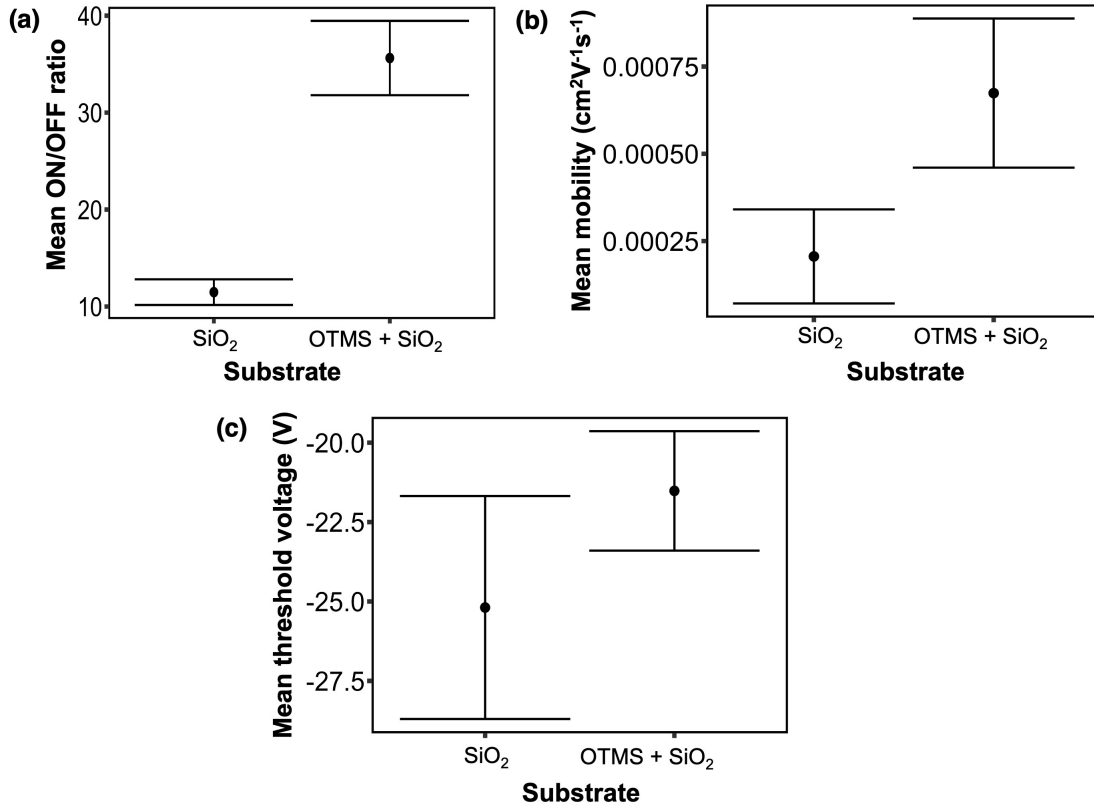


Figure 3.21: Plot depicting the mean (a) ON/OFF ratio, (b) mobility and (c) mean threshold voltage for 5 mg mL^{-1} IDT-BT- N_3 OFET devices of the same size formed on Si/SiO_2 with and without OTMS treatment. $N = 3$. Error bar represents the standard deviation.

Additionally, a hydrophobic surface will help hinder the adsorption of water at the dielectric-semiconductor interface. The extracted figure of merits in Figure 3.21 show and increase in ON/OFF ratio from 11 to 35, an increase in μ from $2 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ to $6.7 \times 10^{-4} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$ and a reduction in threshold voltage from -25 V to -21 V indicating clear performance benefits relating to a reduction in charge trapping, consistent with reports[30].

Characterisation of OFET arrays and deposition methods

The current-voltage results from an array of devices formed from spincoating IDT-BT-N₃ thin films on Si/SiO₂ substrates, are represented in Figure 3.22. The results confirm the drain current I_D dependence on channel-size. The relationship in Equation 2.6 states that I_D scales with W/L , thus increase in the ON/OFF ratio as the device dimensions decrease, as seen in Figure 3.23(a), is expected.

The largest threshold voltage was obtained for the smallest 2.5 μm channel length and is associated with the largest inter-device variation. It is well known in organic electronics that as the channel length decreases, ‘short channel effects’ can arise. This can lead to inaccuracies in the calculated threshold voltage and mobility values, as the effects of contact resistance become more significant in the total device resistance[22]. Underestimated and overestimated figure of merits due to OFET parameter extraction is an ongoing issue in the organic electronics field[31]. As mentioned, the model described in section 2.1.3 was originally derived for MOSFETS, using the gradual channel approximation assuming ohmic contacts between the source and drain electrodes and a band transport mechanism in the semiconductor.

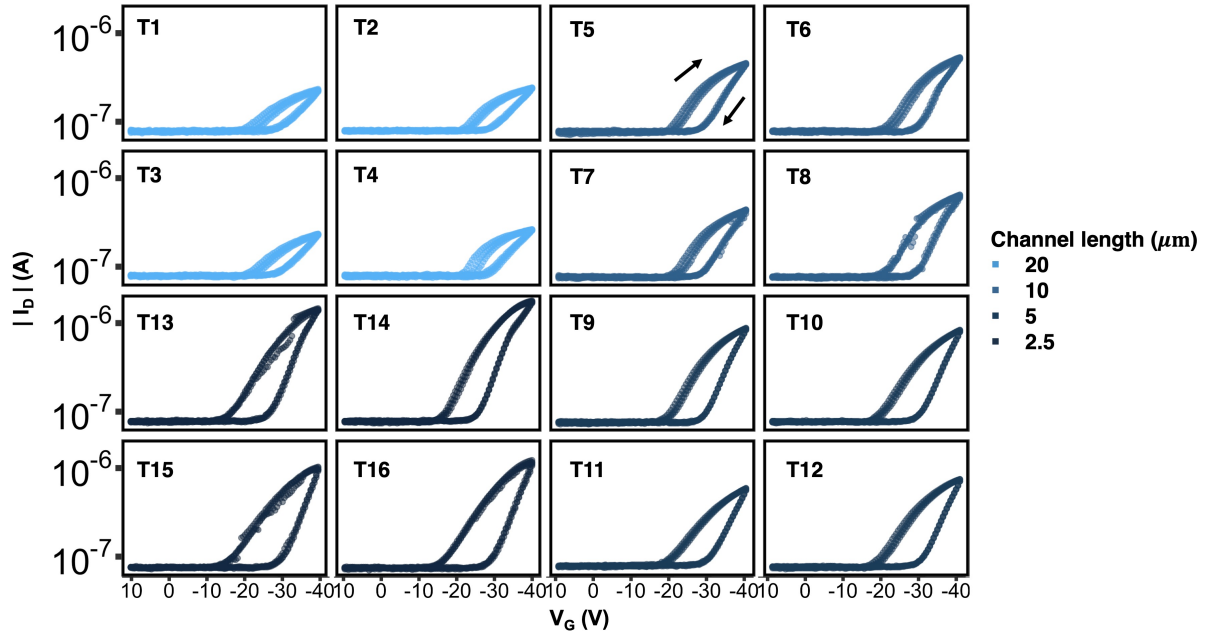


Figure 3.22: Transfer characteristics of 5 mg mL^{-1} IDT-BT- N_3 OFETs formed on Si/SiO₂ substrates via spin coating. Plot depicts devices T1 - T16. Colour gradient from light to dark represents the channel-lengths $20 \text{ } \mu\text{m}$, $10 \text{ } \mu\text{m}$, $5 \text{ } \mu\text{m}$, $2.5 \text{ } \mu\text{m}$. 5 current-voltage sweep replicates are shown. Arrows represent the forwards and backwards sweeps.

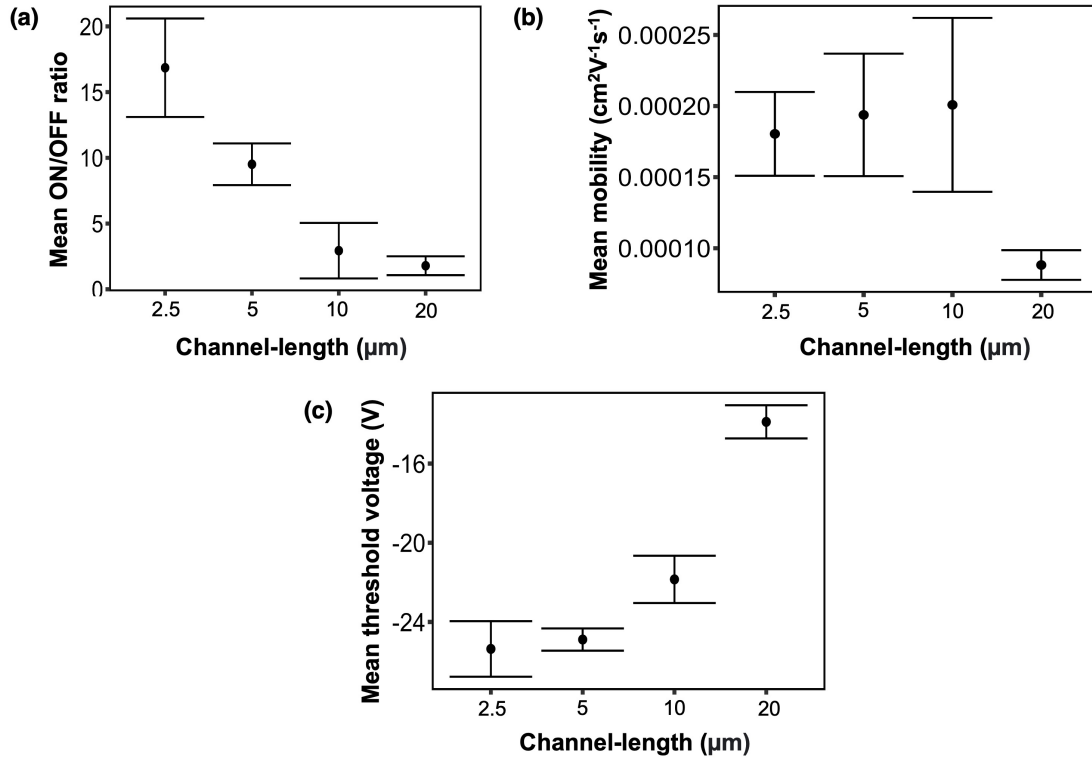


Figure 3.23: Plot depicting the mean (a) ON/OFF ratio, (b) mobility and (c) threshold voltage for 16 devices formed on Si/SiO₂ substrates via spin coating 5 mg mL^{-1} IDT-BT- N_3 , grouped by channel-length. $N = 4$. Error bars represent the standard deviation.

Conversely, OFETs are generally fabricated with disordered organic semiconductors and there are often mismatched energy levels at the contacts for charge injection[32]. This leads to the aforementioned hopping transport and high contact resistance and therefore drastic deviations from the ideal model suggesting that larger channel devices should be relied upon to extract the key performance metrics.

The interdevice variation is relatively low across all sizes, suggesting the benefits of spin coating as a deposition method to fabricate uniform thin films. Comparing to these results to the OFETs formed via automatic dispensing of the organic semiconductor, Figure 3.24 shows the transfer characteristics for a printed device. It's clear from observing the current-voltage curves that these dispensed devices result in a lower drain current I_D across all device dimensions. The hysteresis levels for this printed array also appear to be reduced, however this is not consistent across all devices, perhaps indicating some inhomogeneity in the OTMS SAM deposition for this particular substrate, causing spatially-dependent charge trapping.

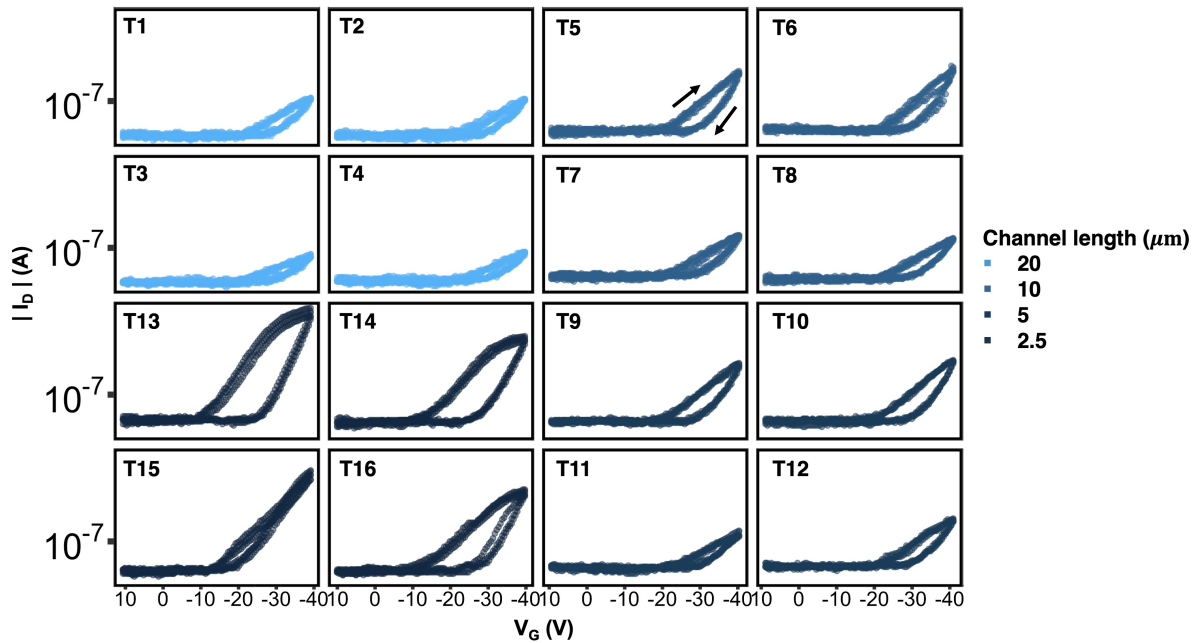


Figure 3.24: Transfer characteristics of 5 mg mL^{-1} IDT-BT-N₃ OFETs formed on Si/SiO₂ substrates via dispensing. Plot depicts devices T1 - T16. Colour gradient from light to dark represents the channel-lengths $20 \mu\text{m}$, $10 \mu\text{m}$, $5 \mu\text{m}$, $2.5 \mu\text{m}$. 5 current-voltage sweep replicates are shown. Arrows represent the forwards and backwards sweeps.

From the ON/OFF ratio values extracted in 3.25, it shows despite the lower drain current I_D reducing these values, the inter-device variation is low across most of the device sizes. Additionally, the current-voltage curves in Figure 3.24 demonstrate that the extracted OFF current I_{OFF} for these printed devices $7.2 \times 10^{-08} \text{ A}$ is lower than for the spin coated devices spin coat $7.8 \times 10^{-08} \text{ A}$. This could be an effect of the organic semiconductor patterning, which is a strategy to reduce leakage current and cross-talk

between neighbouring devices. However, the mobility values are two orders of magnitude smaller than the spin coated devices. For the aims of this work, device mobility values must be sufficient to withstand further modifications of the surface e.g. with biomolecules and still return a stable signal, thus further optimisation may be required.

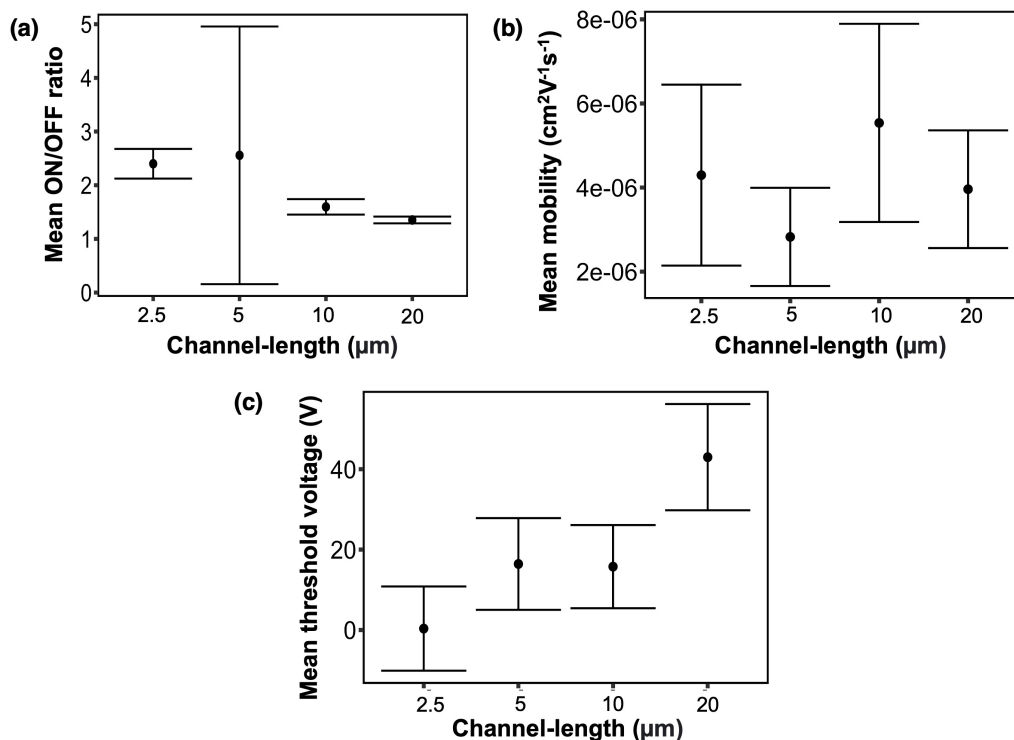


Figure 3.25: Plot depicting the mean (a) ON/OFF ratio, (b) mobility and (c) mean threshold voltage for 16 devices formed on Si/SiO₂ substrates via dispensing 5 mg mL⁻¹ IDT-BT-N₃ droplets, grouped by channel-length. $N = 4$. Error bar represents the standard deviation.

Inkjet printing is likely most analogous to the high-speed automatic dispensing system employed here, and the performances of devices formed via printing are typically lower than that of devices fabricated via spin coating. This is because printing typically induces rough interfaces, resulting in uneven morphology and randomly oriented molecular structures that can affect charge transport at that critical organic semiconductor-dielectric interface. For this reason, much research effort has been focused on strategies to control the morphology of these printed layers through careful selection of solvents, consideration of surface energy mismatches and utilisation of blend formulations[33][34]. Despite the engineering challenges, the economical benefit is stark, with the dispensed OFETs here requiring 3 orders of magnitude less semiconducting ink than the spin coated OFETs. Additionally, removing human error and variation from the experimental process through depositing multiple substrates simultaneously with this high-throughput technique, will likely be beneficial for biosensing assays. Thus despite the drawbacks, this technique was employed for the main biosensor protocol.

Molecular additives for semiconducting polymers

The incorporation of molecular additives to organic semiconductor inks has been investigated as a strategy to boost the environmental and operational stability of OFETs. Ubiquitous moisture in the atmosphere can be absorbed by organic semiconductor films and these environmental species can generate trap states and contribute to non-ideal current-voltage characteristics and poor performance. Nikolka *et al.* reported that incorporating small concentrations of additives into semiconducting polymers can lead to distinct improvements in electrical performance and reproducibility[10]. Here, three small molecule additives were investigated for the semiconducting polymer solutions - 4-aminobenzonitrile (ABN), tetracyanoquinodimethane (TCNQ) and tetrafluoro-tetracyanoquinodimethane (F4TCNQ), the control refers to neat IDT-BT-N₃ without any additive.

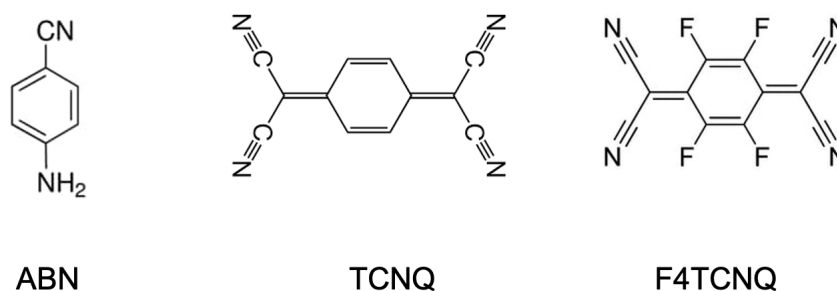


Figure 3.26: Chemical structures of the additives used in this section: ABN, TCNQ and F4TCNQ.

Figure 3.27 shows that in terms of intradevice repeatability the F4TCNQ additive had the most stabilising effect on the transfer characteristics compared to the other inks. This suggests a reduction in bias stress effects, when OFETs operating under continuous electrical bias become unstable performance degradation. Bias stress effects can manifest in different ways, such as a shifts in the threshold voltage V_T , a reduction in the mobility μ and/or increased hysteresis in the device characteristics[35].

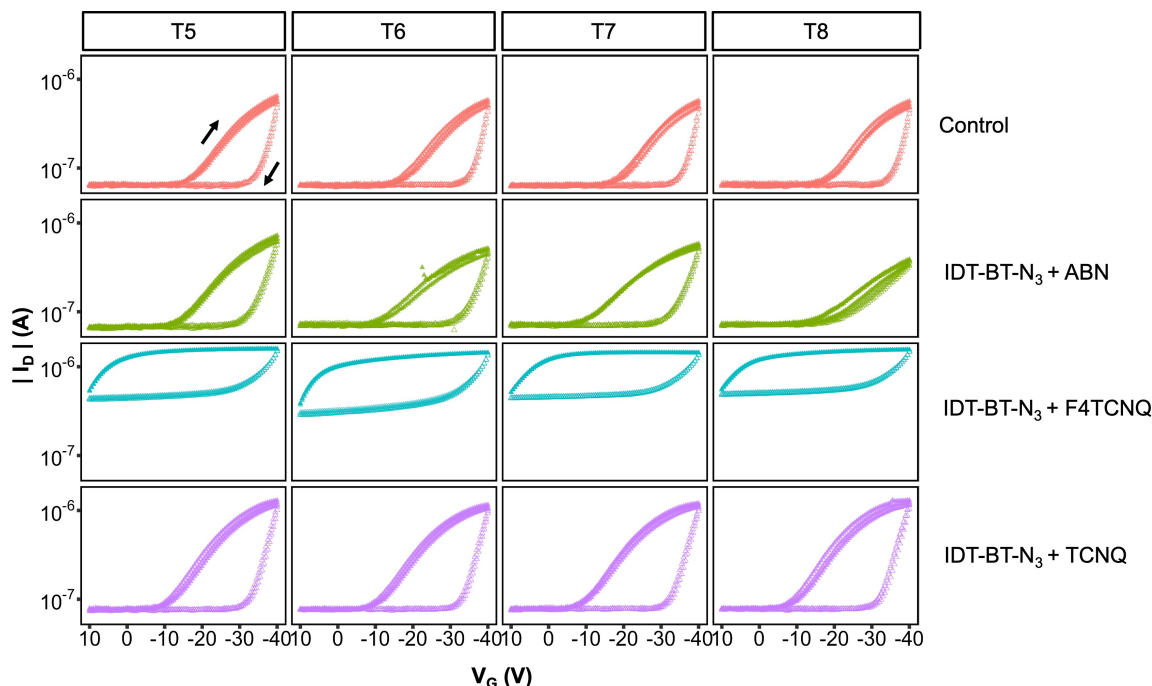


Figure 3.27: Transfer characteristics of devices incorporated with various additives into the IDT-BT-N₃ formulation. Plot depicts 5 current-voltage sweep replicates of four devices T5 - T8 per additive formulation. Each additive was incorporated in the semiconductor at 10 wt%. Arrows represent forward and backwards sweeps.

There are also clear p-doping effects from the F4TCNQ additive at this concentration which can be seen from the large jump in I_{OFF} at low V_G (Figure 3.27 (light blue)). This is to be expected, as the electron affinity for this molecule is at a close enough level to the HOMO of the organic semiconductor to induce some charge-transfer doping which manifests as an increased I_{OFF} . This is not the case for TCNQ and ABN, for which the electron affinities are too low to dope the active layer[10]. Typically large OFF currents are undesirable in organic electronics where a large switching power is desired to compete with inorganic transistors in microprocessors. However, for applications in biosensing, stability and reliability are more appropriate aims. There are degrees of hysteresis in the curves across nearly all devices, indicating no real changes in charge trapping behaviour upon addition of additives.

However, the persistence of this undesirable property could be expected, as these devices are both prepared and tested in ambient conditions as opposed to a nitrogen controlled glove box. This in addition to the chosen geometry, where the organic semiconductor is exposed to the environment, could mean that the incorporation of additives cannot totally displace moisture in the system and some form of encapsulation required needed to hinder this.

It is worth noting that comparing device characteristics to reported literature can be complex and regarding comparing hysteresis levels specifically, papers that report performance improvements through molecular additives may display one current-voltage sweep

direction so the hysteresis cannot be evaluated[10][11][12]. Additionally, many reported devices are in top-gated configuration where the organic semiconductor is protected from the environment so a direct comparison is inappropriate.

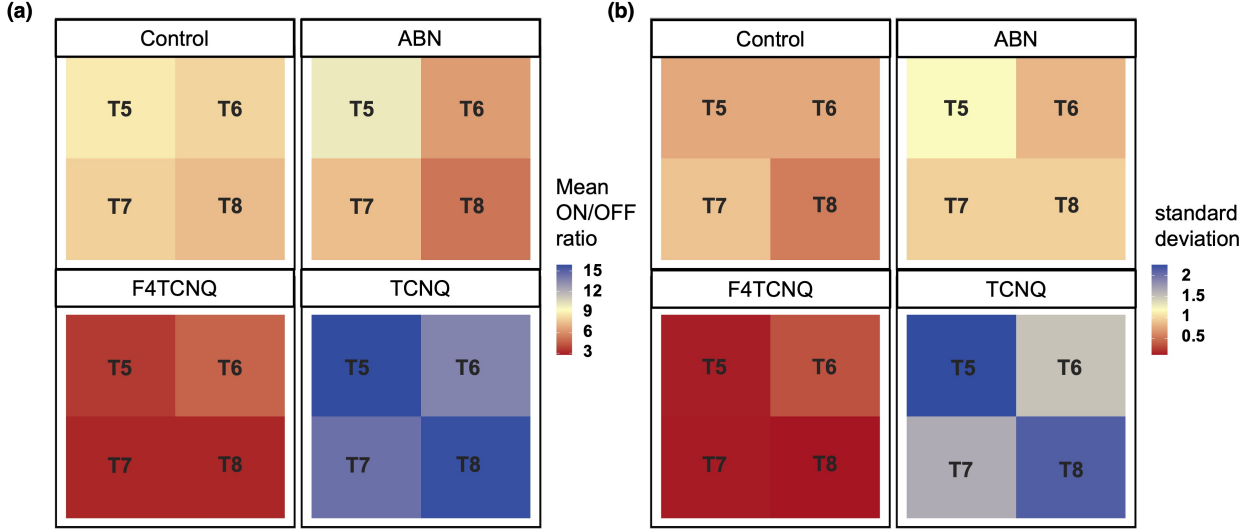


Figure 3.28: Heat maps depicting (a) the mean ON/OFF ratio for devices T5, T6, T7, T8 and (b) the associated standard deviation. $N = 5$.

Extracting the common figures of merits from the current-voltage curves allows assessment of the intra-device and inter-device repeatability. The heat maps in Figure 3.28 demonstrate that for the F4TCNQ formulation, the lowest mean ON/OFF ratio of 3 was measured. However, this is due to the large increase in I_{OFF} reducing the ratio, since the drain current I_D value is the highest of all formulations.

Promisingly, the variation of the ON/OFF ratio between current-voltage sweep replicates is the lowest for the F4TCNQ formulation indicating good intra-device repeatability. Additionally, this value is similar for all four devices T5, T6, T7 and T8 showing good inter-device repeatability.

Figure 3.29 appears to contradict this, showing TCNQ to have the most stable threshold voltage V_T of all formulations. However, the more devices deviate from ideal behaviour, such as they do the significantly for the F4TCNQ blend, the more the MOSFET I_D model used to extract threshold value begins to break down and this could be a source of the V_T variation. Nevertheless, the shift of V_T to more positive values is consistent with reports of F4TCNQ as a molecular dopant[36], which has the effect of reducing the potential barrier at the contact with the electrode.

Molecular doping also decreases interface traps and makes better pathways for charge carriers. An increase in mobility compared to the control appears most for the TCNQ blend and next for the F4TCNQ blend, as seen in Figure 3.30, another effect consistent with reports of molecular dopants in organic semiconductors[36], with the highest intra- and inter-device repeatability for the F4TCNQ dopant.

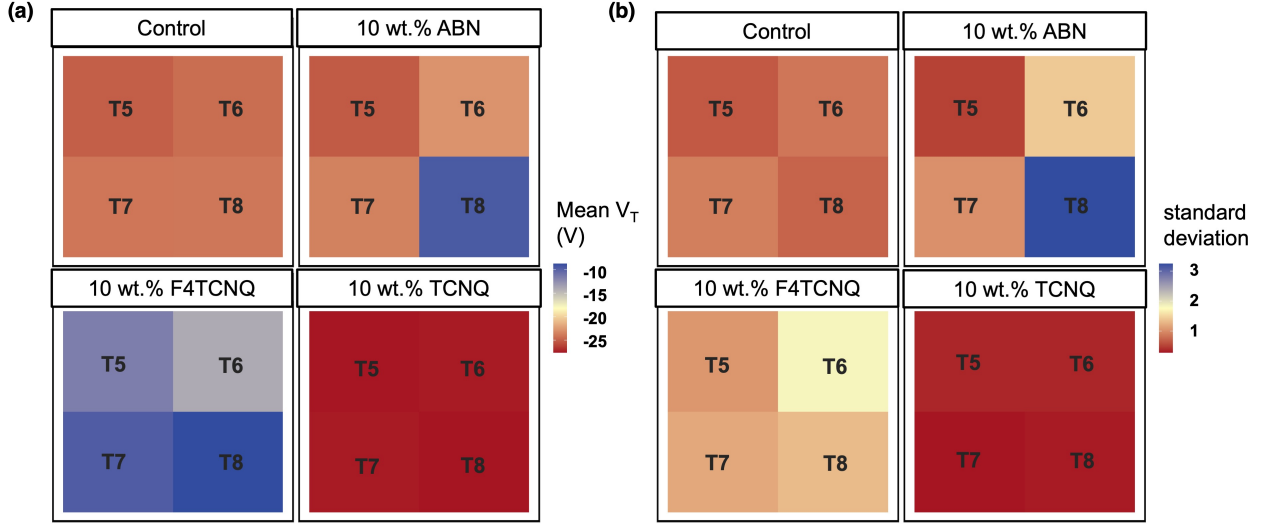


Figure 3.29: Heat maps depicting (a) the mean threshold voltage for devices T5, T6, T7, T8 and (b) the associated standard deviation. $N = 5$

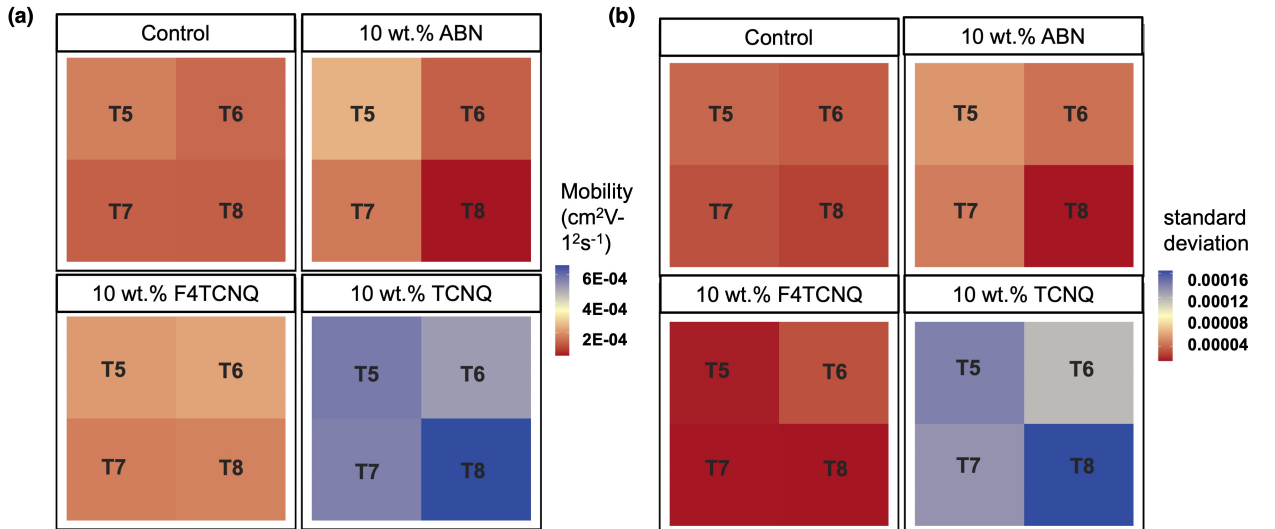


Figure 3.30: Heat maps depicting (a) the mean mobility for devices T5, T6, T7, T8 and (b) the associated standard deviation. $N = 5$

With these results, F4TCNQ was selected to carry about a concentration study and additive formulations were added to the semiconducting ink at concentrations of 1 wt.%, 2 wt.%, 5 wt.% and 10 wt.%. Again, Figure 3.31 shows that the 10 wt.% formulation exhibited the most stabilising effect on the transfer characteristics with the lowest amount of bias stress shifting of V_T , however there are even at the lowest concentration of 1 wt.%. The disappearance of the charge transfer doping effect at lower concentrations can be also be seen, with no significant increases in I_{OFF} .

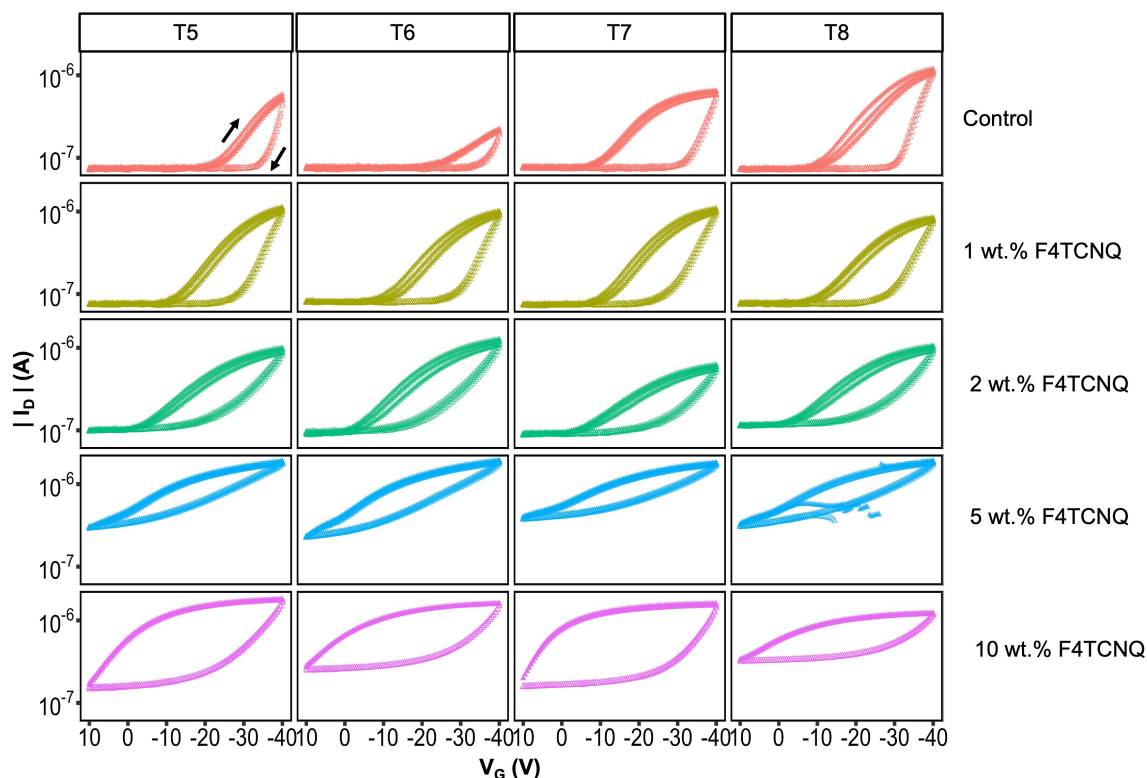


Figure 3.31: Transfer characteristics of devices incorporated with F4TCNQ additives in the IDT-BT-N₃ formulation from 1 - 10 wt.%. Plot depicts 5 x IV sweep replicates of four devices T5 - T8 per chip. Arrows represent forward and backwards sweeps.

However, as there inevitably will be batch variations between chips in terms of the alkyl silane SAM deposition and rough interfaces of printed organic semiconductor droplets drying, comparisons remain difficult. Another source of variation could be differing levels of solubility of additives within the conjugated polymer ink; if some additive blends were not dissolved sufficiently, not all water-induced traps in the thin film would not be efficiently passivated.

Nevertheless, the heatmaps in Figure 3.32 and Figure 3.33 demonstrates that for the 10 wt.% F4TCNQ additive blend, the devices show the intra-device variation of the measurements. Therefore, this was the blend selected to boost device stability before subsequent biofunctionalisation and analyte detection assays.

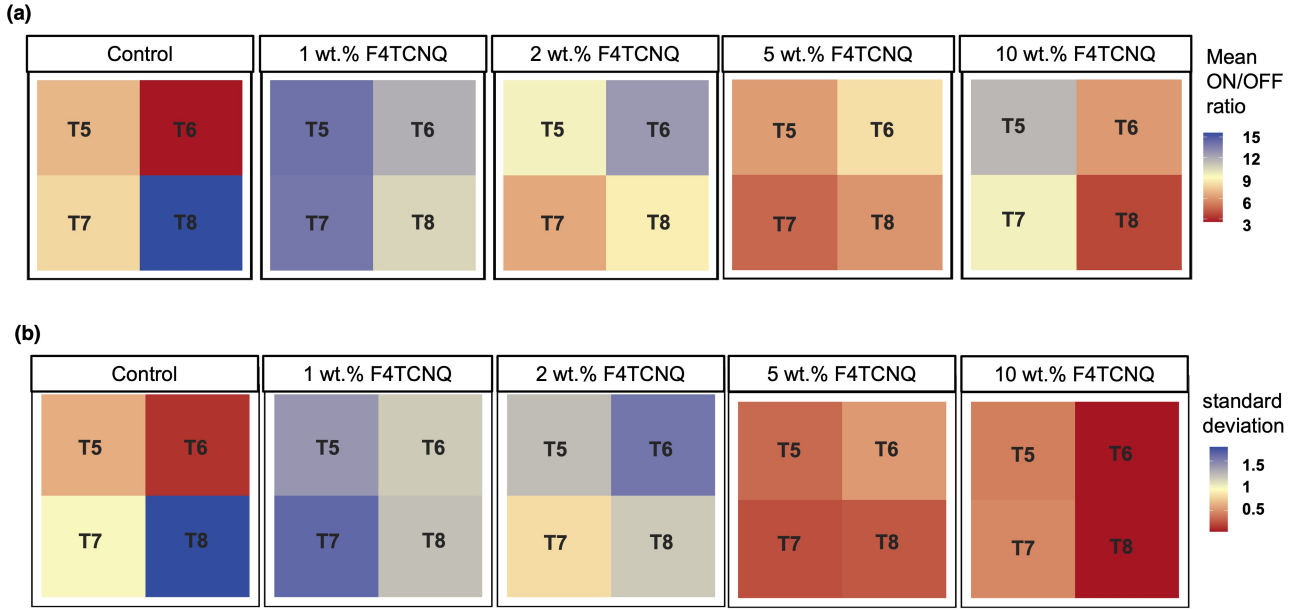


Figure 3.32: Heat maps depicting (a) the mean ON/OFF ratio across 5 replicates for devices T5, T6, T7, T8 and (b) the associated standard deviation.

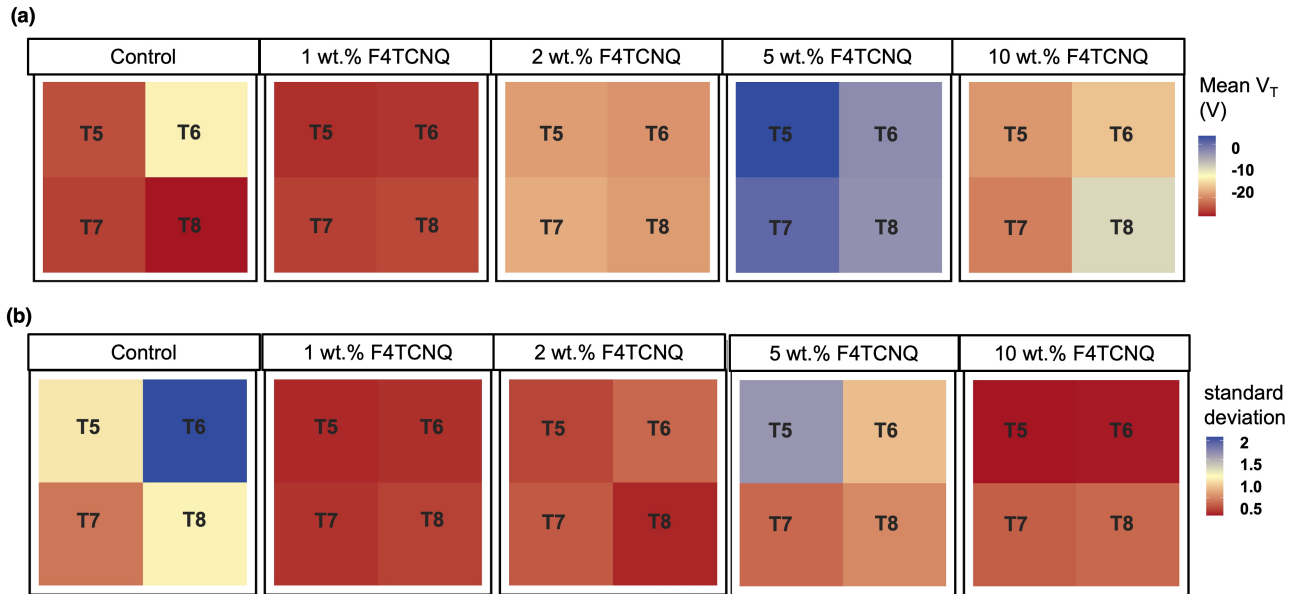


Figure 3.33: Heat maps depicting (a) the mean threshold voltage across 5 replicates for devices T5, T6, T7, T8 and (b) the associated standard deviation.

3.4 Conclusions

This chapter demonstrates the formation of OFETs arrays for application in biosensing platforms. The microfabrication of OFET arrays was successfully carried out entirely in-house. Initial results proved promising, with current-voltage characteristics exhibiting good gain I_D in the μA range and low hysteresis indicating minimal charge trapping. However, low yields were achieved, likely due to defects from crosslinking the bulk polymer dielectric and surface roughness from interfacial mixing of the thinner surface layer with the bulk. Additionally, the desired low-voltage operation was not achieved, likely due to these dielectric defects increasing the leakage current through the device or the high- k fluoropolymer formulation having a lower dielectric constant than presumed. The Covid-19 pandemic halted the progress of the in-house microfabrication optimisation due to lengthy clean room restrictions. Eventually, the project pivoted to utilising commercially-bought BGBC Si/SiO₂ OFET substrates, accepting the trade-off of restricted low-voltage operation ability.

After plasma cleaning, dangling bonds on the SiO₂ can lead to charge trapping which degrade electrical performance thus alkyl-silane SAMs were deposited to passivate these bonds prior to organic semiconductor deposition. However, silane treatments are highly sensitive to the presence of moisture in the system which can lead to issues with reproducibility. There are many differing protocols for alkyl-silane SAM deposition[27] and with more time to screen through the protocols to find the most reproducible would be beneficial to optimise this critical dielectric-semiconductor interface for charge transport.

Spin coating and automatic dispensing were the methods used to deposit the semiconducting inks. Thin films fabricated through spin coating are typically highly uniform and homogeneous leading to improved morphology, however it remains a batch-process and would not be considered appropriate for any large scale process. Dispensed films on the other hand offer versatile, high-throughput substrate fabrication, increasing the potential of these devices to be mass production at low cost. Generally, the resulted demonstrated that in terms of mobility and ON/OFF ratio, the printed films exhibited a lower electrical performance compared to spin coated films, likely due to drying artefacts and rough interfaces. However, placing a higher priority on automated systems with an ability to scale, dispensing was the method chosen to apply in biosensor fabrication, rather than the method that produced the ‘best’ performance metrics.

Three molecular additives ABN, TCNQ and F4TCNQ were investigated in organic semiconductor inks to improve operational and environmental stability. F4TCNQ was found to have the most stabilising effect on the current-voltage sweep replicates, indicating a reduction of the bias stress effect which causes shifts in V_T during operation.

It also caused a strong doping effect with a large increase in I_{OFF} reducing the overall ON/OFF ratio despite increased I_D . Hysteresis was largely unchanged across all additive formulations, suggesting that additives alone are not sufficient to curb all water-induced charge trapping. The 10 wt.% formulation was selected going forward to to stabilise the underlying array prior to the biofunctionalisation and BioFET assays.

References

- [1] A. Dodabalapur, *Materials Today* **2006**, *9*, 24–30.
- [2] V. Raghuwanshi, S. P. Tiwari in *Advanced Micro- and Nano-manufacturing Technologies: Applications in Biochemical and Biomedical Engineering*, (Eds.: S. N. Joshi, P. Chandra), Materials Horizons: From Nature to Nanomaterials, Springer, Singapore, **2022**, pp. 315–333.
- [3] L. Torsi, M. Magliulo, K. Manoli, G. Palazzo, *Chemical Society Reviews* **2013**, *42*, 8612–8628.
- [4] J. Wang, D. Ye, Q. Meng, C.-a. Di, D. Zhu, *Advanced Materials Technologies* **2020**, *5*, 2000218.
- [5] T. Minami, Y. Sasaki, T. Minamiki, S.-i. Wakida, R. Kurita, O. Niwa, S. Tokito, *Biosensors and Bioelectronics* **2016**, *81*, 87–91.
- [6] T. Minamiki, T. Minami, R. Kurita, O. Niwa, S.-i. Wakida, K. Fukuda, D. Kumaki, S. Tokito, *Applied Physics Letters* **2014**, *104*, 243703.
- [7] M.-J. Spijkman, K. Myny, E. C. P. Smits, P. Heremans, P. W. M. Blom, D. M. de Leeuw, *Advanced Materials* **2011**, *23*, 3231–3242.
- [8] W. Tang, J. Zhao, Y. Huang, L. Ding, Q. Li, J. Li, P. You, F. Yan, X. Guo, *IEEE Electron Device Letters* **2017**, *38*, 748–751.
- [9] H. Sirringhaus, *Advanced Materials* **2009**, *21*, 3859–3873.
- [10] M. Nikolka, I. Nasrallah, B. Rose, M. K. Ravva, K. Broch, A. Sadhanala, D. Harkin, J. Charmet, M. Hurhangee, A. Brown, S. Illig, P. Too, J. Jongman, I. McCulloch, J.-L. Bredas, H. Sirringhaus, *Nature Materials* **2016**, *16*, 356.
- [11] M. Nikolka, G. Schweicher, J. Armitage, I. Nasrallah, C. Jellett, Z. Guo, M. Hurhangee, A. Sadhanala, I. McCulloch, C. B. Nielsen, H. Sirringhaus, *Advanced Materials* **2018**, *30*, 1–8.
- [12] E.-S. Shin, J.-Y. Go, G.-S. Ryu, A. Liu, Y.-Y. Noh, *ACS Applied Materials & Interfaces* **2021**, *13*, 4278–4283.
- [13] G. Scarpa, A.-L. Idzko, A. Yadav, E. Martin, S. Thalhammer, *IEEE Transactions on Nanotechnology* **2010**, *9*, 527–532.

-
- [14] J. Mei, Z. Bao, *Chemistry of Materials* **2014**, *26*, Publisher: American Chemical Society, 604–615.
- [15] W. H. Lee, Y. D. Park, *Polymers* **2014**, *6*, 1057–1073.
- [16] S. Han, X. Yu, W. Shi, X. Zhuang, J. Yu, *Organic Electronics* **2015**, *27*, 160–166.
- [17] S. G. Higgins, B. V. O. Muir, J. Wade, J. Chen, B. Striedinger, H. Gold, B. Stadlober, M. Caironi, J.-S. Kim, J. H. G. Steinke, A. J. Campbell, *Advanced Electronic Materials* **2015**, *1*, 1500024.
- [18] J. Golden, H. Miller, D. Nawrocki, J. Ross, *CS MANTECH Conference* **2009**, 5.
- [19] C. Wang, W.-Y. Lee, D. Kong, R. Pfattner, G. Schweicher, R. Nakajima, C. Lu, J. Mei, T. H. Lee, H.-C. Wu, J. Lopez, Y. Diao, X. Gu, S. Himmelberger, W. Niu, J. R. Matthews, M. He, A. Salleo, Y. Nishi, Z. Bao, *Scientific Reports* **2015**, *5*, 17849.
- [20] A. Wadsworth, H. Chen, K. J. Thorley, C. Cendra, M. Nikolka, H. Bristow, M. Moser, A. Salleo, T. D. Anthopoulos, H. Sirringhaus, I. McCulloch, *Journal of the American Chemical Society* **2020**, *142*, 652–664.
- [21] P. Hu, X. He, H. Jiang, *InfoMat* **2021**, *3*, 613–630.
- [22] Z. Bao, J. Locklin, *Organic Field-Effect Transistors*, CRC Press, **2018**, 640 pp.
- [23] X. Sun, C.-a. Di, Y. Liu, *Journal of Materials Chemistry* **2010**, *20*, 2599–2611.
- [24] G. Song, Y. Wang, D. Q. Tan, *IET Nanodielectrics* **2022**, *5*, 1–23.
- [25] A. M. Gaikwad, Y. Khan, A. E. Ostfeld, S. Pandya, S. Abraham, A. C. Arias, *Organic Electronics* **2016**, *30*, 18–29.
- [26] S. Onclin, B. J. Ravoo, D. N. Reinhoudt, *Angewandte Chemie International Edition* **2005**, *44*, 6282–6304.
- [27] M. Singh, N. Kaur, E. Comini, *Journal of Materials Chemistry C* **2020**, *8*, 3938–3955.
- [28] N. R. Glass, R. Tjeung, P. Chan, L. Y. Yeo, J. R. Friend, *Biomicrofluidics* **2011**, *5*, 036501–036501–7.
- [29] S. Lee, T. Ishizaki, N. Saito, O. Takai, *Japanese Journal of Applied Physics* **2008**, *47*, 6442.
- [30] Y. Ito, A. A. Virkar, S. Mannsfeld, J. H. Oh, M. Toney, J. Locklin, Z. Bao, *Journal of the American Chemical Society* **2009**, *131*, 9396–9404.
- [31] E. G. Bittle, J. I. Basham, T. N. Jackson, O. D. Jurchescu, D. J. Gundlach, *Nature Communications* **2016**, *7*, 10908.

- [32] F. Huang, A. Liu, H. Zhu, Y. Xu, F. Balestra, G. Ghibaudo, Y. Noh, J. Chu, W. Li, *IEEE Electron Device Letters* **2019**, *40*, Publisher: Institute of Electrical and Electronics Engineers, 605–608.
- [33] J. A. Lim, J.-H. Kim, L. Qiu, W. H. Lee, H. S. Lee, D. Kwak, K. Cho, *Advanced Functional Materials* **2010**, *20*, 3292–3297.
- [34] L. Feng, C. Jiang, H. Ma, X. Guo, A. Nathan, *Organic Electronics* **2016**, *38*, 186–192.
- [35] S. Park, S. H. Kim, H. H. Choi, B. Kang, K. Cho, *Advanced Functional Materials* **2020**, *30*, eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.201904590>, 1904590.
- [36] W. Lu, J. Cao, C. Zhai, L. Bu, G. Lu, Y. Zhu, *ACS Applied Materials & Interfaces* **2022**, *14*, 23709–23716.

Chapter 4

Surface-functionalisation of conjugated polymers

This chapter reports on the surface functionalisation of organic semiconductor thin films with compounds, proteins and nucleic acid sequences for biosensing purposes, using a novel immobilisation method. The characterisation of these biofunctional surfaces is demonstrated using several characterisation methods. The protocols presented here were subsequently used to fabricate the BioFET devices reported in Chapter 5 and Chapter 6 to target specific analytes.

4.1 Motivation

The surface modification of conjugated polymers is frequently investigated topic due to their promising applications as active layers in optoelectronic devices[1][2] and in semiconducting polymer nanoparticles for targeted imaging, drug/gene delivery and therapeutics[3]. The ability to tune the electronic, physical, chemical and biological properties of conjugated polymers to introduce functional groups onto the backbone is the key to their popularity for these applications. In the context of BioFETs specifically, the attachment of biomolecules to conjugated polymer surfaces can be used to provide biological groups for the specific detection and quantification a target. Therefore, the search for efficient surface functionalisation routes is central towards this aim.

The challenge arises from the fact that the surface properties of polymers are typically correlated to their bulk properties. The delocalised π -system of conjugated polymers provides the material with enhanced stability, meaning they are often poorly reactive and inert in ambient conditions. The traditional method of adapting the bulk structures can be challenging and inefficient. It often relies on the co-polymerisation of suitable monomer materials to provide the desired functionality[4], which can be time-consuming and difficult to control. Variations in molecular weight and dispersity mean it can be difficult

to compare between batches and establish relationships between the material structure and the resulting properties. Concerning BioFETs specifically, adapting bulk structures by co-polymerisation of monomers bearing large functional groups for biorecognition, can disrupt the delocalised π -system. Steric interactions between neighbouring groups can induce backbone twisting which has been shown to hinder charge transport and degrade the electrical performance[5]. Consequently, the search for efficient routes to functionalise conjugated polymer surfaces remains an important focus.

It is notable that the majority of BioFET literature features systems bearing gate electrodes functionalised with biorecognition elements, either immersed in an electrolyte solution[6][7][8][9] or spatially separated from the where the amplification occurs[10][11][12]. In the previous chapter, the benefits of electrolyte-gating were discussed in terms of their ability to operate at very low voltages facilitating real-time sensing and low power consumption. However, the other benefit is the utility of the reliable thiol-gold interaction which occurs spontaneously to attach thiolated-biorecognition elements and to gold gate electrodes.

The work in this chapter aims to explore a much simpler method to covalently bind biomolecules to the surface of conjugated polymer thin films, without the need for expensive equipment to generate functional groups, without the use of intermediary couplings and without any changes to the bulk material to preserve charge transport. The method reported in this chapter builds on the work by Creamer *et al.* in functionalising conjugated polymer nanoparticle backbones with reactive handles for covalent grafting[13]. The starting material IDT-FBT is first synthesised using direct arylation, then undergoes a nucleophilic aromatic substitution, during which the highly electronegative, fluorine leaving group is replaced by a nucleophile, which bears an azide group. The result is an azide-functionalised conjugated polymer IDT-BT-N₃, which can now be used for the strain-promoted azide-alkyne click (SPAAC) chemistry reaction that occurs when dibenzoazacyclooctyne groups are brought together with azides[14].

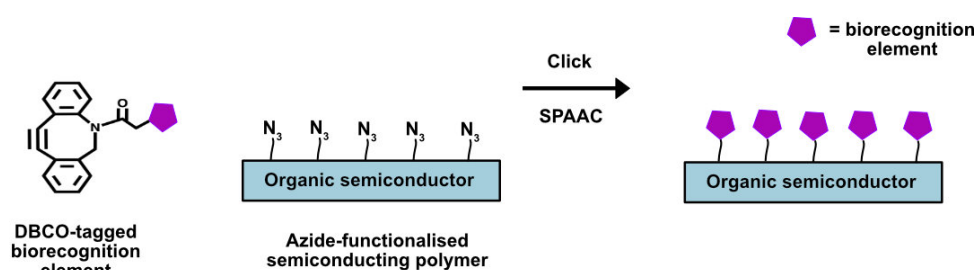


Figure 4.1: Schematic of the conjugated polymer surface functionalisation through strain-promoted azide-alkyne click chemistry reaction.

This is a simple, catalyst-free reaction that occurs spontaneously at room temperature, in a timescale of 1-2 hours. The DBCO tag can be attached to any desired probe, providing a highly modular system with potential for multiplexing. This one-step biofunc-

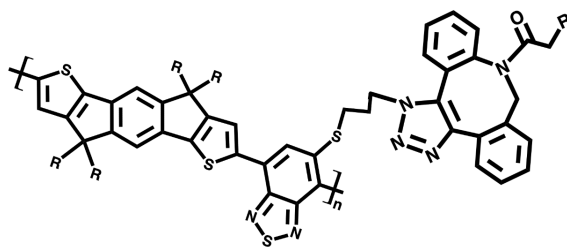


Figure 4.2: Chemical structure of the resulting of conjugated polymer after functionalisation with a DBCO-conjugate through the strain-promoted azide-alkyne click chemistry reaction. R represents the biorecognition element.

tionalisation method markedly simplifies the state-of-the-art of covalent functionalisation strategies to conjugated polymer surfaces, and allows direct coupling of probe and target to the active layer for BioFET sensing.

4.2 Materials and Methods

4.2.1 Azide-functionalisation of IDT-FBT

The starting material, fluorinated IDT-BT (IDT-FBT) was provided by Prof. Martin Heeney or synthesised in-house by Dr. Adam Creamer in the group of Prof. Molly Stevens. IDT-FBT ($M_w = 1314$ g/mol) (5 mg, 0.0038 mmol, 1.0 eq.) and potassium hydroxide (KOH) (8.5 mg, 0.15 mmol, 40 eq.) were placed under argon in a glass vial. A chlorobenzene (CB)/dimethylformamide (DMF) (3:1 v:v) solvent mixture was then added to the vial under argon and the reaction was heated to 120 °C. The solution was stirred and heated for 10 min before adding an excess of the *s*-(3-azidopropyl)thioacetate (6.1 mg, 0.038 mmol, 10 eq.). The vial was left stirring on the hotplate at 120 °C for several hours reaction to take place. After cooling the solution was precipitated into methanol (10 mL) while under stirring for 20 mins. The solution was then passed through through a vacuum filter and washed first x2 with methanol then x2 with acetone.

4.2.2 Surface biofunctionalisation

Thin film deposition

IDT-BT- N_3 was prepared as per section 4.2.1. Solutions of IDT-BT- N_3 (5 mg mL^{-1} or 10 mg mL^{-1}) were prepared by dissolving in DCB and stirring with heat (80 °C). Solutions were filtered through a 0.45 μm PTFE filter, spin coated (2000 rpm, 45 s, 500 rpm^{-1}) on SiO_2 or glass substrates and annealed for 30 minutes at 100 °C and allowed to cool gradually.

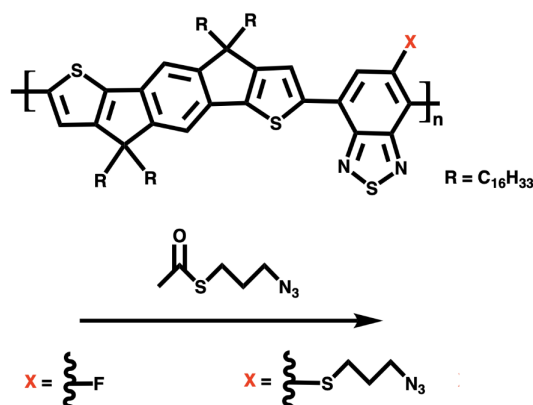


Figure 4.3: Schematic of the nucleophilic aromatic substitution reaction used to functionalise the conjugated polymer with azide groups. The fluorine groups on the backbone are substituted with the nucleophilic group bearing an azide.

PEG immobilisation

Solutions of DBCO-PEG2K (1 mM) (Biochempeg) were prepared by dissolving in DMSO, stirring and filtering through a $0.45\ \mu\text{M}$ PTFE filter. PEG solutions were deposited on the IDT-BT- N_3 covered substrates and left for 1-2 hours. Substrates were subsequently rinsed with DMSO to remove any unbound protein and dried with N_2 .

Biotin immobilisation

Solutions of DBCO-PEG2K-Biotin (0.25 - 1 mM) (Biochempeg) were prepared by dissolving in DMSO, stirring and filtering through a $0.45\ \mu\text{M}$ PTFE filter. Biotin solutions were deposited on the IDT-BT- N_3 covered substrates and left for 1-2 hours. Substrates were subsequently rinsed with DMSO to remove any unbound protein and dried with N_2 .

ssDNA immobilisation

Solutions of single stranded synthetic ctDNA sequences 5' DBCO TTT TTT AGT GAT TTT AGA GAG AGG ATC TCG 3' ($2.5 - 10\ \mu\text{M}$) (Integrated DNA Technologies) were prepared in NaCl-TAE buffer by spinning in a mini centrifuge and vortexing. ssDNA solutions were deposited on the IDT-BT- N_3 covered substrates and left for 1-2 hours. Substrates were subsequently rinsed with DI water to remove any unbound DNA and dried with N_2 .

4.3 Results and Discussion

4.3.1 Azide-functionalisation of IDT-FBT

This section will discuss characterisation of the reaction product IDT-BT-N₃ after the nucleophilic aromatic substitution. As shown in Figure 4.4 (a) and (b), there is a visible colour change of the polymer from a lighter blue starting material to a deeper blue reaction product. This shift is also confirmed by in the UV-Vis spectra in 4.4(c), where the absorption maximum (λ_{max}) undergoes a blue-shift from 658 nm to 600 nm. This shift is likely due to the effect of the newly bound azide group off the backbone, causing steric interactions between molecules which generates backbone twisting. This disruption to the planarity reduces the effective conjugation length of the polymer and manifests in a shortening of λ_{max} [15].

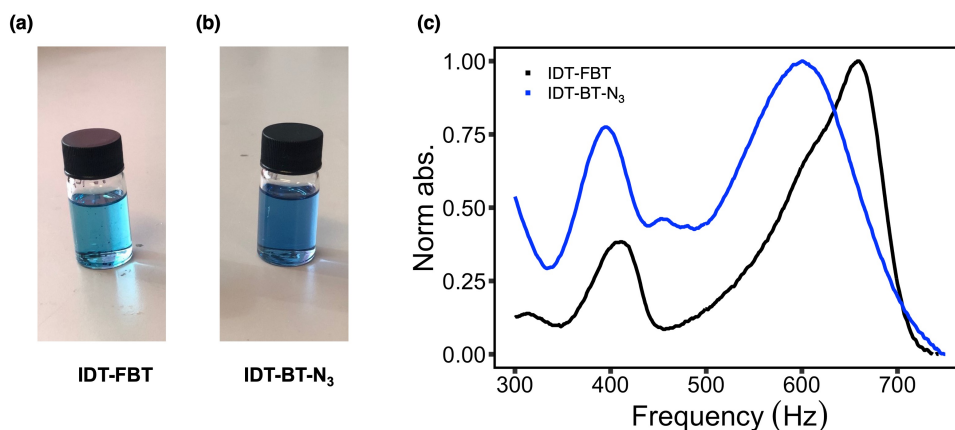


Figure 4.4: Photographs of (a) IDT-FBT solution in THF and (b) IDT-BT-N₃ solution in THF and (c) UV-Vis absorption spectra of IDT-FBT and IDT-BT-N₃ in THF.

The reaction product was also characterised using nuclear magnetic resonance spectroscopy (NMR) with the guidance of Dr. Adam Creamer in the group of Prof. Molly Stevens. There are cooperatively very few reports of conjugated polymer characterisation with proton (¹H) NMR. High molecular weight conjugated polymers are often exhibit poor solubility, which can lead to aggregation in solution and broad featureless ¹H NMR spectra, making analysis difficult[16]. Nevertheless for this material, the presence of azides on the backbone could be determined using ¹H NMR as shown in Figure 4.5, where two peaks (green box) appear in the product spectrum (red spectrum) in the range 3.2 – 3.5 ppm which correspond to the protons next to the azide and the sulphur groups.

Trichloroethylene (TCE) was the solvent used for the starting material spectrum (black spectrum) and it is this solvent peak that hits at the same location at 3.5 ppm. The two peaks that emerge are absent in the starting material spectrum so this appears to have emerged from the substitution reaction. However, to determine the full substitution of the azide groups for the fluorine groups on the backbone, fluorine (¹⁹F) NMR was also

conducted. In Figure 4.6 there are well-defined peaks for the starting material IDT-FBT which corresponds to the fluorine group. No peaks are observed for the product suggesting there are no fluorine groups in the product and a full substitution occurred as desired.

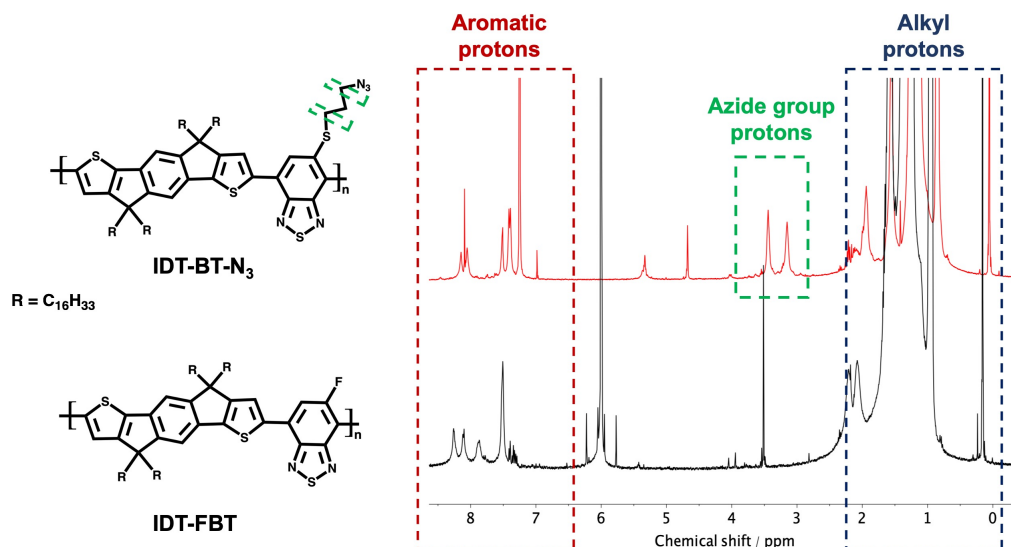


Figure 4.5: Proton NMR of the product IDT-BT-N₃ (red spectrum) and the starting material IDT-FBT (black spectrum). The corresponding structures are provided for reference. Data collected with assistance from Dr. Adam Creamer.

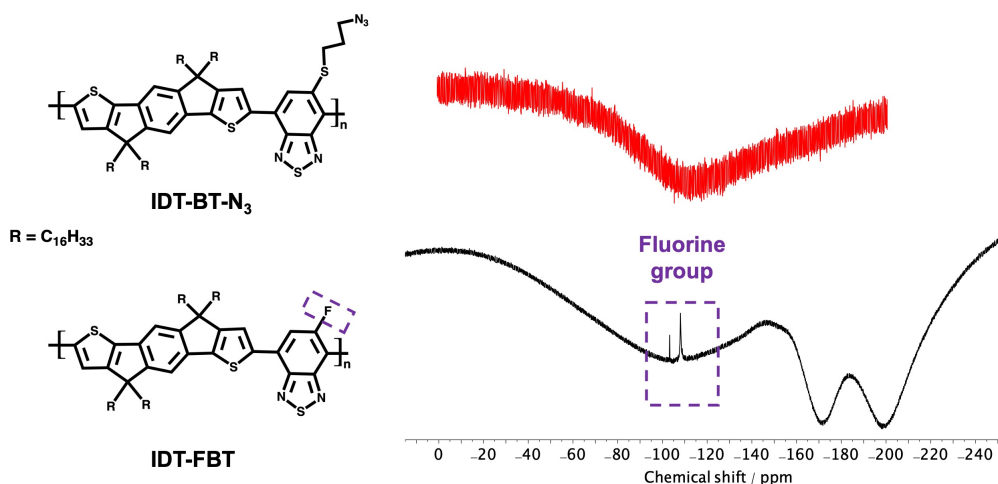


Figure 4.6: Fluorine NMR of the product IDT-BT-N₃ (red spectrum) and the starting material IDT-FBT (black spectrum). The corresponding structures are provided for reference. Data collected with assistance from Dr. Adam Creamer.

4.3.2 PEG immobilisation

This section with present results of conjugated polymer IDT-BT-N₃ thin films immobilised with polyethylene glycol (PEG) molecules. PEG, a neutral, non-toxic hydrophilic

molecule, is an interesting candidate for biosensors for its suggested anti-fouling properties[17] as well as promoting target diffusion to the sensor surface[18].

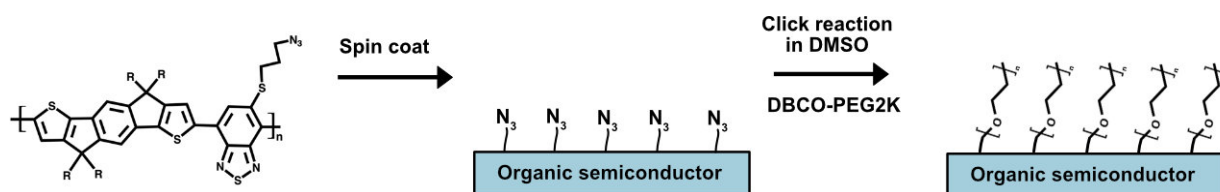


Figure 4.7: Schematic of the surface functionalisation of conjugated polymer thin films with PEG groups.

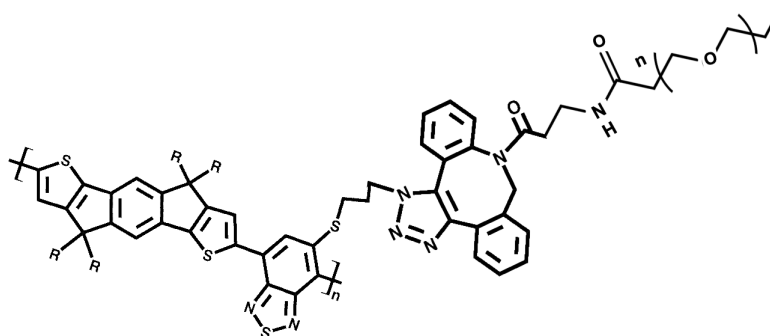


Figure 4.8: Chemical structure of the resulting of conjugated polymer surface after functionalisation with a DBCO-PEG2K conjugate through the strain-promoted azide-alkyne click chemistry reaction. $n = 2000$

Contact angle

Contact angle measurements were initially used to verify PEG grafting to the conjugated polymer thin film surface, as shown in Figure 4.9. The neat polymer which is hydrophobic, is a good system to determine the presence of biomolecules with contact angle measurements. There is a reduction in contact angle as a function of the PEG chain length, indicating the surface becoming more hydrophilic, and the success of the PEG bonding to the surface.

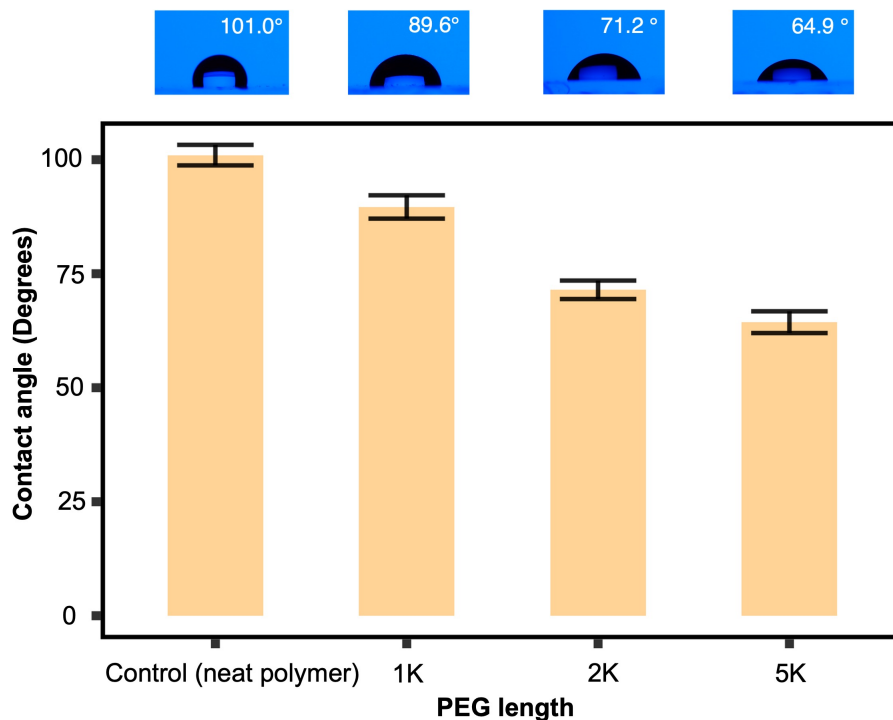


Figure 4.9: Contact angle measurements of DBCO-PEG2K conjugates immobilised on the IDT-BT-N₃ thin film surface. $N = 3$. The errors bars represent the standard deviations.

QCM-D

Quartz crystal microbalance with dissipation monitoring (QCM-D) measurements were also attempted as a method to validate the clicking of PEG groups to the conjugated polymer surface. First the QCMD-D sensor chip was coated with the IDT-BT thin film then solutions of DBCO-PEG5K in DMSO buffer were flown across the surface at a continuous rate (typically $\sim 15 \mu\text{L/s}$). The trend in Figure 4.10 demonstrates a decrease in the frequency of the crystal resonance, appearing to confirm the immobilisation of the DBCO-PEG5K molecules on the sensor surface coated with IDT-BT-N₃. When the frequency reaches saturation after approximately 30 minutes, this indicates no more binding taking place as the thin film surface also becomes saturated. A rinse with the immobilisation buffer DMSO shows that the frequency remains largely unchanged after rinsing, indicating stable bonding of PEG groups to the surface.

However it was observed that the semiconducting polymer underwent significant swelling under constant flow of DMSO measurement buffer, making it difficult to establish a clear baseline. This can be seen in the start of the plot, before the DBCO-PEG5K solution was added. Ideally there should be a flat, stable baseline before the measurement begins. However, this is not achievable for many soft material systems where the time needed to allow the swelling to reach somewhat an equilibrium can be incredibly long and inefficient. Therefore, a reported strategy is to simply select a consistent time to start the measurement and any response greater in magnitude than

the drift response can be attributed to that of the deposited material.[19]. The use of QCM-D as a characterisation system was not continued throughout the project due to the experimental challenges associated with technique. Particularly due to the frequent delamination of the polymer from the gold sensor surface and frequent air bubbles in the flow system which can cause large inaccuracies in the measurement and render the data unusable.

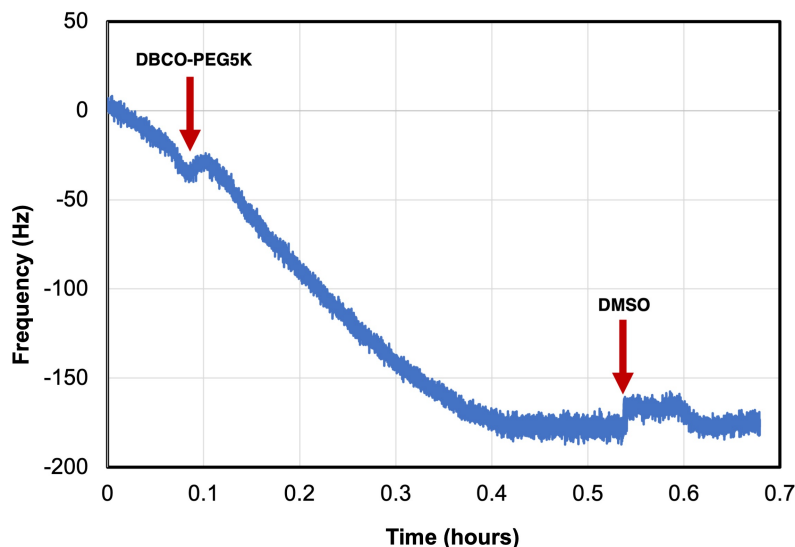


Figure 4.10: The example of the QCM-D frequency signal change upon immobilisation of DBCO-PEG5K to the sensor coated with IDT-BT-N₃ after rinsing with the DMSO immobilisation buffer. The time points at which the PEG solution was introduced to the sensor and when the buffer rinse was introduced are marked with red arrows.

PEG coatings for anti-fouling

This section investigates the use of PEG coatings on conjugated polymer thin films for anti-fouling purposes in a biosensing context. Non-specific protein adsorption of from groups in natural complex media to sensor surfaces can reduce the assay sensitivity and reproducibility, thus various strategies have been considered to counter this[20]. Here, the effectiveness of PEG coatings to resist adsorption of a fluorophore-tagged non-specific protein was investigated. PEG coatings have been thought to resist protein adsorption, either by steric interactions or due to a structured water ‘barrier’ created by the PEG chain[21]. However the effectiveness of such coatings will depend on parameters such as the the chain length (molecular weight) and the chain density.

Existing methods to fabricate PEG coatings involve self-assembly and various graft-to and graft-from synthetic strategies[22]. However, these methods can suffer from drawbacks such as complexity, substrate compatibility, low yield and the potential for generating reactive species. The glass chips presented in Figure 4.11 were coated with IDT-BT-N₃

thin films and then immobilised with DBCO-PEG groups of various chain lengths at the same concentration. On all PEGylated surfaces 100 μL of a streptavidin-AlexaFluor790 conjugate solution was pipetted on the surface, left for 10 minutes, rinsed with DI water then dried with N_2 . All slides were imaged under constant illumination conditions at the same time. ImageJ was used to obtain the average pixel intensity by selecting 3 regions within the area of the deposited fluorophore solution. Pixel intensity values of 40.0 ± 4.0 , 25.2 ± 0.7 , 30.6 ± 0.5 and 17.2 ± 1.4 were calculated for the neat polymer, DBCO-PEG1K, DBCO-2K and DBCO-5K substrate respectively.

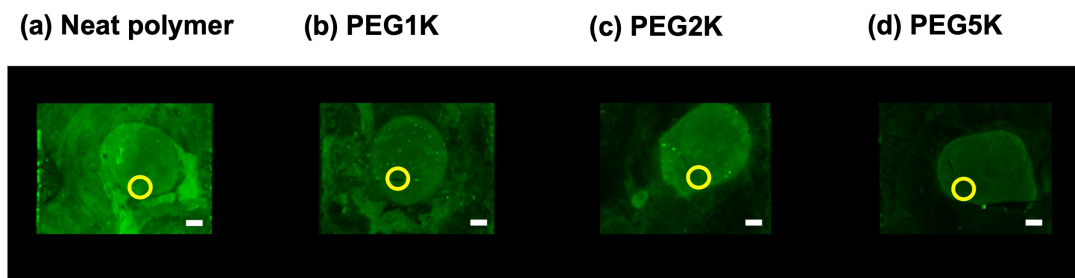


Figure 4.11: The effect of PEGylation on the non-specific adsorption of a streptavidin-AlexaFluor790 conjugate. Examples of region of interests use to measure pixel intensity are highlighted in yellow. Scale bar = 2 mm.

The results appear to demonstrate that the chip bearing the PEG 5K chain length was most effective at blocking the adsorption of the non-specific streptavidin-Alexa790 (Thermo Fisher) conjugate. There are reports that suggest longer PEG chain lengths, with their high degree of flexibility, give rise to an increased excluded-volume suggesting that they occupy a larger space and prevent molecules from approaching as closely[23][24]. However, there is typically an upper threshold above which this effect ceases so further optimisation would required to select the correct molecular weight.

4.3.3 Biotin immobilisation

This section presents the work towards immobilising conjugated polymer IDT-BT- N_3 thin films with biotin molecules. The biotin-streptavidin interaction is commonly utilised as a detection system in biosensor validation studies due of their inherently high affinity for one another. This also applies to BioFET studies, where this is often employed as a model system for an initial proof-of-concept[25]. The binding of these biotin and streptavidin is typically rapid and highly selective. It can also withstand wide ranges of experimental conditions in pH, temperature and organic solvents. Measuring 244 daltons in size, biotin is relatively small making it an attractive choice for bioconjugate development. PEG is often used as a spacer molecule in bioconjugates to increase the the solubility and to provide more conformational freedom to the probe molecules hence the DBCO-PEG2K-Biotin group employed in this system as depicted in Figure 4.12.

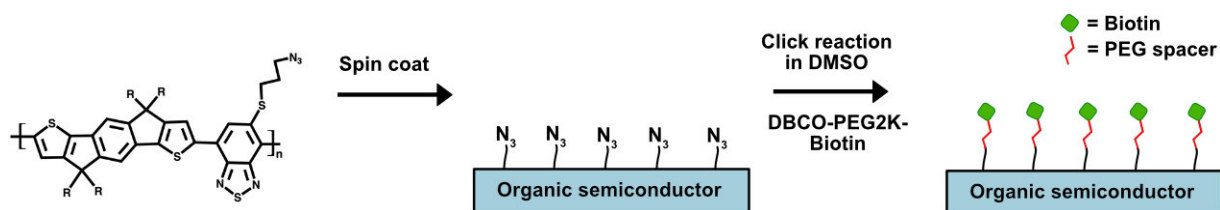


Figure 4.12: Schematic of the surface functionalisation of conjugated polymer thin films with DBCO-PEG-Biotin groups.

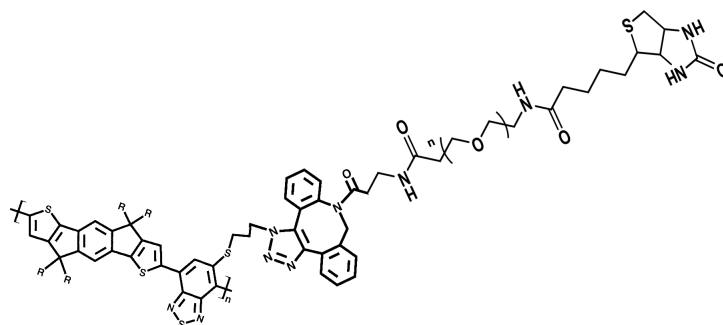


Figure 4.13: Chemical structure of the resulting of conjugated polymer surface after functionalisation with a DBCO-PEG2K-Biotin conjugate through the strain-promoted azide-alkyne click chemistry reaction. $n = 2000$

Contact angle

Contact angle measurements were initially used to verify biotinylation of the conjugated polymer thin film. From the measurements displayed in Figure 4.14, the control sample of neat IDT-BT- N_3 exhibits hydrophobic behaviour. Then after immersion in DBCO-PEG2K-Biotin solutions, the surface is rendered more hydrophilic. This effect also scales with increasing concentration and is expected as a result of increased amounts of the hydrophilic PEG spacer and protein on the surface.

Fluorescence microscopy

Biotin immobilisation on conjugated polymer thin films was also validated with fluorescent microscopy. After biotinylation, a 0.5 mg mL^{-1} solution of an streptavidin-AlexaFluor790 conjugate (Thermo Fisher) was pipetted on the surface, left for 30 minutes for the binding to take place and then imaged with the Licor Imaging System. All slides were imaged under constant illumination conditions at the same time. ImageJ was used to obtain the average pixel intensity by selecting 3 regions within the area of the deposited fluorophore solution. Pixel intensity values of 14.2 ± 0.4 , 34.1 ± 3.4 and 19.1 ± 0.9 were calculated for the neat polymer, 0.5 mM DBCO-PEG2K-Biotin and 1 mM DBCO-PEG2K-Biotin substrate respectively.

To calculate the average intensity, three regions of interest were chosen in the more homogeneous areas of the chip, away from fabrication artefacts that could skew the flu-

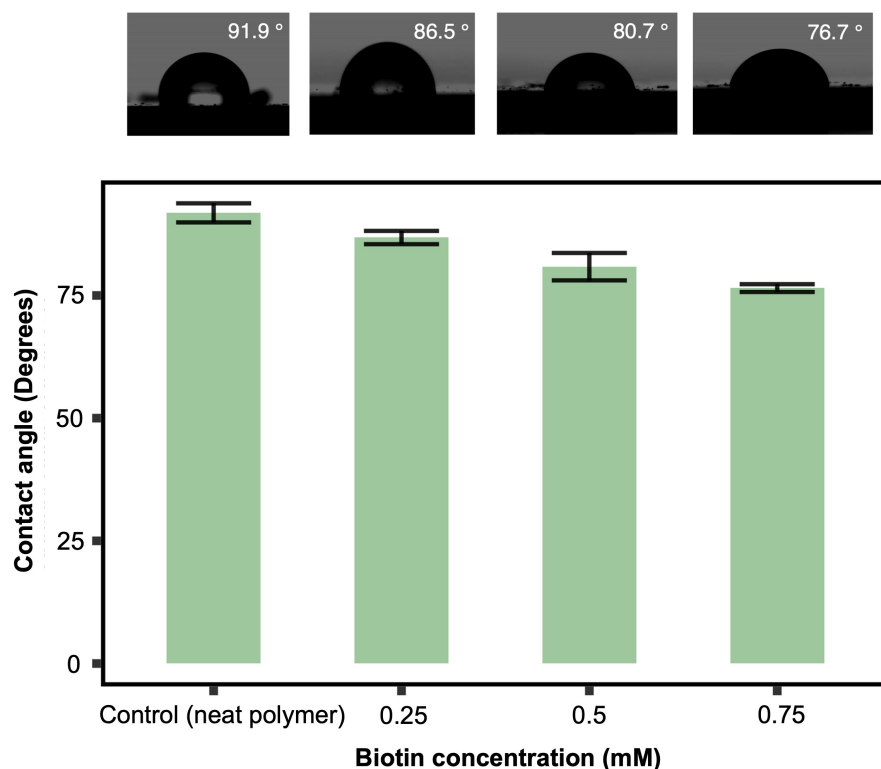


Figure 4.14: Contact angle measurements of DBCO-PEG2K-Biotin conjugates immobilised on the IDT-BT-N₃ thin film surface. N = 3. The error bars represent the standard deviations.

(a) Neat polymer (no Biotin) (b) 0.5 mM DBCO-PEG2K-Biotin (c) 1 mM DBCO-PEG2K-Biotin

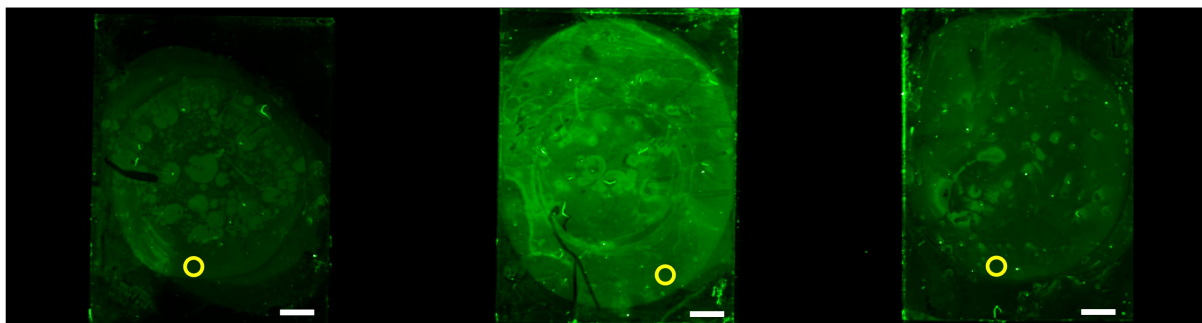


Figure 4.15: Fluorescent microscope images of streptavidin-AlexaFluor790 conjugates bound to biotinylated conjugated polymer thin films. Examples of region of interests used to measure pixel intensity are highlighted in yellow. Scale bar = 1.5 mm

orescent signal, though it is noted that this method can be affected by researcher bias. Nonetheless, the highest average pixel intensity value is measured for the substrate with 0.5 mM DBCO-PEG2K-Biotin immobilised, rather than 1 mM DBCO-PEG2K-Biotin. This indicates that there is less binding of the streptavidin targets at higher biotin densities. This could be expected, as probe density is an important parameter to optimise for biosensors. At too high probe densities, neighbouring biotin probes will start to interact sterically, causing conformational changes and impeding target binding efficiency[26]. The

relatively low target binding at the highest probe concentration here suggests such steric crowding, decreasing the amount of available binding sites for the streptavidin conjugates to bind with. However, the images demonstrate a degree of non-specific adsorption on the control substrates. This suggests a good surface coverage should be achieved to minimise these non-specific interactions and with providing adequate accessibility of the target. In the BioFET context, the results here highlight the need for careful optimisation of the immobilised probe density, to ensure efficient binding to maximise sensitivity and analytical performance.

Time-of-flight secondary ion mass spectrometry

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) was also used to determine biotin immobilisation on conjugated polymer thin film surfaces. The data presented in this section was collected with assistance from Dr. Sarah Fearn (Department of Materials, Imperial College London). This technique is capable of producing chemical images of patterns of biomolecules with high spatial resolution. However, for larger molecules such as proteins, understanding of the molecular structure and what ion fragments are likely to be generated is essential. PEG, the spacer molecule in the biotin probe conjugate, gives rise to characteristic ion fragments such as CH_3O^+ and $\text{C}_2\text{H}_5\text{O}^+$ [27].

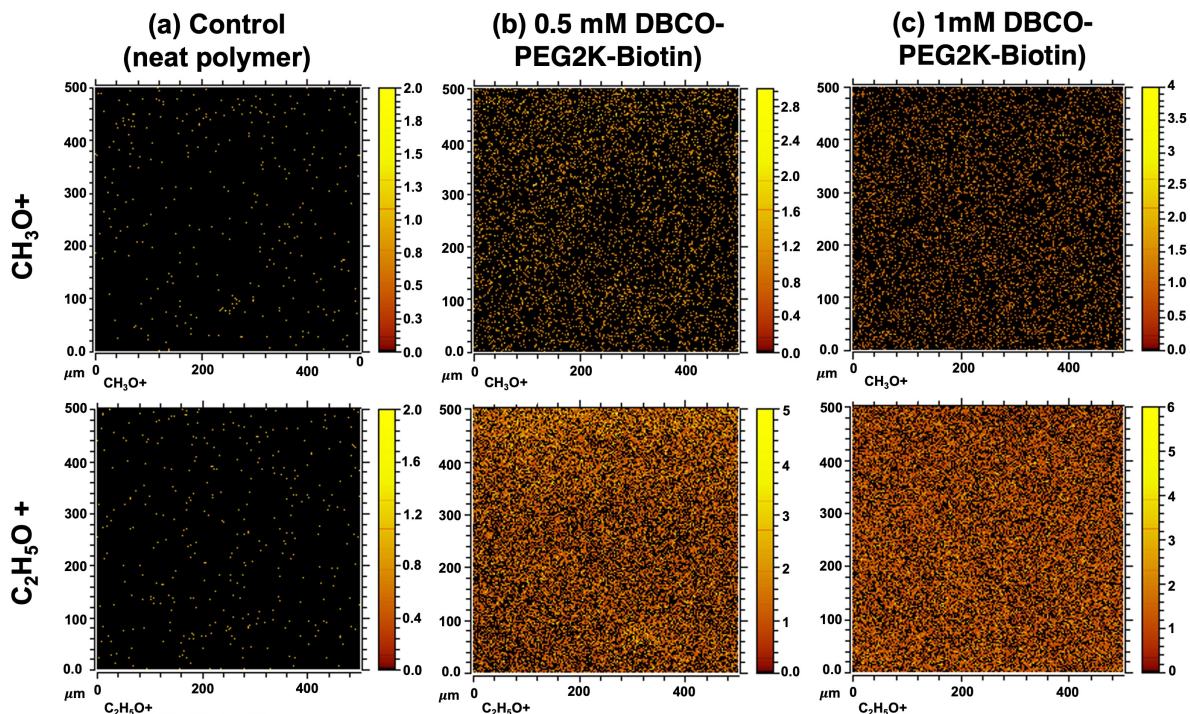


Figure 4.16: TOF-SIMS total positive-ion images of characteristic PEG ion fragments (CH_3O^+ and $\text{C}_2\text{H}_5\text{O}^+$) on IDT-BT- N_3 thin films functionalised with DBCO-PEG2K-Biotin. Colour scale represents the ion fragment signal intensity in arbitrary units. Data collected with assistance from Dr. Sarah Fearn.

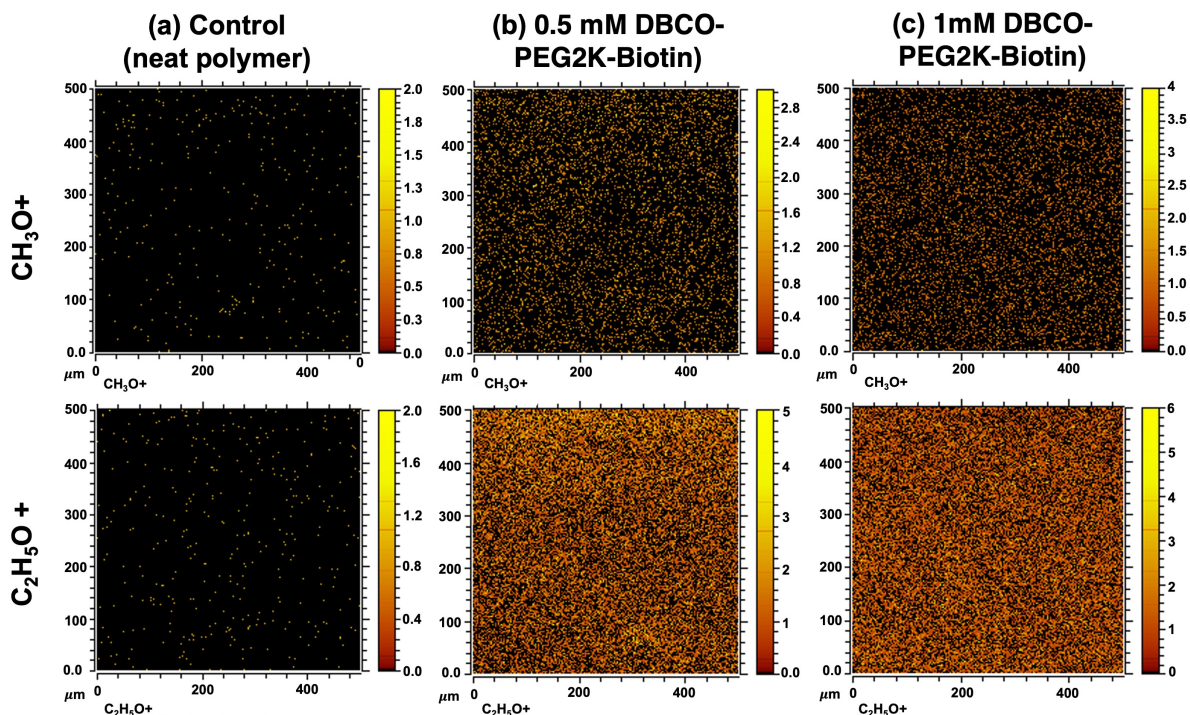


Figure 4.17: TOF-SIMS total positive-ion images of characteristic biotin ion fragments ($C_2H_4N^+$ and $C_3H_8N^+$) on IDT-BT- N_3 thin films functionalised with DBCO-PEG2K-Biotin. Colour scale represents the ion fragment signal intensity in arbitrary units. Data collected with assistance from Dr. Sarah Fearn.

In Figure 4.16, for the biotinylated samples there is a higher spatial intensity of these characteristic ions compared to the neat polymer control. This effect also scales with concentration, with higher signal intensities measured for the samples with higher probe concentrations deposited. This result was supported by the measurements for the characteristic biotin fragments $C_2H_4N^+$ and $C_3H_8N^+$ in Figure 4.17, confirming the success of the surface click reaction with biotin. ToF-SIMS has a few advantages over the commonly-utilised fluorescent imaging, such high signal-to noise-ratio and high sensitivity. However, the complex machinery employed in ToF-SIMS that requires ultra-high vacuum technology, lacks efficiency and practicality.

X-ray photoelectron spectroscopy

Biotin-functionalised conjugated polymer thin films were also analysed using x-ray photoelectron spectroscopy (XPS) to determine the chemical composition of the surface. In this case, a DBCO-PEG4-Biotin conjugate was reacted with IDT-BT- N_3 thin films. The C1s scan is a useful element to scan in this system as there are characteristic groups that are present in the functionalised sample that are not in the neat polymer control. Figure 4.18 shows the emergence of two peaks, a C-O / C-N peak is observed at ~ 286.0 eV and a C=O peak at ~ 288.0 eV which is consistent with molecular structure of the

DBCO-PEG4-Biotin conjugate.

Other elemental species that can be scanned which are present in the biotin probe conjugate are O1s, N1s and S2p however these groups are less indicative of the success of the biotinylation for a few reasons. O1s is unclear because there will always be native oxygen present on samples when working in ambient conditions. N1s and S2p are unclear because these are elements that can also be found in the neat organic semiconductor film. However, Figure 4.19 shows that the relative atomic compositions of the samples are consistent with the expected surface chemistry, particularly with a large increase in the relative oxygen composition of the surface as a result of the PEG and biotin structures.

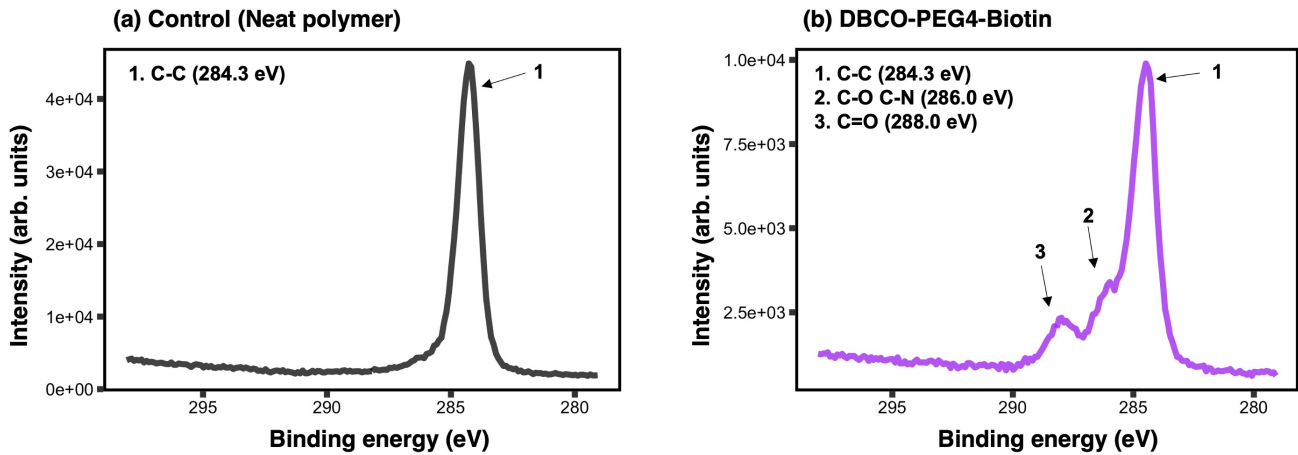


Figure 4.18: XPS spectra of IDT-BT-N₃ thin films (a) before functionalisation and (b) after functionalisation with DBCO-PEG4-Biotin.

Sample	Atomic composition by XPS (%)			
	C	O	N	S
Control	83.95	2.19	11.67	2.19
DBCO-PEG4-Biotin	72.93	11.73	13.75	1.6

Figure 4.19: Relative atomic compositions of IDT-BT-N₃ thin films before and after functionalisation with biotin conjugates.

4.3.4 Single-stranded DNA immobilisation

This section will present results of conjugated polymer IDT-BT-N₃ thin films immobilised with single-stranded DNA (ssDNA) sequences through a surface click reaction. This will form the probe layer in the eventual BioFET device for recognition with its complementary target sequence via hybridisation. DNA probes can be immobilised at one end of their 5' or 3' end chain through modification with a reactive linker.

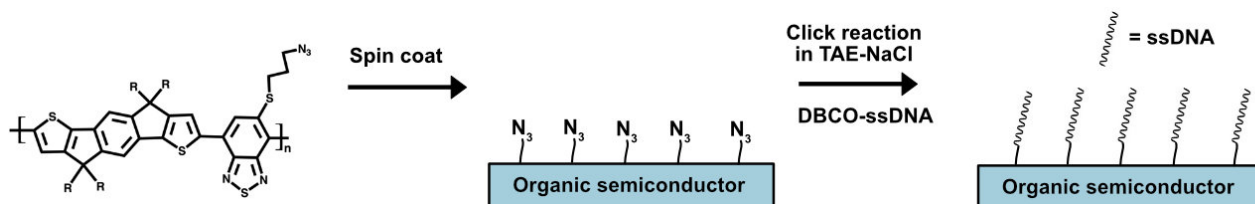


Figure 4.20: Schematic showing biofunctionalisation of conjugated polymer thin films with ssDNA.

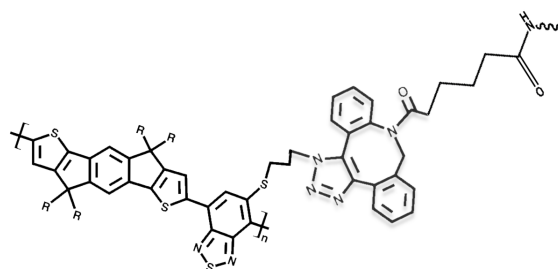


Figure 4.21: Chemical structure of the resulting of conjugated polymer surface after functionalisation with a DBCO-ssDNA conjugate through the strain-promoted azide-alkyne click chemistry reaction.

Contact angle

Contact angle measurements were first used to validate ssDNA immobilisation to the conjugated polymer thin film surface. The measurements in Figure 4.22 demonstrate the hydrophobic unmodified polymer, then upon functionalisation with increasing concentrations of ssDNA solutions, the surface is rendered increasingly hydrophilic. This is likely due to the negatively charged phosphate ions in the DNA backbone which can interact easily with water[28].

Fluorescence microscopy

The immobilisation of ssDNA sequences on dispensed conjugated polymer thin films was also validated using fluorescent microscopy. A solution of DBCO-ssDNA-AlexaFluor790 (IDT) was reacted with IDT-BT-N₃ thin films and then imaged with the Licor Imaging System. All slides were imaged under constant illumination conditions at the same time.

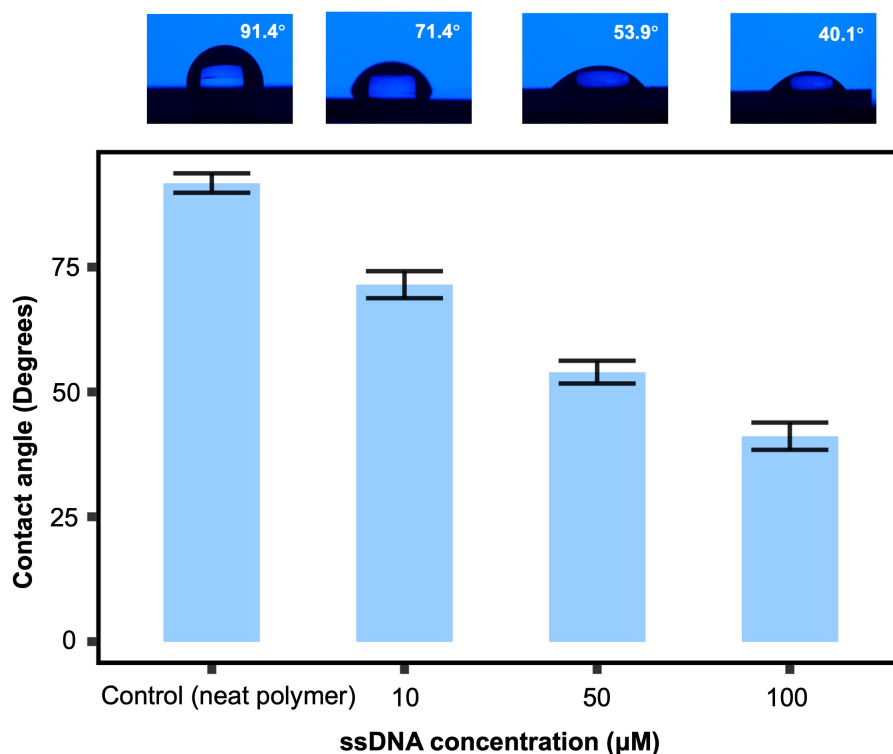


Figure 4.22: Contact angle measurements of DBCO-ssDNA concentrations immobilised on the IDT-BT-N₃ thin film surface. N = 3. Error bars represent the standard deviations.

As a further negative control, a solution that had been pre-reacted with azidoacetic acid (Sigma Aldrich) overnight to bond with all available DBCO groups was also measured.

Three regions of interest were chosen and analysed with ImageJ to obtain average pixel intensity values of 0.03 ± 0.01 , 2.8 ± 1.1 and 33.1 ± 2.3 for the neat polymer control, the pre-reacted ssDNA-Alexa750 control and the ssDNA-Alexa750 substrate respectively as shown in Figure 4.23. The highest pixel intensity is obtained for the sample immobilised with DBCO-ssDNA-Alexa790 indicating the success of binding to the surface. There is evidence of non-specific binding for the pre-reacted substrate, but at a much lower intensity, indicating that the immobilisation of DNA on the surface is primarily through the DBCO-N₃ click handles rather than merely adsorption to the surface indicating a controlled immobilisation process.

X-ray photoelectron spectroscopy

To analyse the chemical composition of ssDNA-functionalised surfaces, XPS was used. Usefully, DNA has distinct functional groups which can be probed with this method. The C1s spectra of a ssDNA-functionalised conjugated film is shown in Figure 4.24(a) (purple spectrum). Peak 1 at ~ 284.3 eV can be assigned to the C-C and C-H bonds, which are also present in the unfunctionalised control sample. However, the emergence of peak 2 at ~ 286.0 eV can be attributed to the C-N and C-O single bonds found in the DNA bases

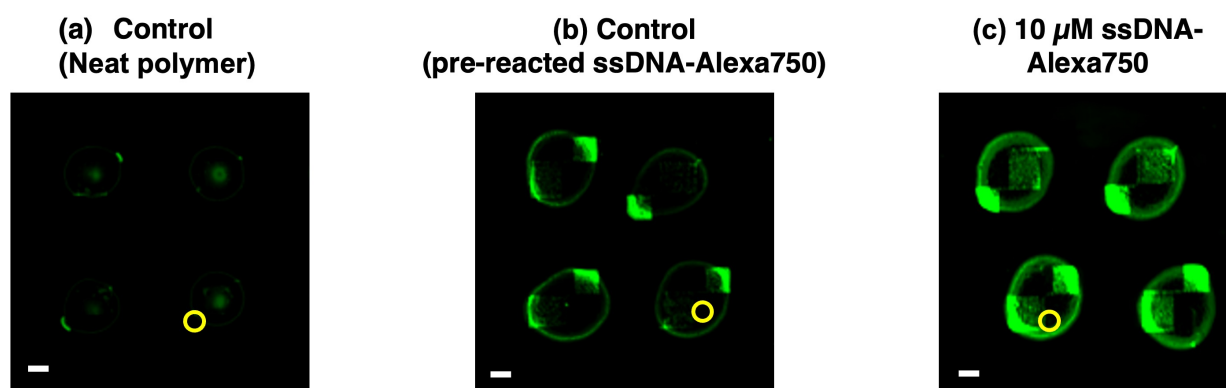


Figure 4.23: Fluorescent microscope images of IDT-BT-N₃ thin films reacted with DBCO-ssDNA-Alexa790 solutions through click chemistry. Examples of region of interests used to measure pixel intensity are highlighted in yellow. Scale bar = 1.5 μ m

thymine and cytosine and peak 3 at ~ 288.0 eV belongs to the C=O groups in guanine, groups that are not present in the control sample. The accuracy of this peak fitting can be evaluated by analysis of the fits against the raw data and the resulting envelope. Figure 4.24(b) it shows that the fitting has good agreement to the raw data with small residuals.

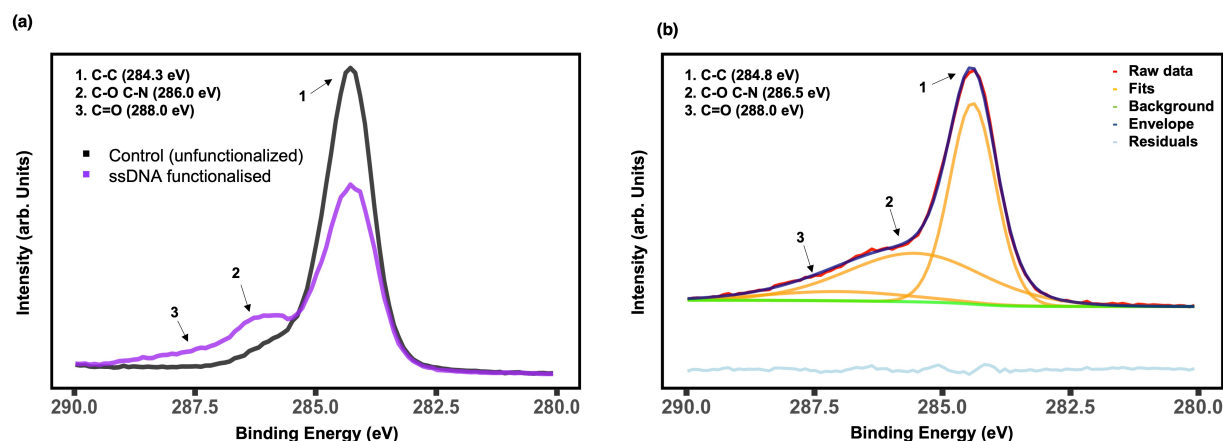


Figure 4.24: XPS C1s analysis (a) Comparison between control IDT-BT-N₃ and ssDNA functionalised spectra, (b) Peak fits of the ssDNA functionalised sample to the raw data with background and residuals plotted.

The N1s scan can also provide useful information on the surface chemistry. In Figure 4.25(a) peak 1 is observed at ~ 399.0 eV for both samples, which can be assigned to the C=N bond. However, for the ssDNA functionalised sample there is the emergence of Peak 2 at ~ 400.5 eV which represents C-NH₂ in agreement with literature represents the amine groups can be found in the adenine, guanine and cytosine bases of DNA[29]. There is also good agreement for these peak fits to the raw data in Figure 4.25(b).

The O1s spectra can be difficult to analyse as many compounds fall within a narrow range with overlapping peaks, however in combination with the other more indicative elements it can provide further validation. In Figure 4.26(a) there are the emergence of

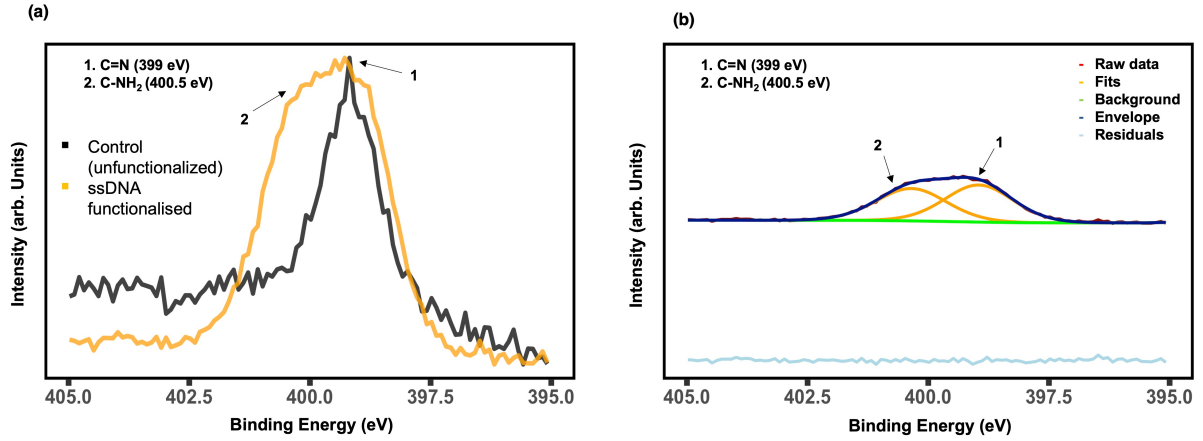
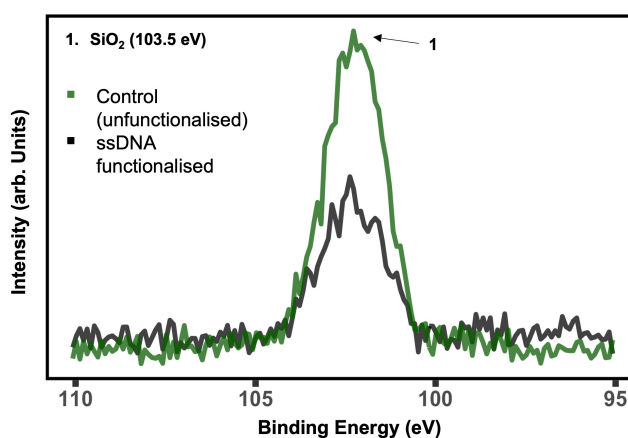
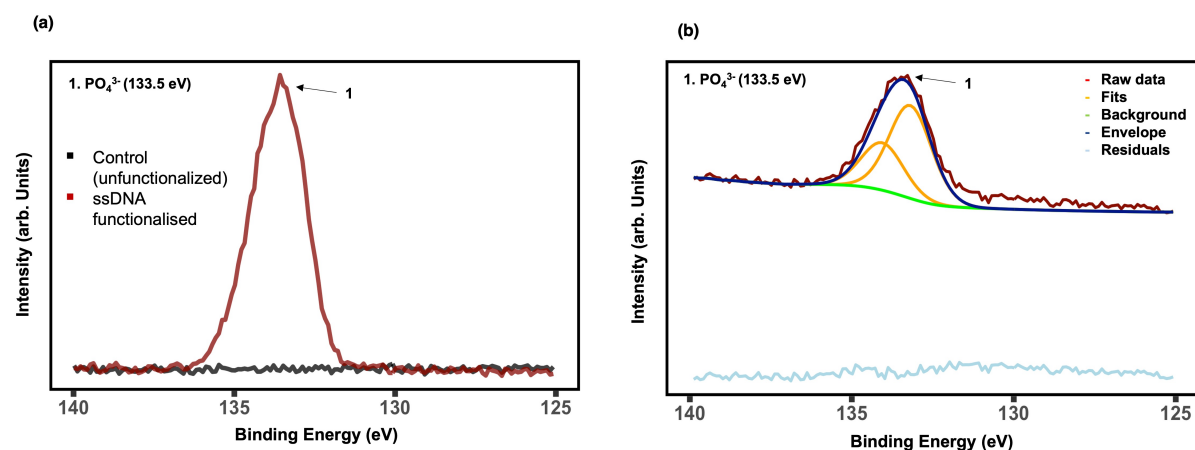
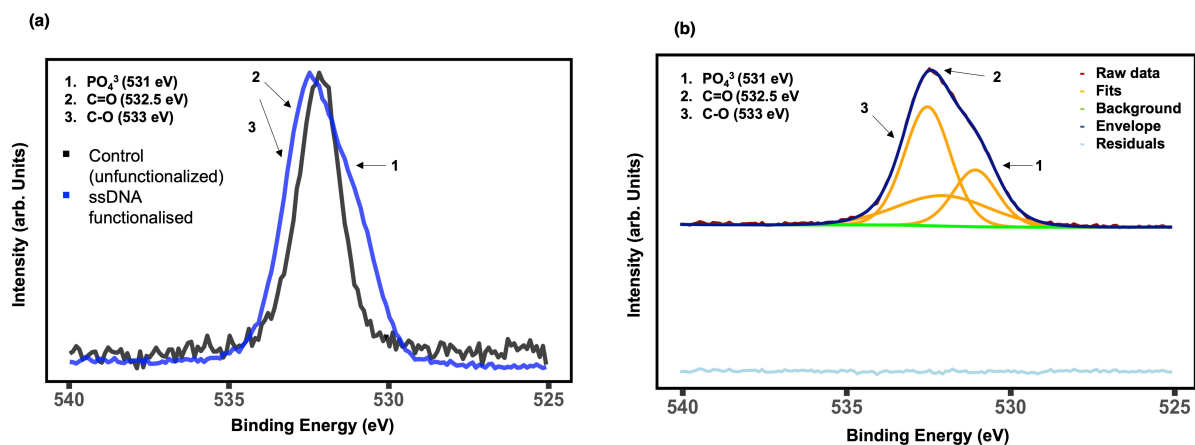


Figure 4.25: XPS N1s analysis (a) Comparison between control IDT-BT-N₃ and ssDNA functionalised spectra, (b) Peak fits of the ssDNA functionalised sample to the raw data with background and residuals plotted.

two peaks for the ssDNA functionalised sample, peak 1 at ~ 531 eV which results from the oxygen bound in the phosphate ion groups and peak 2 at ~ 532.5 eV which is attributed to the C=O bond in the guanine, thymine and cytosine bases. Peak 2 represents the C-O bond, in the control sample this is found at ~ 532 eV however it is slightly shifted to a higher binding energy in the functionalised sample to ~ 533 eV. There will always be some native oxygen on the surface samples that are handled in ambient conditions, which is where this group arises in the control sample. The shift to higher binding energy for the DNA sample is likely due to charging effects of the surface, a common artefact of XPS measurements on non-metal samples, often in nonhomogeneous systems[30]. There is also agreement of these fits to the raw data in 4.26(b).

Perhaps the most indicative element of DNA immobilisation is the P2p scan. No phosphorus groups are present in the unfunctionalised polymer control, however after immobilisation we observe in 4.27(a) clear peak at ~ 133.5 eV in agreement with literature[29][31]. In the DNA structure, a phosphate ion backbone holds the nucleotide bases in place which is the origin of the phosphorus signal here.

Another element that could be scanned for confirmation that is present in the system, is silicon. The underlying substrate is silicon dioxide thus silicon signal should be expected. As XPS is a highly surface sensitive technique (10 nm depth), stronger Si2p signals could perhaps be expected from the control samples than from the ssDNA functionalised samples, for which the silicon dioxide would be further from the surface. This is confirmed by Figure 4.28 where there is a reduction in the SiO₂ peak height at ~ 103.5 eV from 699 before immobilisation to 462 after. XPS is another technique that demands complex machinery and high vacuum operation for analysis, however high-surface sensitivity and lateral resolution can be achieved rendering this technique advantageous.


 Figure 4.28: XPS Si2p analysis of ssDNA functionalised IDT-BT-N₃ thin films.

4.4 Conclusions

This chapter demonstrates a new method of functionalising conjugated polymer thin film surfaces towards biosensor development. It was shown that reactive functional groups can be introduced to thin film surfaces without the need for high vacuum deposition processes or lengthy synthetic protocols, through a nucleophilic aromatic substitution reaction with an azide conjugate. The success of this reaction was verified with UV-Vis spectroscopy and NMR measurements. The anchoring of biomolecules to the surface of conjugated polymer thin films whilst leaving the bulk polymer unmodified was demonstrated by taking advantage of click chemistry.

The versatility of this system to attach different groups to the surface is demonstrated through the immobilisation of PEG, biotin and ssDNA and characteristic through a variety of methods. Contact angle measurements demonstrated that the neat hydrophobic polymer is rendered increasingly hydrophilic in the presence of PEG2K, PEG2K-Biotin conjugates and ssDNA. ToF-SIMS was employed to analyse the spatial mapping of characteristic PEG and biotin ion fragments, which confirmed higher intensities and denser signals for the functionalised samples than the controls. However XPS was favoured as a surface analysis technique due to more access to the equipment and a simpler operation compared to XPS.

QCM-D was employed to detect the immobilisation of PEG5K conjugated on conjugated polymer thin films, however experimental complications such as film swelling and dissolution made this characterisation method inconvenient. Fluorescence microscopy measurements indicated the potential of PEG as an anti-fouling coating to hinder non-specific adsorption, however further investigation is required to optimise important factors such as the probe density and chain length.

The importance of the probe density, in a sensing context, was also demonstrated through fluorescence microscopy. Higher biotin probe densities were shown to bind to less fluorophore-tagged streptavidin than lower probe densities suggesting steric crowding effects reducing the binding efficiency.

The immobilisation of ssDNA through the azide and DBCO bond rather than physical adsorption was confirmed through pre-reacting a fluorophore-tagged DBCO-ssDNA solution with acetic acid and measuring the fluorescence intensity which was significantly less than the non-prereacted solution. XPS was used to confirm biotin immobilisation and ssDNA immobilisation with distinct functional groups appearing in the DNA samples that are not present in the control samples. Considering all characterisation methods discussed in this chapter, the biofunctionalisation method presented appears valid, with potential in biosensor fabrication to provide receptor elements with a simple and highly modular immobilisation method.

References

- [1] S. Inal, J. Rivnay, A.-O. Suiiu, G. G. Malliaras, I. McCulloch, *Accounts of Chemical Research* **2018**, *51*, Publisher: American Chemical Society, 1368–1376.
- [2] J. Mei, Z. Bao, *Chemistry of Materials* **2014**, *26*, Publisher: American Chemical Society, 604–615.
- [3] L. Feng, C. Zhu, H. Yuan, L. Liu, F. Lv, S. Wang, *Chemical Society Reviews* **2013**, *42*, Publisher: Royal Society of Chemistry, 6620–6633.
- [4] Y. Braeken, S. Cheruku, A. Ethirajan, W. Maes, *Materials* **2017**, *10*, Number: 12 Publisher: Multidisciplinary Digital Publishing Institute, 1420.
- [5] Z.-S. Kim, S. C. Lim, S. H. Kim, Y. S. Yang, D.-H. Hwang, *Sensors* **2012**, *12*, 11238–11248.
- [6] E. Macchia, A. Tiwari, K. Manoli, B. Holzer, N. Ditaranto, R. A. Picca, N. Cioffi, C. Di Franco, G. Scamarcio, G. Palazzo, L. Torsi, *Chemistry of Materials* **2019**, *31*, 6476–6483.
- [7] E. Macchia, K. Manoli, B. Holzer, C. Di Franco, R. A. Picca, N. Cioffi, G. Scamarcio, G. Palazzo, L. Torsi, *Analytical and Bioanalytical Chemistry* **2019**, *411*, 4899–4908.
- [8] P. Seshadri, K. Manoli, N. Schneiderhan-Marra, U. Anthes, P. Wierzbowski, K. Bonrad, C. Di Franco, L. Torsi, *Biosensors and Bioelectronics* **2018**, *104*, 113–119.
- [9] M. Magliulo, D. De Tullio, I. Vikholm-Lundin, W. M. Albers, T. Munter, K. Manoli, G. Palazzo, L. Torsi, *Analytical and Bioanalytical Chemistry* **2016**, *408*, 3943–3952.
- [10] T. Minami, Y. Sasaki, T. Minamiki, S.-i. Wakida, R. Kurita, O. Niwa, S. Tokito, *Biosensors and Bioelectronics* **2016**, *81*, 87–91.
- [11] T. Minamiki, T. Minami, R. Kurita, O. Niwa, S.-i. Wakida, K. Fukuda, D. Kumaki, S. Tokito, *Applied Physics Letters* **2014**, *104*, 243703.
- [12] P. Didier, N. Lobato-Dauzier, N. Clément, A. J. Genot, Y. Sasaki, É. Leclerc, T. Minamiki, Y. Sakai, T. Fujii, T. Minami, *ChemElectroChem* **2020**, *7*, Publisher: John Wiley & Sons, Ltd, 1332–1336.

- [13] A. Creamer, C. S. Wood, P. D. Howes, A. Casey, S. Cong, A. V. Marsh, R. Godin, J. Panidi, T. D. Anthopoulos, C. H. Burgess, T. Wu, Z. Fei, I. Hamilton, M. A. McLachlan, M. M. Stevens, M. Heeney, *Nature Communications* **2018**, *9*, Number: 1 Publisher: Nature Publishing Group, 3237.
- [14] Y. Chen, Y. Xianyu, J. Wu, B. Yin, X. Jiang, *Theranostics* **2016**, *6*, 969–985.
- [15] F. Babudri, G. M. Farinola, F. Naso, R. Ragni, *Chemical Communications* **2007**, *10*, Publisher: Royal Society of Chemistry, 1003–1022.
- [16] F. Parenti, F. Tassinari, E. Libertini, M. Lanzi, A. Mucci, *ACS Omega* **2017**, *2*, Publisher: American Chemical Society, 5775–5784.
- [17] S. Lowe, N. M. O’Brien-Simpson, L. A. Connal, *Polymer Chemistry* **2014**, *6*, 198–212.
- [18] J. Song, J. Dailey, H. Li, H.-J. Jang, P. Zhang, J. T.-H. Wang, A. D. Everett, H. E. Katz, *Advanced Functional Materials* **2017**, *27*, 1606506.
- [19] A. D. Easley, T. Ma, C. I. Eneh, J. Yun, R. M. Thakur, J. L. Lutkenhaus, *Journal of Polymer Science* **2022**, *60*, 1090–1107.
- [20] P.-H. Lin, B.-R. Li, *Analyst* **2020**, *145*, Publisher: Royal Society of Chemistry, 1110–1120.
- [21] Q. Yu, Y. Zhang, H. Wang, J. Brash, H. Chen, *Acta Biomaterialia* **2011**, *7*, 1550–1557.
- [22] X. Chen, A. Noy, *APL Materials* **2021**, *9*, Publisher: American Institute of Physics, 020701.
- [23] W. R. Gombotz, W. Guanghai, T. A. Horbett, A. S. Hoffman, *Journal of Biomedical Materials Research* **1991**, *25*, 1547–1562.
- [24] S. R. Benhabbour, H. Sheardown, A. Adronov, *Macromolecules* **2008**, *41*, Publisher: American Chemical Society, 4817–4823.
- [25] B. M. Lowe, K. Sun, I. Zeimpekis, C.-K. Skylaris, N. G. Green, *The Analyst* **2017**, *142*, 4173–4200.
- [26] L. M. Bonanno, L. A. DeLouise, *Langmuir* **2007**, *23*, Publisher: American Chemical Society, 5817–5823.
- [27] P. Kingshott, J. Wei, D. Bagge-Ravn, N. Gadegaard, L. Gram, *Langmuir* **2003**, *19*, Publisher: American Chemical Society, 6912–6921.
- [28] T. Böcking, K. A. Kilian, K. Gaus, J. J. Gooding, *Langmuir* **2006**, *22*, Publisher: American Chemical Society, 3494–3496.
- [29] S. Ptasinska, A. Stypczynska, T. Nixon, N. Mason, D. Klyachko, L. Sanche, *The Journal of chemical physics* **2008**, *129*, 065102.

REFERENCES

- [30] D. R. Baer, K. Artyushkova, H. Cohen, C. D. Easton, M. Engelhard, T. R. Gengenbach, G. Greczynski, P. Mack, D. J. Morgan, A. Roberts, *Journal of Vacuum Science & Technology A* **2020**, *38*, 031204.
- [31] S. Kumaravel, P. Thiruvengadam, K. Karthick, S. S. Sankar, S. Kundu, *ACS Sustainable Chemistry & Engineering* **2019**, *7*, Publisher: American Chemical Society, 18463–18475.

Chapter 5

Streptavidin-targeting BioFET

This chapter reports on the development and characterisation of a streptavidin-targeting BioFET. Here, the design of the device is illustrated and the experimental methods for fabrication and characterisation are briefly explained, as this was covered in more detail in Chapter 3 and Chapter 4. The chapter concludes with a discussion of BioFET response analysis methods and the scope for further study is considered.

5.1 Motivation

As the method of BioFET fabrication employed in this work is novel, much validation is required and the streptavidin-biotin interaction was chosen for an initial proof-of-concept system. Being one of the strongest naturally occurring non-covalent binding events with an affinity $K_A \approx 10^{15} \text{ M}^{-1}$, it is one of the most popular biosensor validation systems[1]. There are a few reported examples of streptavidin detection with BioFETs. Devices featuring semiconducting layers with a biotinylated backbone often involve lengthy multi-step synthetic protocols which negatively affects the end yield[1]. Additionally, the incorporation of large protein groups to the backbone in the bulk of the semiconducting polymer can cause steric twisting, disrupting the planarity of the backbone. This tends to hinder interchain packing, reducing the mobility and results in OFET devices with poor electrical performance[2].

The aim here was to demonstrate that with this simple biofunctionalisation method, biotin probes can be attached to the surface of the semiconducting polymer, leaving the bulk unmodified and thus retaining the charge transport properties. This biotinylated semiconducting polymer surface can then be employed in BioFET platforms to target streptavidin molecules in solution and the electrical modulation can be observed. The hypothesis is that this direct connection of binding event to the active layer where the charge transport occurs, could provide a increased sensitivity compared to other architectures where the binding event is spatially separated from the active layer[3][4].

5.2 Materials and Methods

The bottom-gate bottom-contact Fraunhofer substrates were prepared as per section 4.2.2 then the biotin functionalisation was conducted as per section 5.2.2 to fabricate the BioFET. Serial dilutions of streptavidin were prepared in phosphate buffered saline (PBS) and dispensed onto the BioFET surface, covering all devices. The devices were left for 10 mins for the target binding to take place, rinsed several times with DI water, then dried with N_2 before electrical testing.

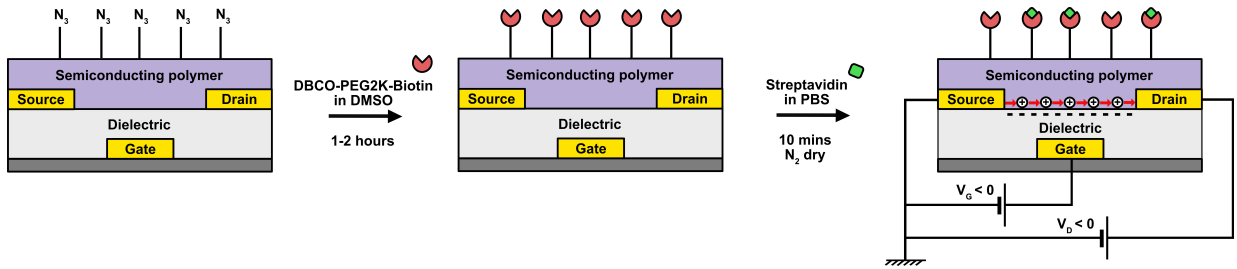


Figure 5.1: Schematic representation of the fabrication of a streptavidin-targeting BioFET and the resulting assay operation.

For the BioFET assays, a pure PBS sample without any target was used for the reference measurements. The obtained currents I_D for the target are normalised to the current obtained for the reference sample I_0 . These currents are extracted from the data at a constant gate voltage V_G used in the following equation for the normalised response R :

$$R = \frac{(I_D - I_0)}{I_0} \quad (5.1)$$

The calibration curve is constructed by plotting the response R versus the analyte concentrations.

5.3 Results and Discussion

5.3.1 Effect of biotin probe density on the sensor response

The density of capturing probes on a biosensor surface is a key parameter to optimise for sensing performance. Figure 5.2(a) shows the transfer characteristics of a sensor functionalised with 1 mM of biotin probes and exposed to a serial dilution of streptavidin in PBS. For this concentration of probes, no discernible trend in the transfer curves were observed. A very minimal but overall reduction in the normalised current response was observed with increasing in concentration, as shown in Figure 5.2(b), which suggests no detection and likely simply noise or degradation from the assay protocol.

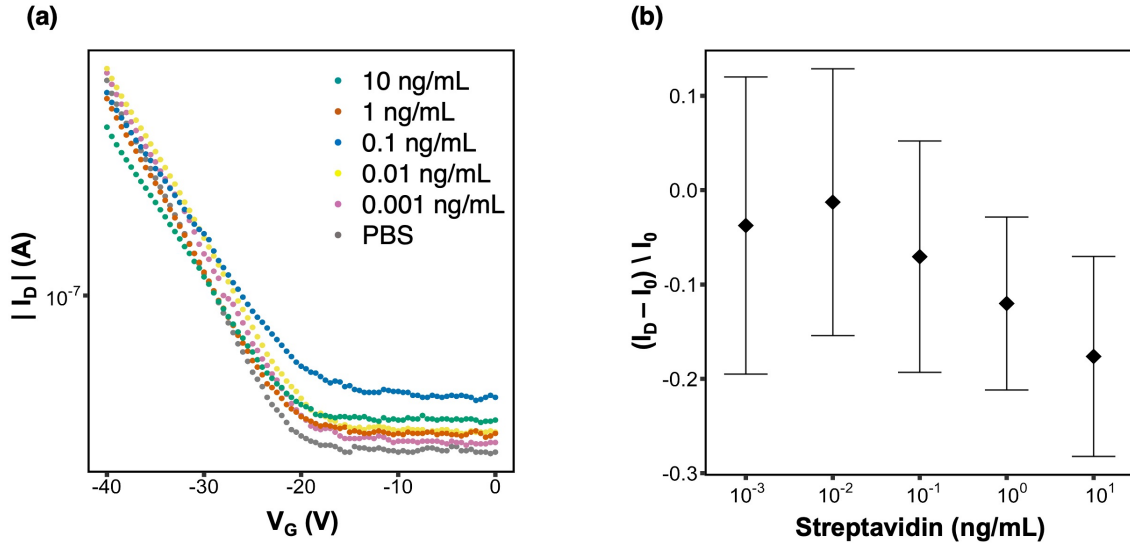


Figure 5.2: (a) Transfer characteristics (back sweep) of a single streptavidin BioFET device functionalised with 1 mM biotin in the saturated regime $V_D = -40$ V with IDT-BT- N_3 as the semiconducting polymer. Each data point I_D is the mean value of $N = 5$ current-voltage sweep replicates, (b) Normalised response of the streptavidin BioFET to increasing streptavidin concentrations. I_D and I_0 are taken at -35 V for each concentration. $N = 30$ for 6 devices of different dimensions. Error bars represent the standard deviations.

In contrast, Figure 5.3(a) shows the transfer curves of a sensor functionalised with 0.5 mM of biotin capture probes. Here, a clear systematic shift to more positive voltages of the transfer curves is observed and a dose-dependent increase in the response was measured as shown in Figure 5.3(b). The sensing mechanism of this device could be described by an extra-gating effect produced by the bound streptavidin target. With an acidic isoelectric point (pI) ≈ 5 , streptavidin is negatively charged at the pH of the PBS measurement buffer. In an OFET system with a gate potential applied to a dielectric, this would result in increased positive charge accumulation in the p-type semiconductor film, and a resulting increase in the measured drain current I_D . The biosensor could be approximated as a dual-gated OFET system, in which negative top-gate biases cause threshold voltage shifts to more positive values as a result of an increased hole density throughout the film causing the filling of traps at lower bottom gate voltages[5][6][7].

In Figure 5.3(b), there also appears to be an eventual saturation at the 1 ng mL^{-1} dose. This indicates a gradual accumulation of charge on the sensor surface directly connected with the complex formation, then a saturation in the signal when the available binding sites become increasingly occupied. The observed variation in the response could be attributed to increasing noise in the measurements as the assay progressed. Each chip being subjected to rinsing and drying prior to testing could introduce defects which affect the electrical properties. Additionally, here the mean response was taken on 6 devices of different dimensions, and the response of devices of different sizes could be expected to give rise to responses of varying magnitude.

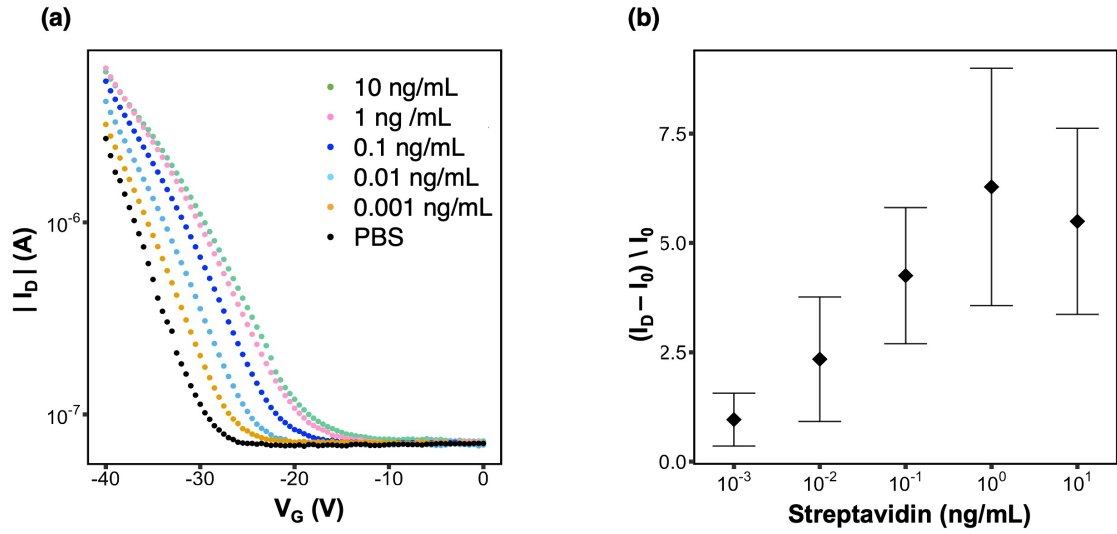


Figure 5.3: (a) Transfer characteristics (back sweep) of a single streptavidin BioFET device functionalised with 0.5 mM biotin in the saturation regime $V_D = -40$ V with IDT-BT- N_3 as the semiconducting polymer. Each data point I_D is the mean value of $N = 5$ current-voltage sweep replicates. (b) Normalised response of the streptavidin BioFET to increasing streptavidin concentrations. I_D and I_0 are taken at -35 V for each concentration. $N = 25$ for 5 devices. Error bars represent the standard deviations.

However, the dose-dependent trend is clear and this response can be attributed to a more optimal biotin probe density at the sensor surface leading to reduced steric crowding. This is a consequence that has been reported with biotin-streptavidin systems on a solid surfaces, which is a reason why long linker molecules are often found in the probe conjugates to extend the distance between the immobilised probe and the sensor surface[8]. This effect has also been characterised in detail by Pérez-Luna *et al.* with biotin-terminated SAMs on gold substrates, where it was observed that too high concentrations of biotin can sterically hinder the binding of streptavidin[9]. Jung *et al.* also investigated this in further detail and found that surface-bound biotin coverage in the range 10-40% led to the most the streptavidin binding. Over 40% biotin coverage led to a reduction in streptavidin binding, which was attributed again to steric crowding resulting in poor orientation of receptors thus inhibiting streptavidin binding[10].

The biosensing results here appear to support the fluorescence microscopy data presented in Section 4.3.3 Figure 4.12 where higher biotin concentrations led to a lower fluorescence intensity suggesting reduced binding of streptavidin-Alexa790 conjugates compared to lower concentrations, indicating steric crowding inhibit efficient binding. This highlights the need for careful optimisation of the probe densities, to ensure enough receptors to provide a stable signal response but not too many to induce steric crowding and reduce the binding efficiency. Reported streptavidin BioFETs in the field adopt various architectures and operating mechanisms though to-date detection limits in the low pM range have been stated[11][12]. A common estimate of the LOD evaluates this value as

the lowest target concentration that can provide a detectable sensor response observed visually based on the calibration curve[4]. Here, this is for the 0.001 ng mL^{-1} concentration of the 60 kDa streptavidin molecule, which results to an estimated LOD in the low fM range.

5.3.2 Evaluating the selectivity of the sensor in response to a non-specific protein

It is paramount in biosensor development to confirm that the response is due to solely target binding rather than other interfering proteins such as those present in natural matrix components. This section aims to evaluate the selectivity of the sensor in response to a non-specific protein, bovine serum albumin (BSA) with a tendency to non-specifically adsorb to surfaces. Increasing concentrations of BSA solutions were tested on an identical biotin-functionalised device as was presented in Figure 5.3 and compared with the streptavidin response. In Figure 5.4, from the 0.01 ng mL^{-1} dose, the streptavidin response clearly exhibits a higher than that produced by non-specific BSA, confirming the selectivity of the response. The lowest concentration, however, is more difficult to distinguish from the BSA response. In a true plasma sample with interfering proteins in a complex matrix, this indicates that further intervention is likely required to distinguish lower concentrations of target. A substrate passivisation step could perhaps be introduced to actively prevent the interfering proteins from adsorption to the surface. Physical passivisation methods with materials such as BSA other milk proteins are often used as blocking agents for ELISA and similar assays, however it can be difficult to achieve uniform layers thus suffers from low reproducibility[13].

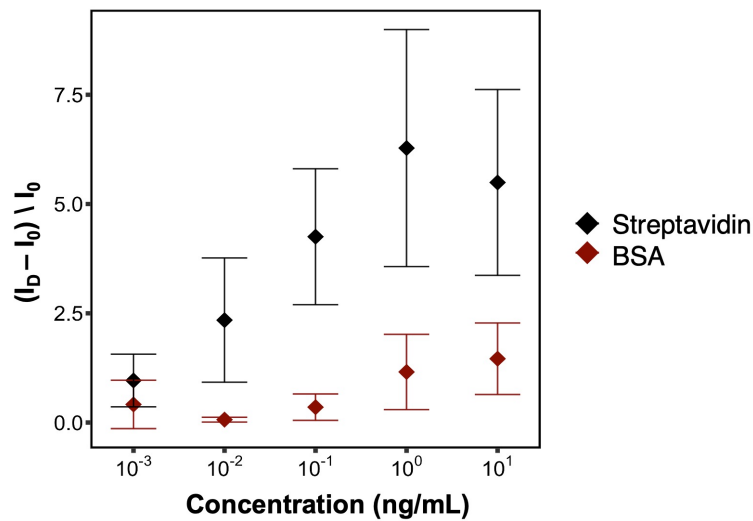


Figure 5.4: Response comparison of a biotin-functionalised device exposed to increasing streptavidin (black) and bovine albumin serum concentrations (red). $N = 20$ for 4 devices. Error bars represent the standard deviations.

5.3.3 Effect of the gate voltage extraction value and device dimensions on the sensor response

As stated, the method of constructing concentration-response curves from the raw data was done using Equation 5.1 at a constant gate voltage V_G as depicted in Figure 5.5. This is a typical method reported in BioFET literature[14][12] and the gate voltage value chosen likely falls in the saturation regime of OFET operation.

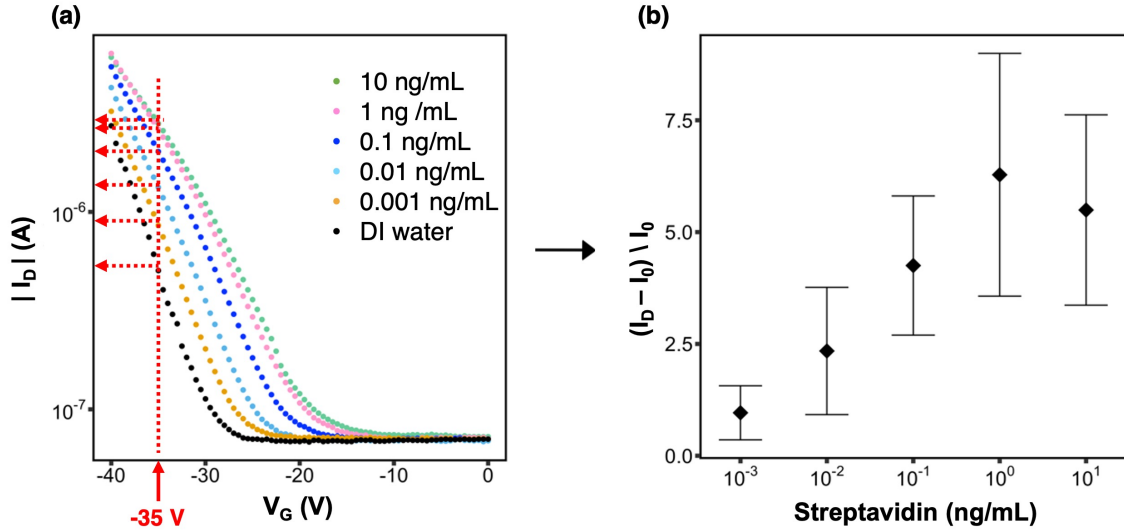


Figure 5.5: Depiction of the response extraction method used so far in this results chapter. (a) The I_D and I_0 values are extracted from the raw data at $V_G = -35$ V. (b) The extracted values are inputted into the response equation and to result in normalised response values that can be plotted against the dose.

With custom R scripts developed by Dr. Stuart G. Higgins in the group of Prof. Molly Stevens, the data was analysed to investigate the effect of the gate voltage extraction values on the sensor response. At present, there are likely variations in response extraction protocols, due to the wide variation in systems, however at times details about what gate voltage value is chosen and why are absent. Figure 5.6 shows the effect of extracting I_D and I_0 for Equation 5.1 at various gate voltages for the streptavidin samples. A dependence of the magnitude and the variation of the sensor response on the V_G extraction is observed. The results suggest that the chosen extraction V_G of -35 V in Figure 5.6 leads to a dose response curve balanced between signal and noise. The curves perhaps suggest that an optimal value exists in the range -30 V and -35 V to achieve a minimum signal-to-noise.

The effect of device dimensions on the sensor response was also investigated. Responses of devices with different dimensions perhaps could be expected to vary. Expressively, the transistor drain current I_D in Equation 2.6 scales with W/L , however exactly how the sensor response scales with device dimensions is less clear. There have been reports of inorganic silicon nanowire BioFETs exhibiting a greater current response to streptavidin with increased device surface areas, likely due to a larger number of bound analytes im-

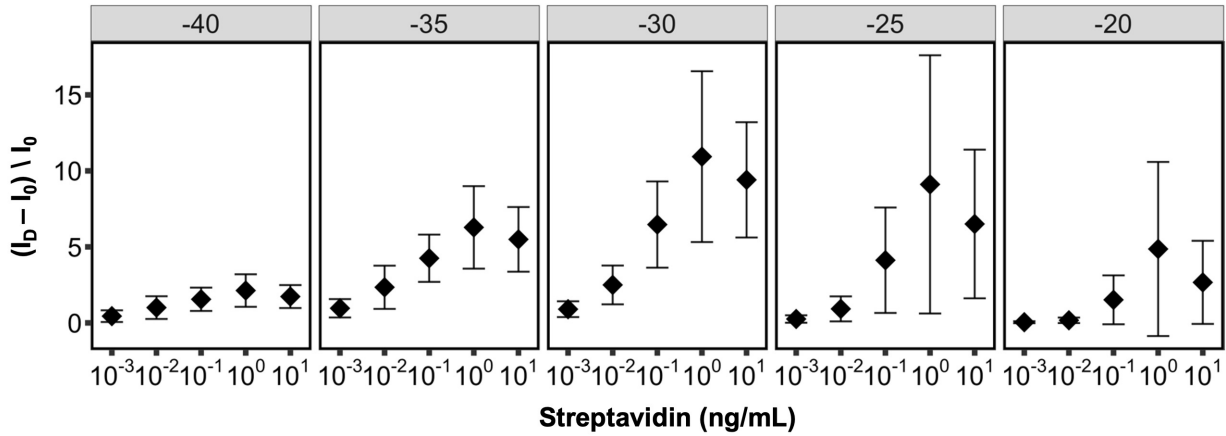


Figure 5.6: Effect of the gate voltage extraction value V_G on the normalised current response. The optimal value for the calibration curve was chosen at $V_G = -35$ V for a balance between signal and noise.

proving the measurement signal and stability[15] however an analysis of this effect in organic BioFETs is yet to be seen. The response curves presented in 5.7(a) was calculated by averaging over devices of two different dimensions, $L = 20\mu m$ (purple points) and $L = 10\mu m$ (green points). It appears that gathering the responses of devices of different dimensions is contributing to variation in the measured responses, as seen by the emergence of populations, with the longer channel devices $L = 20\mu m$ resulting in a larger overall response. This could perhaps be due to larger device dimensions providing larger effective area for target binding to occur upon, however the exact correlation is likely complex.

Normalising the data by the device dimensions by incorporating an additional scaling factor L/W , Figure 5.7(b) shows the data points begin to converge. This indicates the necessity to additionally normalise for the device geometry if combining devices of different sizes for the response analysis, or rather, to simply measure devices of identical geometries to minimise the variation in the response. Such analysis can help to guide future device designs with the aim of including device geometries which exhibit the which maximum response.

5.3.4 Expanding the streptavidin BioFET dynamic range and the effect of PEGylation on non-specific interactions

The dynamic range is an important consideration in biosensor development. A larger dynamic range means the biosensor is able to detect a wider range of analyte concentrations, however, an ideal biosensor would have a clinically relevant dynamic range that includes the target range found in the body. Here, the dynamic range was expanded to increase the accuracy of the calibration curve for analysis. Figure 5.8 shows a streptavidin BioFET operational for concentrations spanning five orders of concentration magnitude

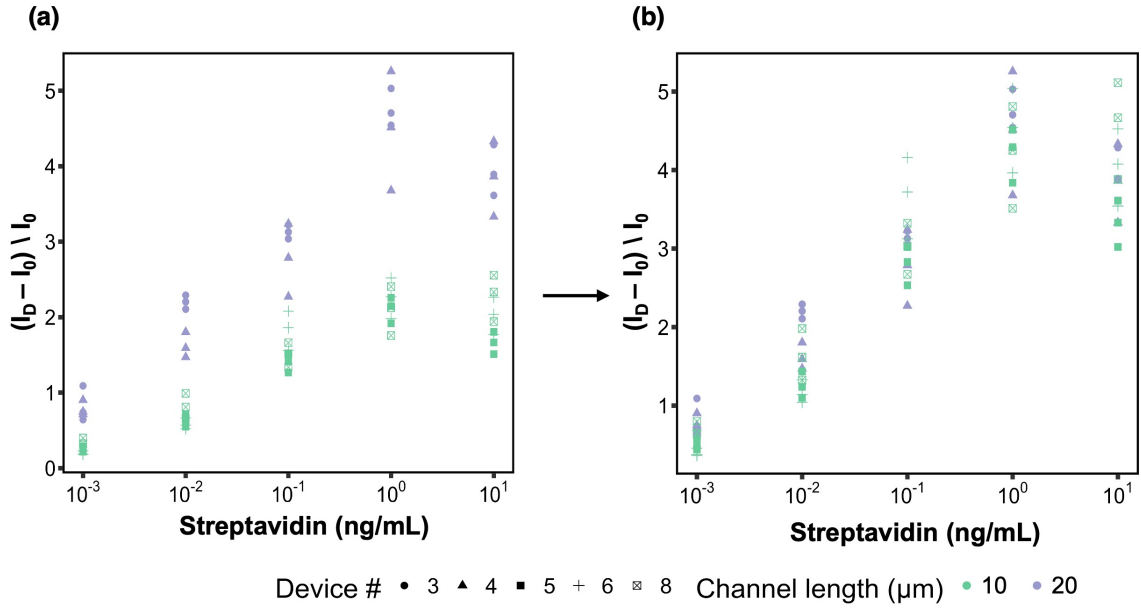


Figure 5.7: (a) The response plotted per device showing populations of device dimensions $L = 20\mu\text{m}$ (purple points) and $L = 10\mu\text{m}$ (green points) and (b) the response normalised for the device dimensions with a scaling factor L/W showing a reduction in the variation.

in a PBS buffer solution. From at the 0.01 ng mL^{-1} concentration (purple points), a concentration-dependent shift of the transfer curves is to higher drain current I_D values observed is before an eventual saturation as the biotin receptors become increasing unavailable. The similar behaviour to the results presented in Figure 5.3, suggesting the reproducibility of this BioFET fabrication method.

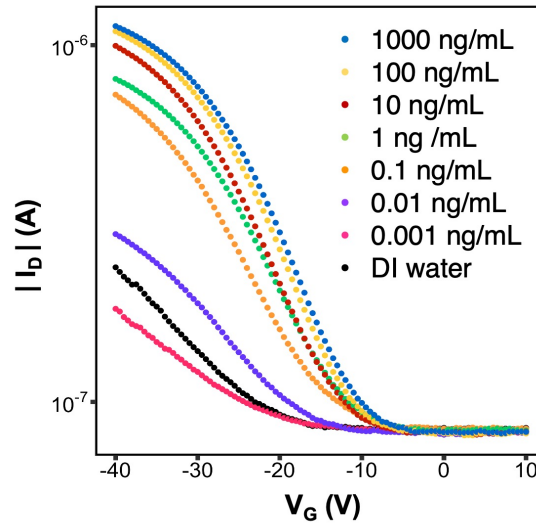


Figure 5.8: Transfer characteristics (back sweep) of a streptavidin Bio-FET device ($W/L = 1000/5$) functionalised with 0.5 mM biotin in the saturation regime $V_D = -40 \text{ V}$, with a 1:1 IDT-BT- N_3 as the semiconducting polymer. Mean I_D is plotted. $N = 5$.

Another control experiment was performed to confirm that the measured BioFET response is due to complex formation of target streptavidin with surface-bound biotin probes, rather than the non-specific adsorption of streptavidin molecules on the surface. Here, the organic semiconductor surface was functionalised with PEG molecules and exposed to increasing concentration of streptavidin, then the response was measured. This was also done to evaluate PEG coatings as a potential anti-fouling strategy and optimisation step, to increase resistance to protein adsorption whilst increasing surface hydration[16].

In Figure 5.9, for the biotin-functionalised device (black points) a clear concentration-dependent increase in the response to streptavidin is observed, with saturation beginning to occur towards the upper bound of the dynamic range as the biotin-probes become increasingly unavailable. This, again, could be an expected consequence of negatively-charged streptavidin molecules becoming increasingly bound to the surface and electrostatically inducing more charge carriers in the organic semiconductor film. This has the effect of increasing the effective gate voltage that the sensor experiences and increases the current response, until no more streptavidin can no longer bind and the response saturates. For the PEG-functionalised device (red points) there is no discernible response observed. The measured responses appear to be noise fluctuations from the assay protocol with successive washing and drying steps. Along with the BSA control experiment in Figure 5.4, these results suggest that the response observed from the biotin-functionalised device is from selective binding to streptavidin. The results also point to PEG coatings as a potentially effective strategy to passivate sensor surfaces and reduce the non-specific adsorption of interfering biomolecules.

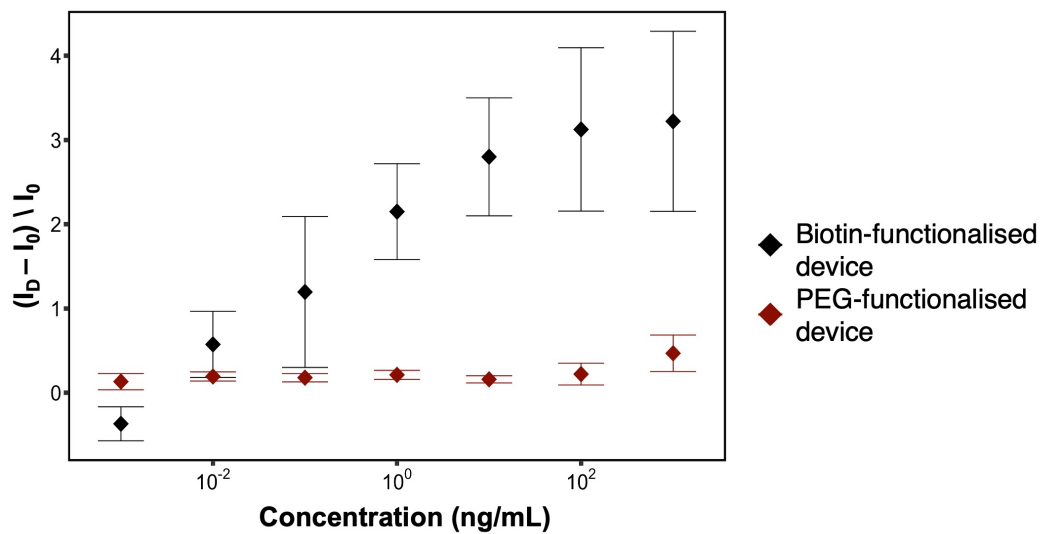


Figure 5.9: Response comparison of a biotin-functionalised and PEG-functionalised BioFET exposed to different streptavidin concentrations. I_D is taken at -35 V for each sample. $N = 20$ for 3 devices of identical dimensions. Error bars represent the standard deviations.

5.3.5 Effect of dispensing or spin coating the organic semiconductor on the sensor response

This section concerns the effect of the semiconducting polymer deposition method on the sensor response. Dispensing and thus patterning the semiconducting polymer layer leads to patterned areas of biotin probes, rather than poorly defined probe areas that arise from depositing biotin solutions on spin coated thin films. Figure 5.10 shows the transfer characteristics of an BioFET fabricated in the exact conditions as in Figure 5.8 but with a dispensed semiconducting polymer layer. A similar, but increased overall response compared to that of the spin coated device is detected, further suggesting the reproducibility of this biosensor fabrication method.

Comparing the dose-responses in Figure 5.11 shows that for the dispensed configuration, the LOD or the lowest target concentration as estimated visually from the calibration curve, is reduced by one order of magnitude in comparison to the spin coated sensor, at a value of 0.001 ng mL^{-1} . The results indicate that patterning the organic semiconductor through dispensing, and hence patterning the resulting probe layer, can enhance the sensitivity of the system and increase the magnitude of the response across the concentration range, perhaps by reducing the noise and cross-talk between across devices. Additionally, the results indicate the potential of throughput deposition methods for rapid additive manufacturing of organic bioelectronic sensors.

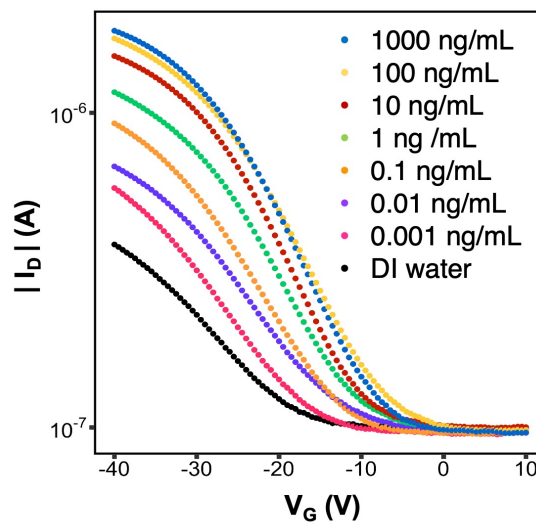


Figure 5.10: Transfer characteristics (back sweep) of a streptavidin Bio-FET device ($L/W = 5/1000$) with a printed semiconducting polymer functionalised with 0.5 mM DBCO-PEG2K-Biotin in the saturated regime $V_D = -40 \text{ V}$, with 1:1 IDT-BT- N_3 :IDT-BT blend as the semiconductor. Mean I_D is plotted. $N = 5$.

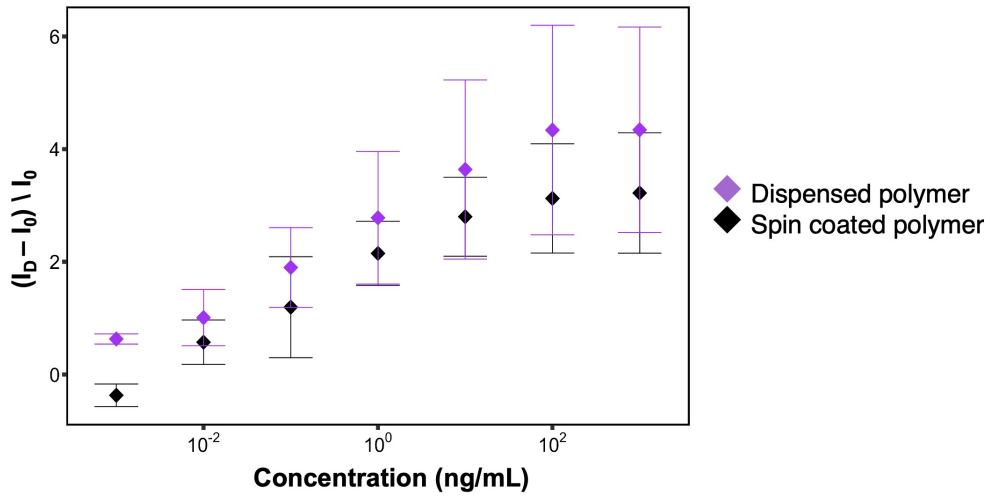


Figure 5.11: Response comparison of a BioFET fabricated with a dispensed OSC and spincoated OSC exposed to different streptavidin concentrations. I_D is taken at -35 V for each sample. $N = 15$ for three devices. Error bars represent the relative standard deviations.

5.3.6 Fitting sigmoidal models to BioFET concentration-response curves

This section will discuss the modelling of logistic functions to BioFET concentration-response curves. Regression analysis was performed using a four-parameter logistic model (4PL) (Prism, GraphPad Software). Considering the example data set in Figure 5.3, it appears to follow a classic sigmoidal ‘S’ shape with bottom and top plateaus, thus the nonlinear regression model commonly employed in bioassay analysis was chosen here. The 4PL equation based on least-squares fitting was used as follows:

$$y = \text{bottom} + \frac{(\text{top} - \text{bottom})}{1 + 10^{(\log EC_{50} - x) * \text{hillslope}}} \quad (5.2)$$

Where bottom refers to the minimum response, top refers to the maximum response, EC_{50} is the concentration value that produces a response 50% between the bottom and top and hillslope refers to the steepness of the curve. This model applied to a streptavidin BioFET data set (fabricated via spin coating) is shown in Figure 5.12 and the four parameter values extracted are $1.316E-7$, $5.319E-7$, 0.4517 and 0.6482 for the minimum, maximum, EC_{50} and hillslope respectively, with an R^2 value of 0.90 indicating good agreement with the raw data.

From this model, values for the LOD can be interpolated, however there are different strategies reported to calculate this. Often, the LOD is simply estimated from the concentration-response curve by observing the lowest concentration that produces a visual response[3]. A common strategy to numerically determine the LOD concerns the response from the target that is reliably above the signal coming from the blank sample (reference).

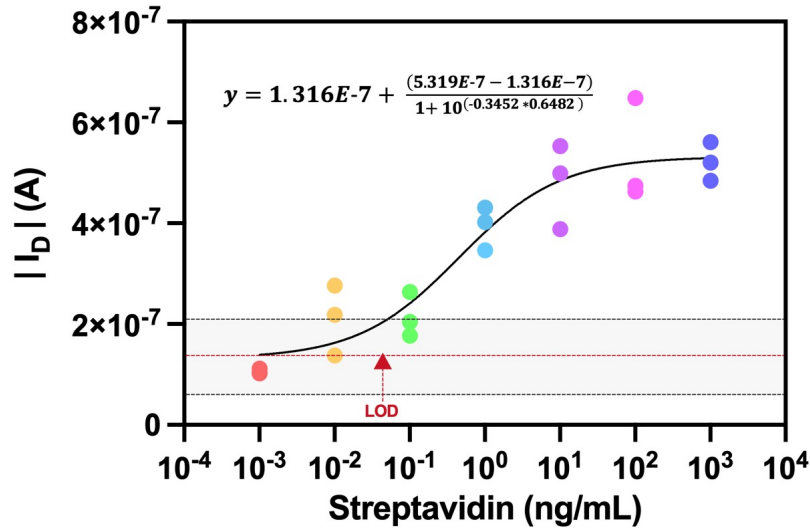


Figure 5.12: Concentration-response curve of a streptavidin BioFET (fabricated via spin coating) with a four-parameter sigmoidal function modelled to the average response data. $N = 3$. The mean blank response level is indicated (red line) along with the error (grey area) and LOD.

The signal is measured from the blank samples, then the mean response B_{mean} and associated standard deviation σ_B are calculated. Next, the concentration corresponding to a response that is $B_{mean} \pm k\sigma_B$ is evaluated, where k is a numerical factor chosen according to the level of confidence ($k = 3$ is a recommended value)[14][4][17]. To perform this on the BioFET data, the absolute current values must be used, as the blank response results in zero when using in the normalised response. Interpolating this mean blank response value of $1.35\text{E-}7 \pm 7.20\text{E-}8$ A from the calibration curve results in a LOD of $0.0448 \text{ ng mL}^{-1}$ or 745 fM .

Another method of LOD evaluation involves determining the average noise level of the negative control experiment in the whole concentration range of the experiment and adding this to three times the noise standard deviation[18]. Taking into account the negative control experiment conducted using a PEGylated chip in response to streptavidin (Figure 5.13), the mean response of control was calculated as $1.16\text{E-}7 \pm 1.59\text{E-}8$ A. The LOD value interpolated from the curve in this method was calculated as 0.011 ng mL^{-1} or 183 fM . The LOD value calculated here is approximately 4 times lower than that of the previous method, highlighting the need to stay consistent with the calculation method in order to compare experimental conditions and data sets accurately.

Despite the variations in the LOD calculations, a consistent numerical method taking into account the blank response is likely a more robust method of comparing data sets rather than relying on visual estimation from the curve. For example, the same 4PL model was applied to a streptavidin BioFET data set (fabricated via dispensing) and is shown in Figure 5.14. The four parameter values extracted are $1.83\text{E-}7$, $9.37\text{E-}7$, 1.405

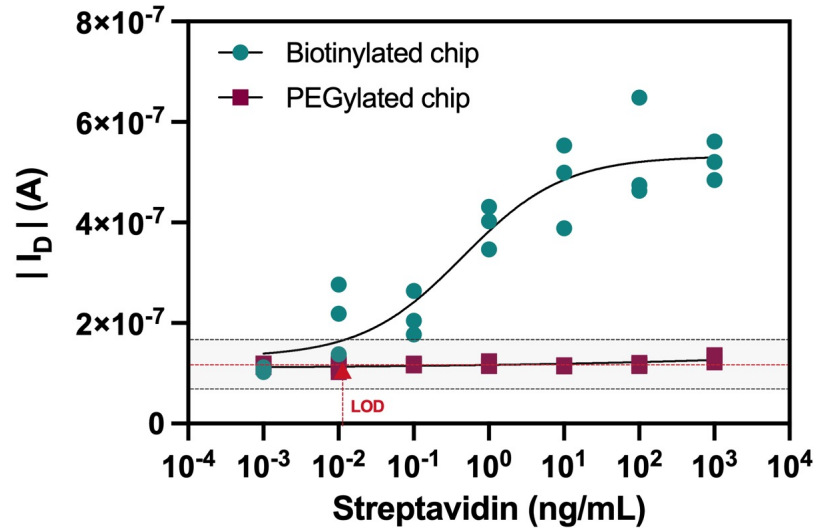


Figure 5.13: An alternative method of LOD calculation taking into account the average noise level of the negative control experiment. The mean blank response level is indicated (red line) along with the error (grey area) and LOD.

and 0.2794 for the minimum, maximum, EC50 and hillslope respectively, with an R^2 value of 0.83 indicating a fairly good agreement with the raw data. Taking the LOD again as the concentration corresponding to $B_{mean} \pm k\sigma_B$ where $k = 3$ results in a blank response of $1.87E-7 \pm 1.43E-7$ A. This results in a LOD value as interpolated from the 4PL model of value of 0.009 ng mL^{-1} or 150 fM, numerically confirming a 5 fold reduction in the LOD by an order in magnitude compared to the BioFET fabricated via spin coating as was presented in Figure 5.12.

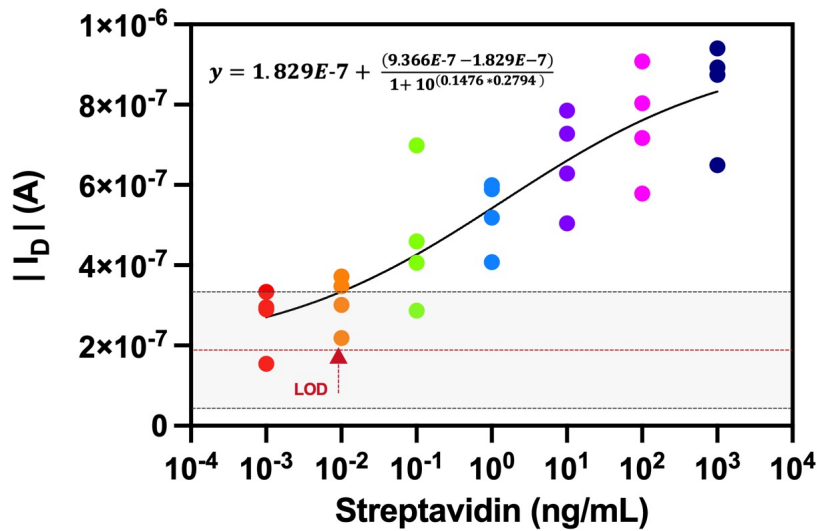


Figure 5.14: Concentration-response curve of a streptavidin BioFET (fabricated via dispensing) with a four-parameter sigmoidal function modelled to the average response data for 3 devices of identical dimensions. The mean blank response level is indicated (red line) along with the error (grey area) and LOD.

There are only a few reports of streptavidin-biotin BioFET systems to put the calculated LOD value into context, with some systems adopting streptavidin as the probe and biotin as the target. Picca *et al.* reported a BGTC-OFET configuration with a functional biological interlayer sandwiched between the dielectric and organic semiconductor. The interlayer was composed of streptavidin molecules for biorecognition and zinc oxide nanoparticles as a molecular additive to hinder environmental degradation[12]. Detection of biotin down to the low pM range was achieved. Mulla *et al.* reported an EGOFET biosensor for streptavidin detection through depositing carboxyl groups and anchoring biotinylated phospholipids. Electronic detection of streptavidin in PBS down to 10 pM was observed[19]. These results indicate that the biosensor presented here, in most cases, achieves a superior sensitivity with a lower LOD by approximately three orders of magnitude. A reason for this could be the direct coupling of the bound target analyte and probe complex to the organic semiconductor in this system, which could result in a higher sensitivity than other configurations which are more spatially separated. The target does not first have to diffuse through the active layer to reach the probes as is required in a system with an functional inter-layer. Additionally, the signal from the target does not have to couple across a phospholipid membrane to the active layer to provide the transduction.

5.4 Conclusions

This chapter reported on a proof-of-concept system for fabricating BioFETs through direct surface functionalisation of conjugated polymer thin films via click chemistry. The effect of biotin probe density on the binding efficiency of target streptavidin molecules was demonstrated. Higher biotin densities appeared to result in sensors with essentially zero response, which was attributed to steric crowding effects reducing the accessible binding sites. In contrast, lower biotin probe densities resulted in the expected dose-dependent normalised current response, indicating the significance of optimising this parameter for successful BioFET operation.

The selectivity of target response was evaluated against BSA to ensure that the signal was due solely to streptavidin-biotin complex formation as opposed to non-specific interactions. The results demonstrated a lower limit of 0.001 ng mL^{-1} below which the response between streptavidin and non-specific BSA is difficult to differentiate. However, from this limit the response of streptavidin binding was found to be significantly larger than that of BSA, demonstrating the selectivity of the sensing mechanism.

The data was also analysed to determine the effect of the gate voltage extraction value on the resulting response curves. The value appears to have a significant effect on the curves with an apparent trade-off between signal and noise. The results also validated the gate voltage choice of -35 V for the response, which results in a calibration curve with a balanced signal-to-noise. This analysis also has implications in the potential simplification

of BioFET operation, with the prospect of choosing an optimal value of gate bias to apply to the electrode and measuring the ‘best’ response rather than sweeping the bias from low to high.

The effect of the device dimensions on the response was also studied. In this system, devices with larger channel areas result in the larger responses. This could perhaps be attributed to due the larger effective area of biotin-streptavidin complexes above the channel area which induce the response, however the exact correlation is unclear. Nonetheless, such analysis informs the data processing protocol and highlights the requirement to compare the responses of devices with identical dimensions to reduce the variation of the calibration curve. Such studies could also help guide BioFET development from the initial design, with longer channel devices favoured over smaller devices, contrasting with traditional transistor design goals.

Another control experiment to determine selectivity was performed using a PEG-functionalised BioFET. This system was also used to determine the effectiveness of PEG as a potential anti-fouling coating to reduce non-specific interactions. The results revealed that the normalised current response of streptavidin on this PEG-functionalised devices is was essentially zero across the concentration range. Whereas from the 0.01 ng mL^{-1} the streptavidin signal on biotin-functionalised devices was significantly higher, indicating selective sensing and low levels of non-specific adsorption on the PEGylated sensor. This could also have consequences in future biosensor designs, where a mixed immobilisation of PEG and probe molecules could be implemented together for target binding and anti-fouling properties, of which the latter will become crucial when sensing in complex media and real patient samples.

The effect of the organic semiconductor deposition method on the sensor response was also evaluated. The BioFETs with patterned organic semiconductor areas exhibited an increased overall normalised current response in addition to a reduction in the LOD. This suggests that patterned organic semiconductor areas, and thus patterned biotin-streptavidin probe-target complexes, could improve biosensor performance perhaps by improving the bare device array performance. These results again have implications for guiding biosensor fabrication protocols which could enhance biosensor sensitivity.

Finally, regression analysis was performed whole concentration-response curves using the four-parameter logistic equation. Different methods of LOD calculation were considered, however generally, values in the high fM range were achieved, indicating a high sensitivity with respect to reported BioFETs in literature. The results demonstrated that the method of LOD calculation will affect the calculated value. Currently in the field there is no universal standard, however remaining consistent one method and applying it to various data can be useful for comparison and to guide experimentation.

References

- [1] C. Suspène, B. Piro, S. Reisberg, M.-C. Pham, H. Toss, M. Berggren, A. Yassar, G. Horowitz, *Journal of Materials Chemistry B* **2013**, *1*, 2090–2097.
- [2] Z.-S. Kim, S. C. Lim, S. H. Kim, Y. S. Yang, D.-H. Hwang, *Sensors* **2012**, *12*, 11238–11248.
- [3] T. Minamiki, T. Minami, R. Kurita, O. Niwa, S.-i. Wakida, K. Fukuda, D. Kumaki, S. Tokito, *Applied Physics Letters* **2014**, *104*, 243703.
- [4] C. Baldacchini, A. F. Montanarella, L. Francioso, M. A. Signore, S. Cannistraro, A. R. Bizzarri, *Sensors* **2020**, *20*, 6364.
- [5] M. Morana, G. Bret, C. Brabec, *Applied Physics Letters* **2005**, *87*, 153511.
- [6] B. B. Patil, Y. Takeda, S. Singh, A. Singh, T. T. Do, K. (Ostrikov, S. P. Singh, S. Tokito, A. K. Pandey, P. Sonar, *Materials Research Express* **2021**, *8*, 096301.
- [7] G. H. Gelinck, E. van Veenendaal, R. Coehoorn, *Applied Physics Letters* **2005**, *87*, 073508.
- [8] L. M. Bonanno, L. A. DeLouise, *Langmuir* **2007**, *23*, Publisher: American Chemical Society, 5817–5823.
- [9] V. H. Pérez-Luna, M. J. O’Brien, K. A. Opperman, P. D. Hampton, G. P. López, L. A. Klumb, P. S. Stayton, *Journal of the American Chemical Society* **1999**, *121*, 6469–6478.
- [10] L. S. Jung, K. E. Nelson, P. S. Stayton, C. T. Campbell, *Langmuir* **2000**, *16*, 9421–9432.
- [11] M. Magliulo, A. Mallardi, M. Y. Mulla, S. Cotrone, B. R. Pistillo, P. Favia, I. Vikholm-Lundin, G. Palazzo, L. Torsi, *Advanced Materials* **2013**, *25*, 2090–2094.
- [12] R. A. Picca, K. Manoli, A. Luciano, M. C. Sportelli, G. Palazzo, L. Torsi, N. Cioffi, *Sensors and Actuators B: Chemical* **2018**, *274*, 210–217.
- [13] J. Y. Lichtenberg, Y. Ling, S. Kim, *Sensors* **2019**, *19*, 2488.
- [14] L. Torsi, M. Magliulo, K. Manoli, G. Palazzo, *Chemical Society Reviews* **2013**, *42*, 8612–8628.

-
- [15] B. M. Lowe, K. Sun, I. Zeimpekis, C.-K. Skylaris, N. G. Green, *The Analyst* **2017**, *142*, 4173–4200.
- [16] A. Frutiger, A. Tanno, S. Hwu, R. F. Tiefenauer, J. Vörös, N. Nakatsuka, *Chemical Reviews* **2021**, DOI 10.1021/acs.chemrev.1c00044.
- [17] M. Berto, E. Vecchi, L. Baiamonte, C. Condò, M. Sensi, M. Di Lauro, M. Sola, A. De Stradis, F. Biscarini, A. Minafra, C. A. Bortolotti, *Sensors and Actuators B: Chemical* **2019**, *281*, 150–156.
- [18] E. Macchia, L. Sarcina, R. A. Picca, K. Manoli, C. Di Franco, G. Scamarcio, L. Torsi, *Analytical and Bioanalytical Chemistry* **2020**, *412*, 811–818.
- [19] M. Y. Mulla, P. Seshadri, L. Torsi, K. Manoli, A. Mallardi, N. Ditaranto, M. V. Santacroce, C. D. Franco, G. Scamarcio, M. Magliulo, *Journal of Materials Chemistry B* **2015**, *3*, 5049–5057.

Chapter 6

Circulating tumour DNA-targeting BioFET

This chapter describes the application of BioFETs to nucleic acid detection using circulating-tumour DNA (ctDNA) as the model system for targeting. This proof-of-concept platform first demonstrates how single-stranded DNA (ssDNA) probes functionalised on BioFET surfaces can modulate the device electrical properties, which are further modulated upon hybridisation with the complementary target. The immobilisation density on the target binding efficiency was also investigated. The effect of non-specific binding on the device response was assessed and the selectivity against a non-complementary target was analysed. Then, the work towards boosting the sensing performance through the use of toehold domains on the probe and target sequences is introduced. Finally, the chapter concludes with a critical discussion of the limitations and opportunities for BioFET sensing of ctDNA and the response analysis.

6.1 Motivation

Global cancer cases are increasing. Particularly breast cancer, which has overtaken lung cancer as the world's most commonly diagnosed cancer, and represents 1 in 4 cancers diagnosed among women globally[1]. This increase could be partly attributed to the fact that, on average, our world population is ageing rapidly and this increases the risk factor for many diseases, including cancer[2]. It could also be partly due to growing outreach and education on cancer screening, leading to increased awareness among the public and an higher numbers of diagnoses. Despite the multifaceted nature of the origin of the increase in cases, it nonetheless highlights the greater importance of reliable cancer detection methods.

The breast cancer patient journey can vary considerably, however a typical breast cancer patient journey could involve the following: (i) finding a symptom, (ii) going to

a GP assessment for an analysis of family history and/or blood tests, (iii) a referral for an MRI or a mammogram, (iv) a referral for a tumour biopsy, (v) treatment in the form of surgery and/or chemotherapy/radiotherapy. Combinations of the detection methods in steps (i) to (iv) have been successful in diagnosing breast cancer for millions of people around the world, but they do have drawbacks in terms of speed, accuracy and invasiveness. Additionally, after the patient has received treatment, it is paramount to be able to detect breast cancer recurrence accurately and reliably.

Furthermore, the ideal post-treatment cancer monitoring would cause as little distress and discomfort for the the patient as possible, and this is where the BioFET technology could fit in as depicted in Figure 6.1. BioFETs are proposed as platforms for post-treatment monitoring of cancer biomarkers to determine the state of the disease and/or the response to treatment, something very difficult for conventional imaging and tumour biopsies to provide sufficient information on, in real time.

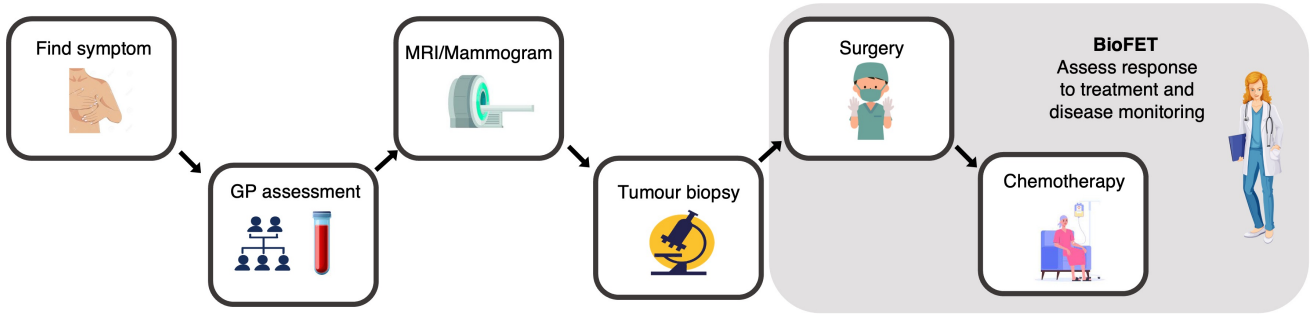


Figure 6.1: Illustration of a typical breast cancer patient journey from detection to treatment, highlighted in grey with the location where BioFET technology is proposed to enter.

In particular, ctDNA has emerged as a cancer biomarker with notable diagnostic and prognostic potential across a number of cancer types[3]. Tumour cells release fragments of DNA into circulation in the bloodstream as they die, along with other biological materials such as circulating tumour cells (CTCs) and extracellular vesicles (EVs) to name a few. The term cell-free DNA (cfDNA) refers to all non-encapsulated DNA in the blood stream. In cancer patients, a fraction of the total cfDNA is the ctDNA, which carries mutations that are specific to the primary tumour[4].

To this point, such genetic mutations have been primarily detected from blood samples using genomic techniques such as PCR. However, the analysis of ctDNA in a so called ‘liquid biopsy’ could be a cost-effective and minimally-invasive method to track tumour progression. The half-life of ctDNA in the bloodstream is under 2 hours[5], meaning that such a method could be dynamic, providing real-time correlations between disease status/response to treatment to a measurable biomarker in patient samples. However, to detect a relatively small amount of ctDNA in a background of cfDNA and other biological components requires a device with high sensitivity and selectivity.

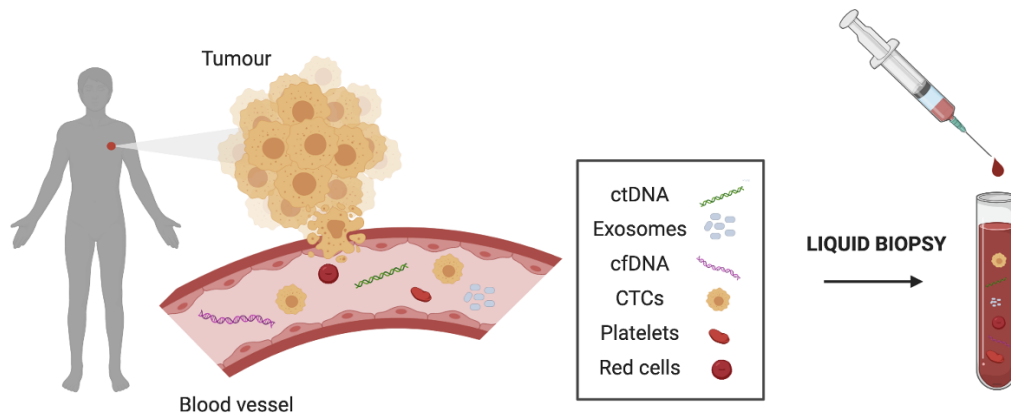


Figure 6.2: Illustration of the liquid biopsy procedure. Tumour cells release fragments of DNA into the bloodstream as they die along with also biological materials. Such components can be isolated from blood samples and used for analysis.

The concentration of cfDNA found in the bloodstream varies considerably, typically ranging from 0 > 100 ng/ml in healthy patients which can increase up to 1000 ng/ml in cancer patients. Of the total cfDNA in the blood, the fraction of ctDNA can range from as low as 0.01% to as high as 90%[6]. These very low concentrations of ctDNA can make detection challenging, meaning that this biomarker is not particularly suited for the early stages of tumour development where extremely low concentrations are present.

Nonetheless, a recent study led by Coombes *et al.* demonstrated ctDNA monitoring in the blood plasma of cancer patients after treatment. They found ctDNA positive samples ahead of clinical diagnosis in 16 of the 18 patients that went on to clinically relapse and none of the 31 non-relapsed patients were ctDNA-positive at any time point across the plasma samples[7]. This demonstrates that ctDNA monitoring post treatment may provide a critical window for additional therapeutic intervention to improve patient outcomes and save more lives.

The aim of this work was to demonstrate that with this simple biofunctionalisation method applied to sensing, previously demonstrated with a streptavidin-biotin system, can be applied to a ssDNA capturing probe hybridising with a ssDNA complementary target. The PIK3CA mutation was chosen as a model target sequence with guidance by Ryan Wong in the group of Prof. Charles Coombes, as this is one of the most prevalent mutations in breast cancer[8]. Of all the PIK3CA mutations, specifically the E542K hotspot (Exon 9) was chosen due to it's frequency of occurrence, and this mutation involves a single nucleotide variation G > A from the wild type (non-mutated DNA). To the best of our knowledge, this is the first demonstration of ctDNA targeting with a BioFET fabricated through direct functionalisation of the conjugated polymer via click chemistry.

6.2 Materials and Methods

6.2.1 BioFET fabrication and operation

The bottom-gate bottom-contact Si/SiO₂ substrates were prepared as per section 3.2.2. The ssDNA probe functionalisation was prepared as per section 4.2.2 to fabricate the BioFET. Dilutions of ssDNA targets were prepared in NaCl-TAE buffer (typically 1 M) and dispensed onto the BioFET surface, covering all functionalised devices. A blank sample of buffer containing no target was used for the reference sweep before testing the target sample (1 M or 50 mM). The devices were left for 10 mins for the target binding to take place before being rinsed several times with DI water and dried with N₂. Unless stated otherwise all the electrical characterisation was performed in air using a semiconductor characterisation system (Keysight B2902A) and probe station setup. The transfer characteristics were measured by sweeping the V_G , typically from ranges of +10 V to 40 V at a constant V_D of -40 V.

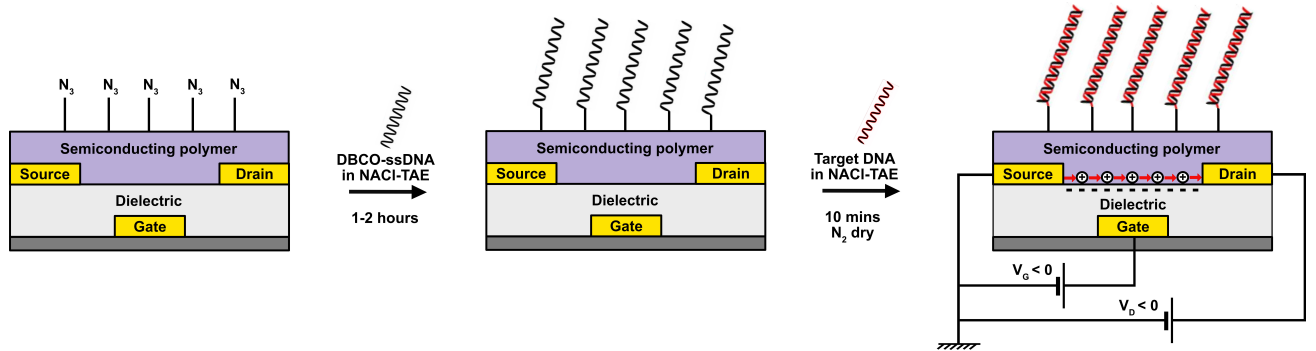


Figure 6.3: Schematic representation of the fabrication of the ctDNA-targeting BioFET and the resulting assay operation.

6.2.2 Fluorescence assay

In this section (i) probe-DNA refers to the substrate strand with a toehold domain, (ii) weak-DNA refers to the partially-complementary strand that can form a duplex with the probe-stand, (iii) target DNA refers to the fully-complementary strand that acts as the invader strand and (iv) - (vi) refers to the base-pair mismatches (bpm) from the target sequence. ctDNA sequences based on the PIK3CA E542K hotspot were designed and obtained commercially (Integrated DNA Technologies). The mutations from the target are highlighted in bold to form the base-pair mismatch sequences:

- (i) Probe-DNA: 5' TTT TTT AGT GAT TTT AGA GAG AGG ATC TCG Cy5 3'
- (ii) Weak-DNA: 5' BHQ-2 CGA GAT CCT CTC TCT AAA 3'
- (iii) Target DNA (SNV): 5' CGA GAT CCT CTC TCT AAA ATC ACT 3'

- (iv) 3bpm-DNA: 5' CGA GAT CCT **TTC GCT GAA** ATC ACT 3'
- (v) 2bpm-DNA: 5' CGA GAT CCT CTC **GCT GAA** ATC ACT 3'
- (vi) 1bpm-DNA (wild type): 5' CGA GAT CCT CTC TCT **GAA** ATC ACT 3'

Solutions were prepared in NaCl-TAE buffer by spinning in a mini centrifuge and vortexing. For the double-stranded DNA (dsDNA) probe complex, the probe-DNA (200 nM) and weak-DNA (240 nM) were combined in an eppendorf tube, then placed in a heated block (95 °C for 7 mins) before being left to cool down over several hours to room temperature. Target and mismatch sequences (15 nM) were added to wells with the dsDNA complexes after 10 mins. The positive control well contains only the probe-DNA fluorophore solution. The first negative control well contains only the double-stranded probe complex. The second negative control well contains only measurement buffer. A plate reader (Spectramax M5) was used to record the fluorescence signal.

6.3 Results and Discussion

6.3.1 Electronic response of devices immobilised with single-stranded DNA in comparison with bare devices

This section will describe the work towards functionalising certain devices on the BioFET chip with ssDNA whilst leaving others unmodified for reference, a form of patterning the device with probes in specific areas. This also has interesting implications for future work in developing multiplexed biosensors with the ability to target multiple analytes. The yellow dotted box in Figure 6.4 depicts a chip quadrant with 4 devices, the devices labelled T5 and T6 have been printed with a solution of 10 μ M ssDNA undergoing functionalisation, whilst T7 and T8 are left blank.

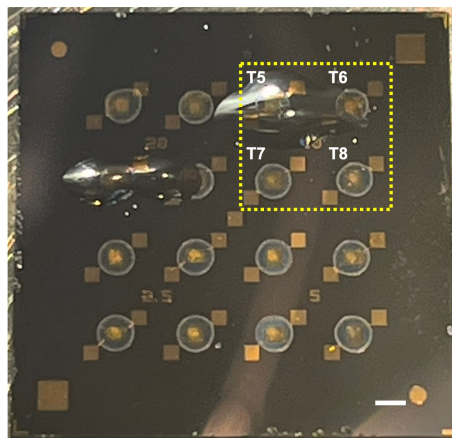


Figure 6.4: Photograph of the OFET chip during immobilisation with ssDNA. The yellow box signifies the devices which were tested. T5 and T6 were printed with the ssDNA solution whilst T7 and T8 were left blank. Scale bar = 1 mm.

For the electrical characterisation, T5 and T6 were tested prior to ssDNA immobilisation and after, with a buffer rinse. T7 and T8 were tested prior to being left blank and after rinsing with buffer for reference to determine any unspecific variation in the sensor response.

Figure 6.5 shows the associated transfer characteristics of the devices highlighted above during each experimental/measurement stage. Prior to any immobilisation (grey points), the devices exhibit uniform behaviour with a mean threshold voltage V_T across the four devices of -25.1 ± 1.0 V. After ssDNA immobilisation of the top row (T5 and T6), a clear shift in the transfer characteristics (orange points) is observed, whilst the characteristics of the bottom row which was left unmodified (T7 and T8) remain largely unchanged. This demonstrates clear differences in the device electrical properties based on whether ssDNA is present at the surface.

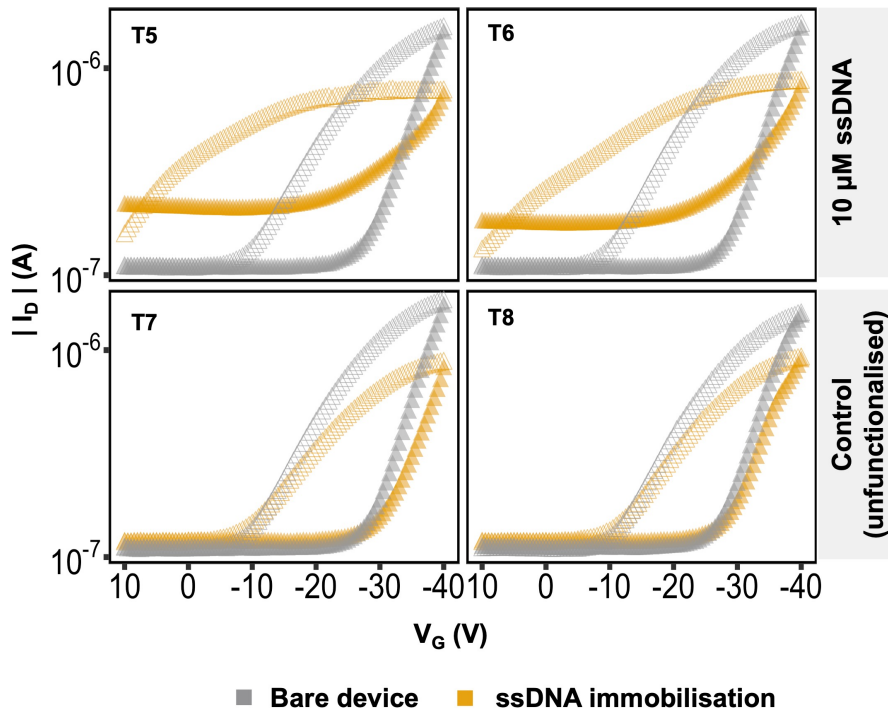


Figure 6.5: Transfer characteristics of four OFETs before (grey points) and after (orange points) immobilisation with $10 \mu\text{M}$ single-stranded DNA probe sequence (T5 and T6). For the control devices that were left blank/without DNA (T7 and T8) the bare device was first tested (grey points) and then again (orange points) for comparison. Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.

Extracting the threshold voltage values V_T from the curves after functionalisation, distinct populations from the top row of devices can be observed easily, such as in Figure 6.6. The threshold voltages for T5 and T6 shift to significantly more positive values as stated in the summary of Figure 6.7. For the bottom row there are comparatively minor changes in the threshold voltage, which are most likely attributed to noise induced from

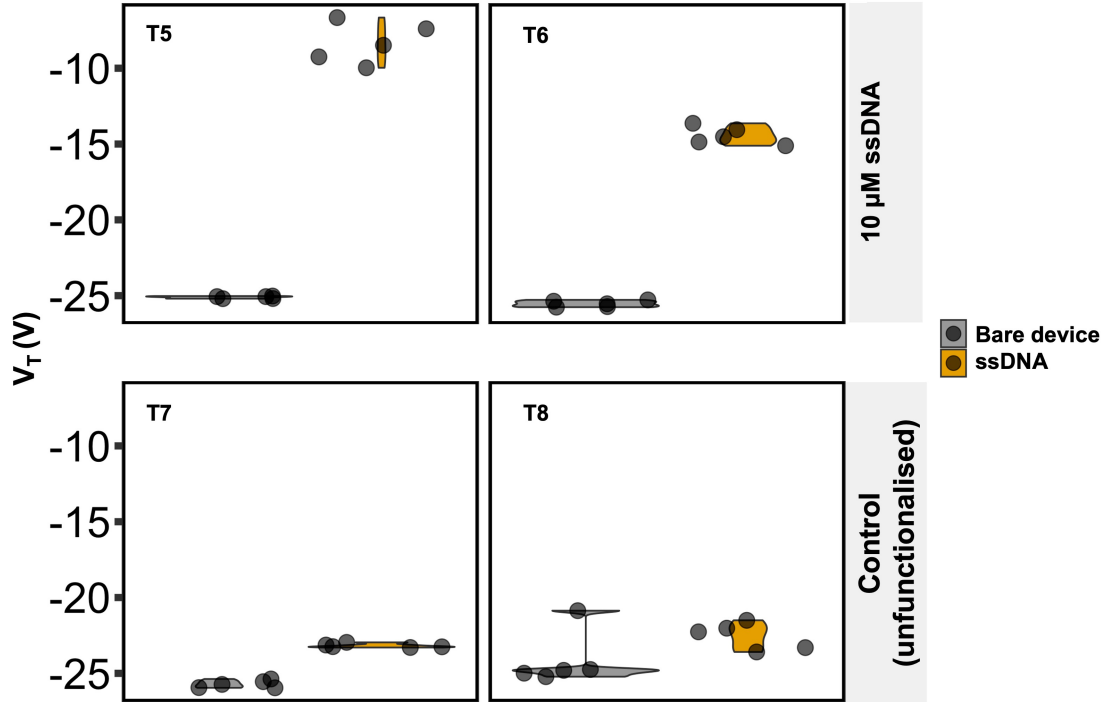


Figure 6.6: Extracted V_T values of OFETs (T5 and T6) before (grey violin) and after (orange violin) immobilisation with 10 μ M single-stranded DNA probe sequence. For the devices that were left blank/without DNA (T7 and T8) these first tested (grey violin) and then again (orange violin) for comparison. $N = 5$.

the assay protocol. Despite not being exposed to any ssDNA solution, the bottom row were still subjected to the same washing and drying steps as the top row which slightly affects the device properties. However, the little changes in the bottom row demonstrate low levels of non-specific binding to the control areas during the immobilisation process.

Furthermore, the device T5 demonstrates a larger positive V_T shift than T6, shifting to -8.4 V compared with the latter to -14.4 V. Looking again at Figure 6.4, the deposited ssDNA has complete coverage of T5 whilst the solution doesn't cover the complete device area of T6, due to a flaw from the printing. However, this provides support to the notion that the surface-functionalised ssDNA is causing the positive shift of V_T .

This characteristic shifting of the threshold voltage to more positive values is consistent with reports of DNA immobilisation on OFETs[9][10][11] however the exact nature of these variations are diverse, which is to be expected due to differences in the devices and experimental conditions.

Device	Threshold Voltage (V)	
	Bare device	ssDNA
T5 (ssDNA functionalised)	-25.1 ± 0.08	-8.4 ± 1.3
T6 (ssDNA functionalised)	-25.5 ± 0.2	-14.4 ± 0.6
T7 (Control)	-23.7 ± 0.3	-23.2 ± 0.1
T8 (Control)	-24.1 ± 1.8	-22.5 ± 0.9

Figure 6.7: Table summary of the data shown in Figure 6.6.

Nonetheless Zhang *et al.* reported pentacene OFETS immobilised with DNA sequences which exhibited positive V_T shifts of -12.5 V to 6.6 V [12], a shift of 19.1 V which is comparable to the shift observed for T5 of 16.7 V. This threshold voltage shift was attributed to the negatively-charged phosphate groups on the DNA backbone causing increased hole accumulation and charge density in the conducting channel, with an effective p-doping effect. This is likely the same mechanism underway with the system reported here, as there is a p-type semiconducting polymer employed as the active layer in a OFET device operated in accumulation mode.

The fact that this shift was observed in the top row indicated p-doping within only local regions of the channel where the ssDNA was present. The good inter-device reproducibility prior to immobilisation appears to have resulted in easier comparison across devices and assay statuses by reducing the overall noise. Considering other common figures of metrics such as the mobility and drain current, there are less indicative changes in these parameters so this is not included here.

Reports tracking the drain current upon DNA sensing vary, with some attributing a decrease in current to increase in disorder and resistance of the conducting channel[9] and some reporting increases in current due to the doping effect[12]. In this system, the drain current appears sensitive to the experimental protocol, as reductions are observed across all devices, so this was not deemed to be a useful parameter to distinguish between analytes. However the results indicate, in corroboration with previous reports, that for that this system the threshold voltage is likely a key indicator of DNA detection.

6.3.2 Electrical detection of DNA hybridisation

This section concerns the work towards detecting complementary target ssDNA sequences with the ssDNA-immobilised BioFET chip. Again, the top row of devices (T5 and T6) were functionalised with the ssDNA probe and tested, whereas the bottom row (T7 and T8) were left unfunctionalised and tested along with the other devices for reference to evaluate any unspecific variation in the sensor response.

Figure 6.8 shows the results from each assay stage and for clarity the bare device curves are omitted. Electrical characterisation was performed in the dry state after the ssDNA probe immobilisation (orange points), after the devices were subject to a reference sample containing only the measurement buffer (1M NaCl+TAE) (blue points) and after the device was immersed in 100 nM of the target sample (green points). The results demonstrate clear shifting of the curves for the top row across all assay stages while the bottom row remains largely unchanged. The extracted threshold voltage values plotted in Figure 6.9 display some differences between the two rows of devices. The top row exhibit a shifting of V_T to increasingly more positive values with an increase in the related variation.

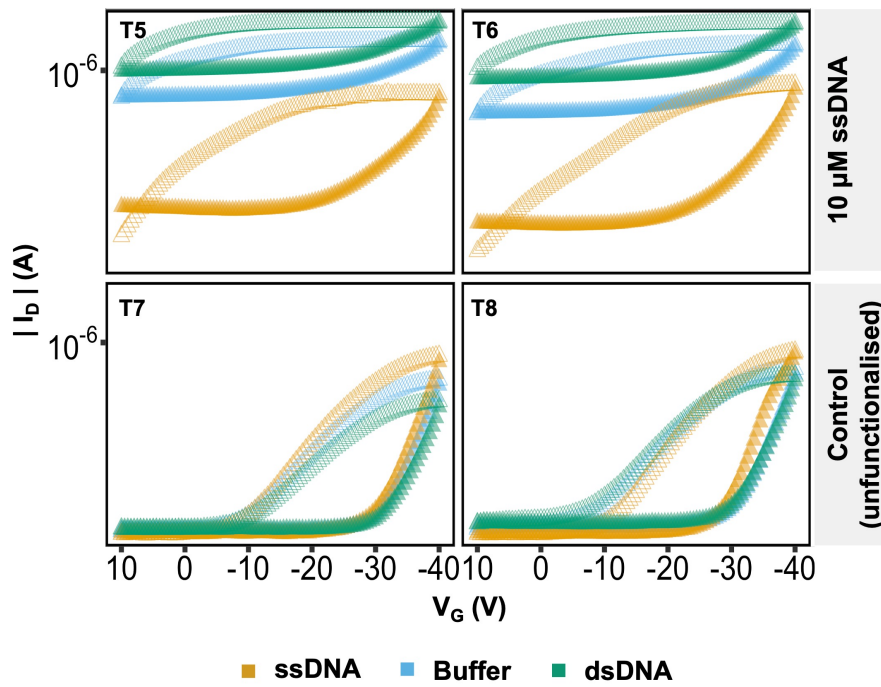


Figure 6.8: Transfer characteristics of four OFETs after ssDNA immobilisation (T5 and T6) (orange points), after the buffer reference sample (blue points) and after hybridisation with 100 nM of the target strand solution (green points). For the devices that were left blank/without probe DNA (T7 and T8) these were tested at the same time as the other devices for reference. Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.

However, this shift is not present in the bottom row which experiences very minimal changes in V_T across the assay steps, suggesting this is likely noise generated from washing and drying steps in the protocol. This increase in noise of the top row as the surface becomes increasingly biologically-active with nucleic acid sequences is to be expected when dealing with charged probes on an electrically sensitive surface such as an OFET.

As stated previously, typically BioFET dose-response data is normalised to a baseline, which in this case would be the buffer sample V_T . It appears that there may be a more positive average V_T for the dsDNA status than for the the buffer status however the variation is also too high for this shift to be reliable. Comparing the V_T of the dsDNA to the ssDNA, however, the shift is clear of the margin of error, indicating that modifications to the experimental protocol may be required to stabilise the system for the buffer reference measurement and reduce the variation of this baseline.

The origin of the shift in the V_T before and after exposure to the buffer solution was considered. The buffer used for reference in this case, 1M NaCl+TAE buffer, was kept the same as the immobilisation buffers; the high salinity of which was chosen to screen the DNA charges and promote immobilisation and target binding. The buffer shift perhaps suggests conformational changes to the nucleic acid sequences immobilised on the surface that arise when the chip is re-immersed in the buffer, altering the effective surface charge density and the resulting electrical response and increase in noise.

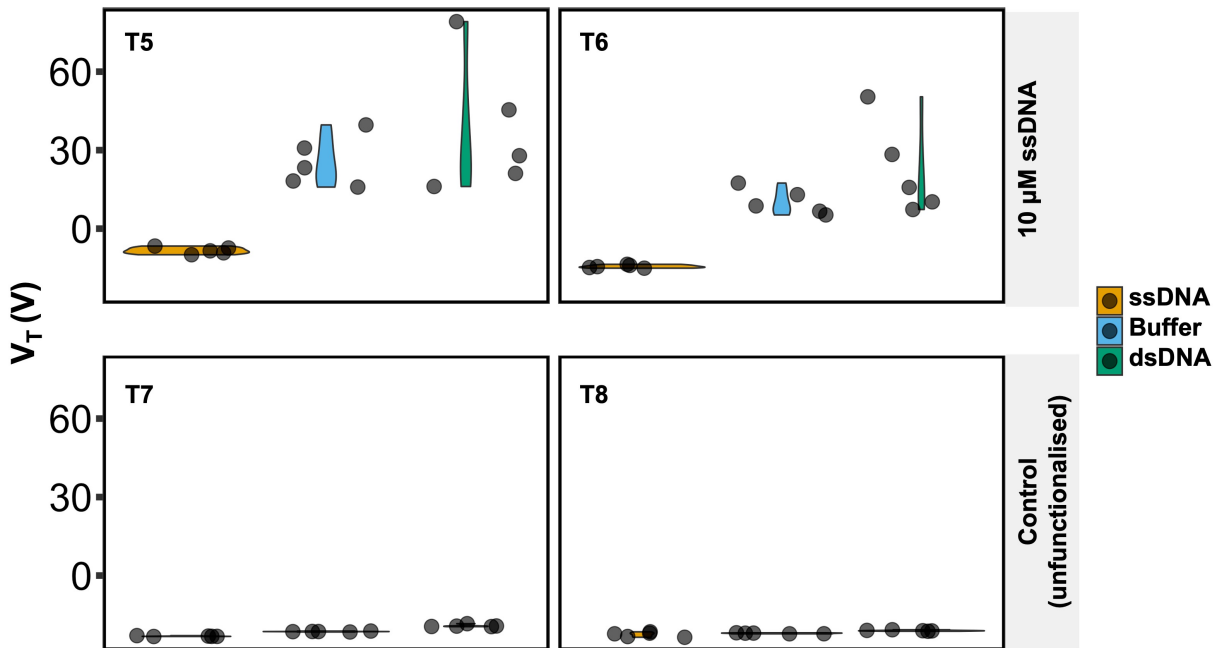


Figure 6.9: Extracted V_T values of four OFETs after ssDNA immobilisation (T5 and T6) (orange points), after the buffer reference sample (blue points) and after hybridisation with 100 nM of the target strand solution (green points). For the devices that were left blank/without probe DNA (T7 and T8) these were tested at the same time as the other devices for reference. $N = 5$.

Device	Threshold Voltage (V)		
	ssDNA	Buffer sample	dsDNA
T5 (ssDNA functionalised)	-8.35 ± 1.3	25.6 ± 9.7	37.9 ± 25.6
T6 (ssDNA functionalised)	-14.4 ± 0.6	10.1 ± 5.9	22.4 ± 17.6
T7 (Control)	-23.2 ± 0.1	-19.2 ± 0.5	-21.4 ± 0.1
T8 (Control)	-23.5 ± 0.9	-21.0 ± 0.2	-22.1 ± 0.2

Figure 6.10: Table summary of the data shown in Figure 6.9.

Many studies have investigated the biophysical properties of DNA tethered to solid surfaces in different ionic strength solutions. In high ionic strength solutions such as 1M NaCl+TAE buffer, the abundant ions will screen other charges efficiently so that the electrostatic potentials decay within sub-nanometre distances. This effect will have greater significance for ssDNA because it is inherently less mechanically rigid than the dsDNA helix, causing ssDNA strands to adopt a compacted state[13]. This could likely increase the effective surface charge density that the device experiences and further shift the threshold voltage.

However, the biophysical factors affecting DNA immobilised on surfaces are complex and such properties are also affected by factors such as the sequence length and temperature. This effect of a buffer reference altering the electrical properties has been reported previously; Boileau *et al.* fabricated DNA-targeting BioFETs based on a small molecule organic semiconductor and reported positive shifts of the V_T after the addition of a salt buffer sample however to a smaller magnitude[14].

6.3.3 Effect of the ssDNA probe density on target strand hybridisation efficiency

The immobilisation density of capture probes is an important parameter in many biosensing systems. This factor becomes further critical when dealing with charged probes such as nucleic acid sequences where electrostatic interactions effect the functionalisation process. The concentration of the ssDNA probe solution will determine properties of this layer which will affect the the target binding efficiency.

This section aims to investigate the dependence of the ssDNA probe density on these two factors. A range of ssDNA probe solution concentrations (10 μ M, 5 μ M, 2.5 μ M and 1.25 μ M) were immobilised on four chips prepared in the same conditions, covering 4 devices of identical dimensions per chip. Figure 6.11 shows representative electrical characteristics of four devices before (grey points), and after ssDNA immobilisation (orange points) for each concentration.

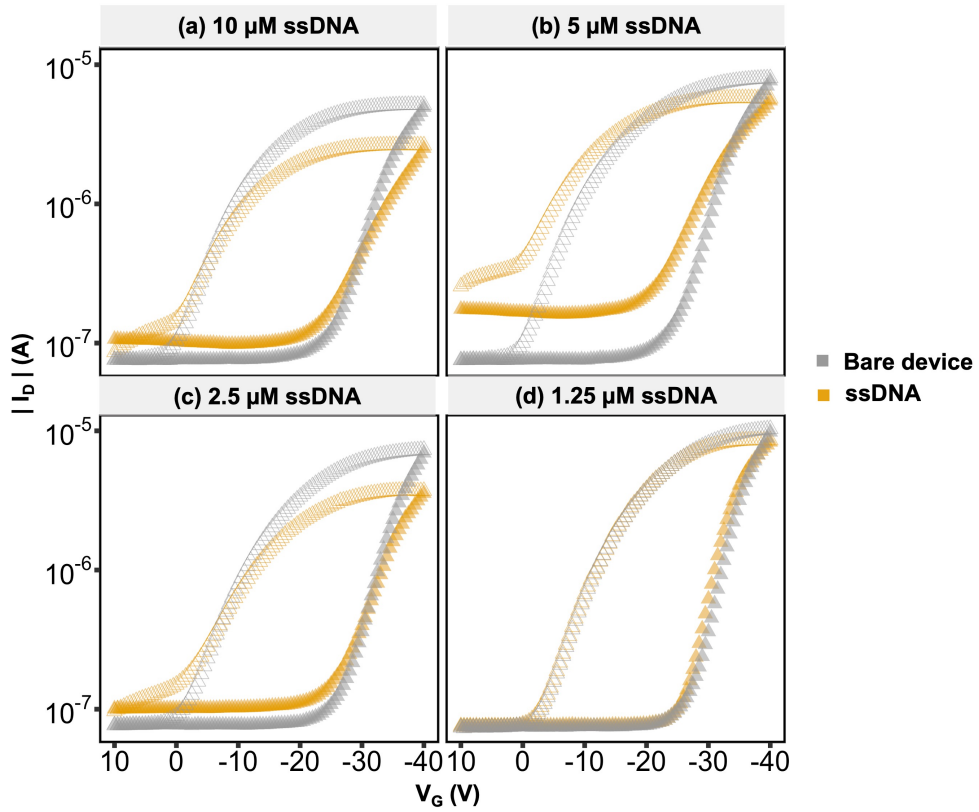


Figure 6.11: Transfer characteristics of four OFETs before (grey points) and after immobilisation with (a) 10 μ M, (b) 5 μ M, (c) 2.5 μ M and (d) 1.25 μ M ssDNA (yellow points). Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.

The data indicates that the degree to which the curve shifts after immobilisation depends on the concentration of ssDNA probe solution, with the greatest shift observed for the 5 μ M chip. Considering again the threshold voltage as the detection parameter for ssDNA immobilisation, the values were extracted for three devices per chip (5 replicates per device) and plotted in Figure 6.12.

The immobilisation concentration of the 5 μ M chip demonstrates the clearest positive V_T shift from -24.9 ± 1.9 V to -18.4 ± 2.9 V. The second largest shift was for the 10 μ M concentration whereas for the lowest concentration of 1.25 μ M, V_T was largely unchanged as listed in Figure 6.13. If we attribute positive V_T shifts to increasing negative charge from the surface-bound DNA leading to increased charge accumulation in the channel, we could perhaps presume the highest capture probe density for the 5 μ M chip.

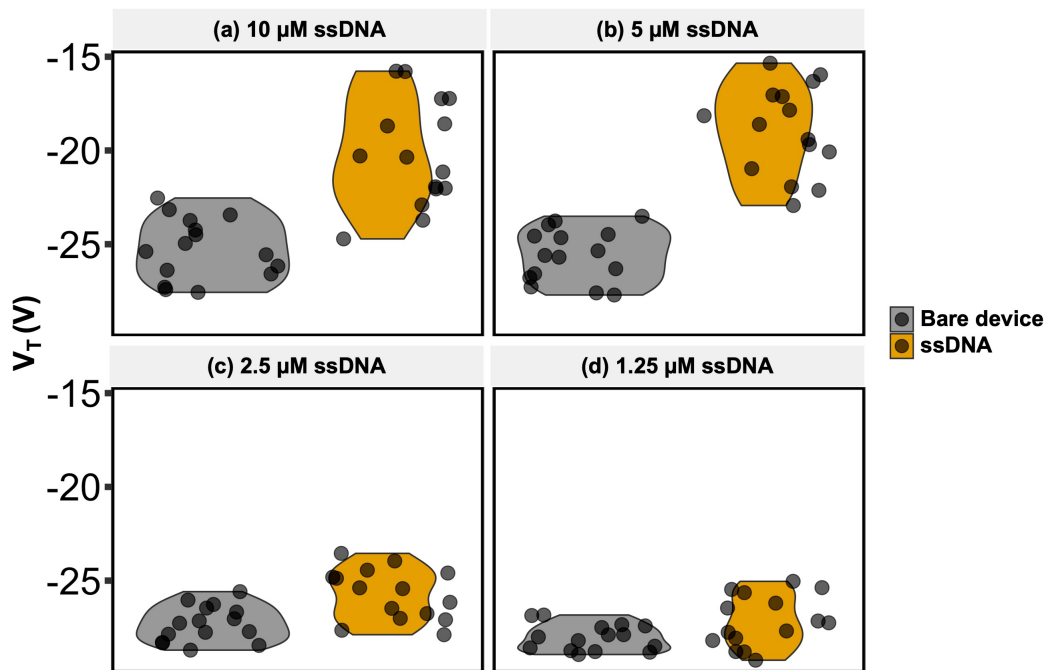


Figure 6.12: Extracted V_T of OFETs before (grey) and after immobilisation with (a) 10 μ M, (b) 5 μ M, (c) 2.5 μ M and (d) 1.25 μ M ssDNA (orange). Each point represents a replicate. $N = 15$ per concentration.

ssDNA immobilisation concentration (μ M)	Threshold Voltage (V)	
	Bare device	ssDNA
10	-24.2 ± 2.4	-20.4 ± 3.7
5	-24.9 ± 1.9	-18.4 ± 2.9
2.5	-27.1 ± 1.7	-24.6 ± 2.0
1.25	-27.7 ± 1.3	-25.5 ± 3.5

Figure 6.13: Table summary of the data shown in Figure 6.12.

The results could also suggest that at a point after the 5 μ M concentration the amount of ssDNA immobilisation begins to decrease, due to inefficient click binding to the surface from the electrostatic and steric effects. Or rather these effects could modulate the effective charge that the sensor experiences as the surface becomes increasingly disordered. Nonetheless, it suggests a limit where electrostatic factors and steric factors begin to dominate and affect the capturing probe layer which modulates the V_T shift at higher concentrations. The smaller V_T shifts at the lower concentrations likely indicate simply a lower degree of probe immobilisation and thus less effects from the bound negative charge.

At this stage, the aim was to investigate how these chips with different capture probe densities affect the binding efficiency with the complementary target. Figure 6.14 shows representative electrical characteristics of four devices after ssDNA immobilisation (orange points), after exposure to a buffer reference solution (50 mM) and after exposure to 100 nM of the complementary target (blue points), for each concentration (a)-(d). The bare device characteristics are omitted for clarity. The largest shift of the curves from the buffer status to the dsDNA status appears for the 5 μM sensor. The second largest shift occurs for the 10 μM sensor whereas the shifts for the chips with the lowest concentrations are relatively minimal.

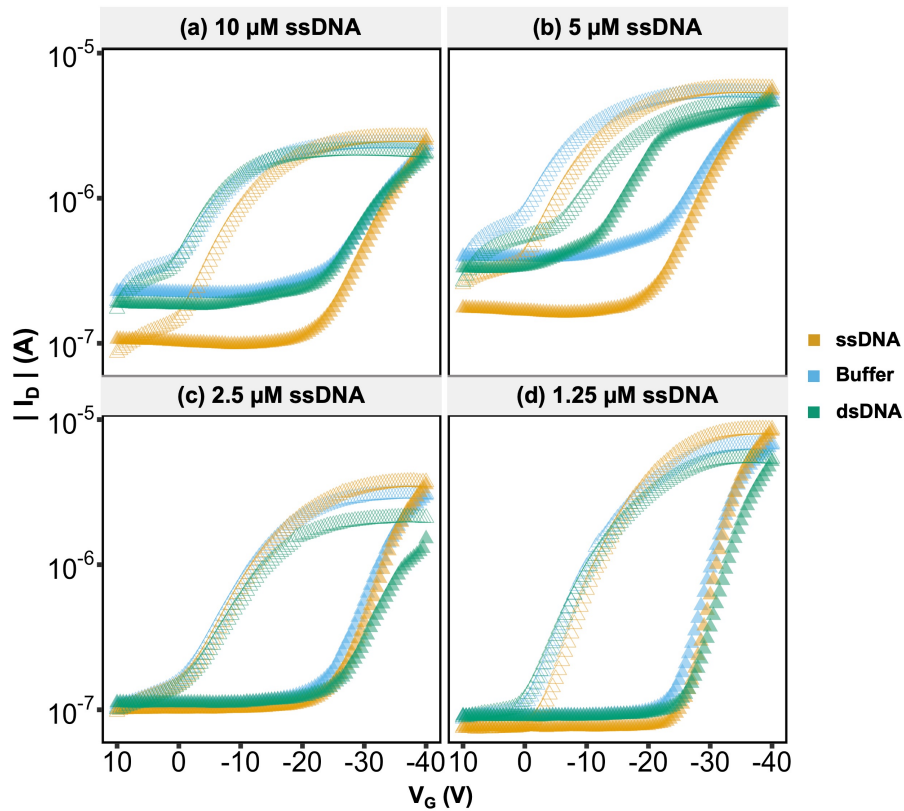


Figure 6.14: Transfer characteristics of OFETs after immobilisation with (a) 10 μM , (b) 5 μM , (c) 2.5 μM and (d) 1.25 μM ssDNA (orange points), also after the buffer reference sample (blue points) and after hybridisation with 100 nM of the target strand solution (green points). Plot depicts mean current-voltage sweep $N = 5$. Filled and empty shapes represent the backwards and forwards sweeps respectively.

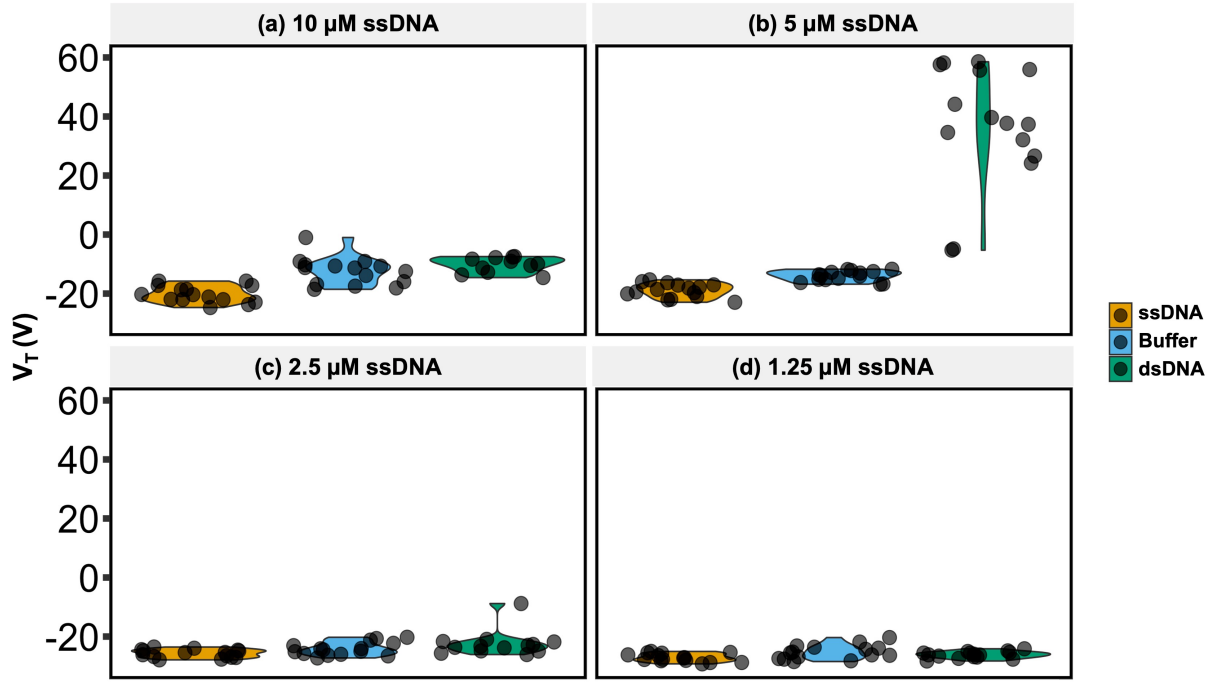


Figure 6.15: Extracted V_T of four OFETs immobilised with ssDNA (orange), after the buffer sample (blue), and after exposure to the target sample (green) for (a) 10 μM , (b) 5 μM , (c) 2.5 μM and (d) 1.25 μM ssDNA probes. $N = 15$ per concentration and status.

ssDNA immobilisation concentration (μM)	Threshold Voltage (V)		
	ssDNA	Buffer sample	dsDNA
10	-20.4 ± 3.7	-11.2 ± 6.9	-8.3 ± 3.0
5	-18.4 ± 2.9	-13.3 ± 2.0	47.7 ± 26.9
2.5	-24.6 ± 2.0	-21.9 ± 4.6	-23.7 ± 7.3
1.25	-25.5 ± 3.5	-22.4 ± 7.0	-25.7 ± 1.8

Figure 6.16: Table summary of the data shown in Figure 6.16.

Interpreting positive V_T shifts again, as increased charge density in the channel induced by the accumulation of negative charge on the surface, the larger shift for the 5 μM probe chip compared to the 10 μM probe chip is noted. This is consistent with reports found in hybridisation simulations[15] and biosensing experiments[10] where at too high probe densities the electrostatic repulsion and steric hindrance between neighbouring probe strands becomes larger and the target binding efficiency is reduced. This concept has been described as ‘the Goldilocks principle’ which describes an optimal capture probe density of nucleic acids at surfaces[16]. Above this density, overcrowding of probes can lead to steric and electrostatic barriers which result in incomplete hybridisation. Below this density, while hybridisation may be efficient, the resulting signal may be too weak due to too few capture probes. The ideal scenario is in the middle of these two extremes where

sufficient signal is produced with reasonable efficiency. The results indicate that there is a narrow window of ssDNA immobilisation density, outside of which target binding becomes much more difficult to differentiate, either due to not enough probe being available to provide a sufficient signal or too much probe causing steric overcrowding and inefficient target binding. Further optimisation could reveal where the local maxima values for V_T occur within the concentration range, either towards the upper or the lower limits. As mentioned previously, threshold voltage shifts reported for BioFETs upon target DNA hybridisation vary widely. However, the positive shifts of the threshold voltage is the consistent factor in many reports[9][10][12][11] including the results reported here. Whilst far from conclusive, the data appears to indicate the ability of the device to detect a fully complementary target.

6.3.4 Non-specific binding evaluation with sensors formed with pre-reacted ssDNA probes

It is imperative in biosensing to determine that the response arises from the target binding to the probe only, as opposed to interacting non-specifically with the sensor surface. A control experiment was carried out in which the ssDNA probes were first pre-reacted overnight with acetic acid in order to bind all the DBCO-linkers in advance. This was done to prevent them from binding to the azide-functionalised semiconducting polymer through click chemistry and form a sensor with essentially no probes.

The electrical characteristics in Figure 6.17(a) show the transfer curve shifting the opposite direction of that typical for ssDNA immobilisation, suggesting a lack of functionalisation. The negative curve shift and increased hysteresis also perhaps indicates increased disorder/degradation induced by the immobilisation protocol, leading to increased charge trapping and increasing the threshold voltage and hysteresis.

The results in Figure 6.17(b) also appear to confirm a lack of probes on the surface, with little change to the current-voltage characteristics after exposure to the target. This can also be observed clearly in Figure 6.18, where contrary to the typical shift of V_T to positive values, they remain largely negative. Average values of $9.7 \text{ V} \pm 3.6 \text{ V}$, $-17.1 \text{ V} \pm 9.3 \text{ V}$, $-11.8 \text{ V} \pm 3.6 \text{ V}$, $-6.2 \text{ V} \pm 14.7 \text{ V}$ were extracted for the bare device, ssDNA, buffer sample and dsDNA stages respectively. This further validates the observation of a clear positive V_T to confirm the presence of immobilised DNA. However, the relatively minor increase in V_T and the associated variation from buffer stage to the dsDNA stage perhaps implies some degree non-specific binding at the surface modulating the response of some devices. This positive shift of 5.6 V , however, remains much lower than the shift observed in Figure 6.16 of 61 V which suggests the latter originates from a hybridisation event.

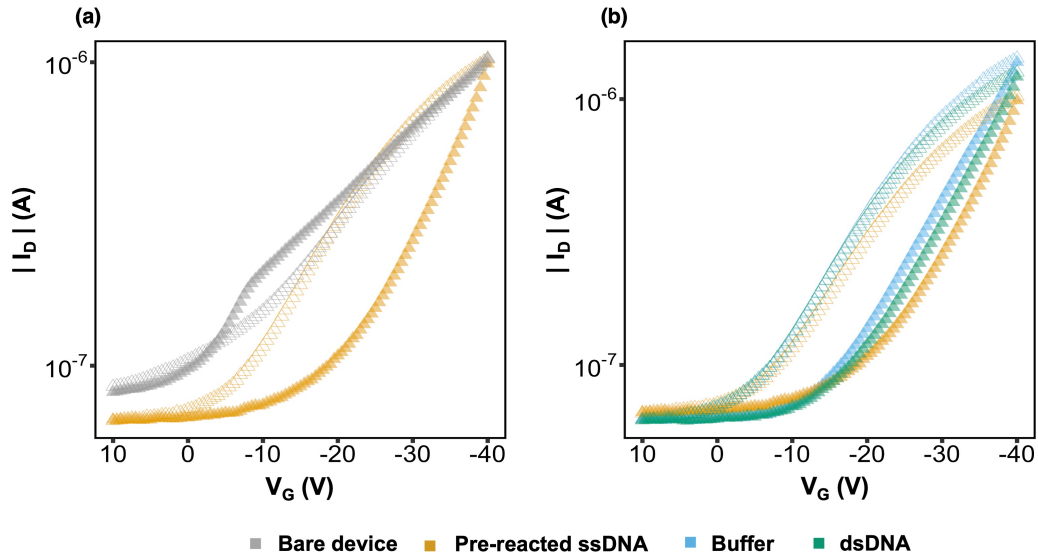


Figure 6.17: (a) Transfer characteristics of OFETs before (grey points) and after immobilisation with 5 μ M pre-reacted DBCO-ssDNA solution (orange points) and (b) the device after exposure to the buffer reference sample (blue points) and after exposure to 100 nM of the target strand solution (green points). $N = 5$ current-voltage sweep replicates for a single device. Filled and empty shapes represent the backwards and forwards sweeps respectively.

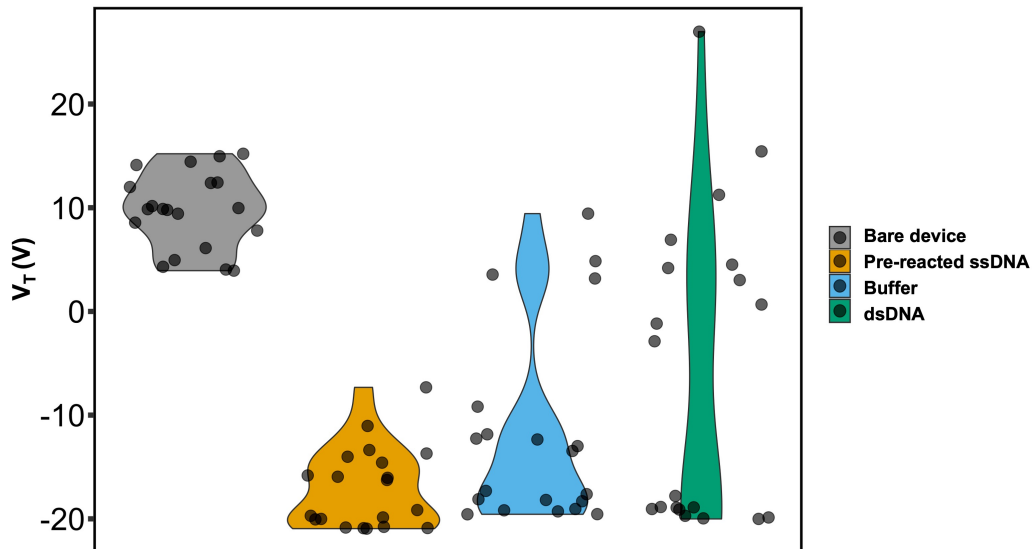


Figure 6.18: Extracted V_T of OFETs before (grey) and after immobilisation with pre-reacted ssDNA (orange), after the buffer reference sample (blue), and after exposure to the target sample (green). $N = 20$ per status.

6.3.5 Response comparison between the target and a mismatch sequence exposed to the sensor

Ultimately, an essential goal of a DNA sensor is to distinguish point mutations in nucleic acid sequences that could mean the difference between healthy and cancerous DNA. The control experiment described here was carried out to determine selectivity of the response by evaluating the electrical characteristics in response to a non-complementary containing 3 base-pair mismatches from the target as described in Section 6.2.1. A second sensor prepared in the same conditions was used for exposure to the target. Figure 6.19 shows the extracted threshold voltage values across the assay steps. Taking into account the shift from the buffer to the target there is a clear positive V_T shift in response to the target of 26.7 ± 23.1 V to 75.7 ± 71.4 V, however this is again associated with a large variation making relative shift comparisons less reliable. For 3 bpm sequence a very slight shift of V_T from 10.5 ± 22.9 V to 21.3 ± 10.8 V is observed. However, the relatively smaller shift of the 3 bpm mismatch sequence compared to results from target binding in Figure 6.16 indicates a degree of selectivity from the sensor.

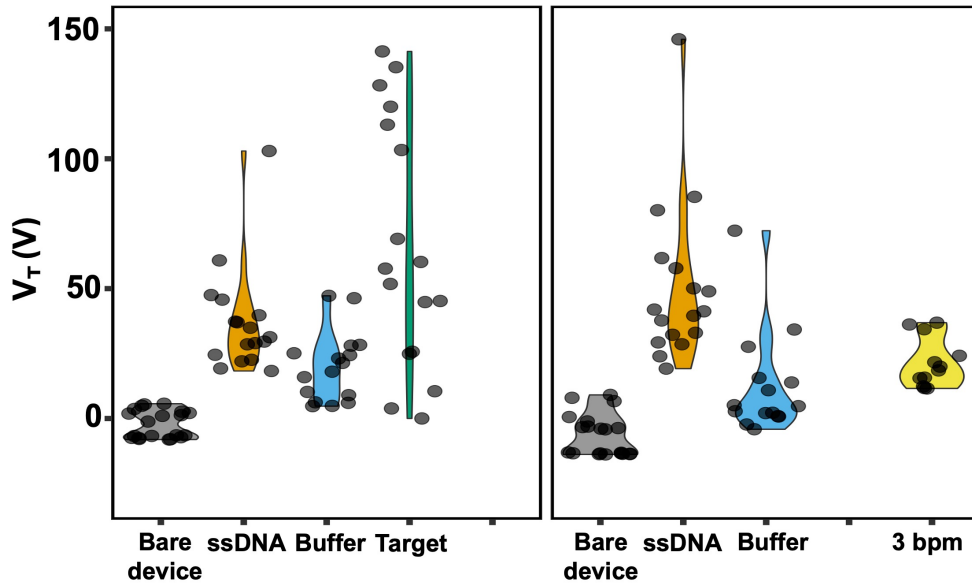


Figure 6.19: Extracted V_T of OFETs before (grey points), after immobilisation with pre-reacted ssDNA (orange), after the buffer reference (blue), and after exposure to the 3 base-pair mismatch sequence (yellow points). $N = 20$ per assay stage.

The probe and target sequences for this system were designed to be 18 base-pairs long to allow for room temperature BioFET operation and DNA solubility and stability simultaneously. However, it was considered that this may cause a trade-off with the selectivity, where the hydrogen-bonding between neighbouring sequences of this length may be too strong enough to perceive a few base-pair mismatches. Intervention may be required to boost selectivity, especially when concerning sequences with fewer mismatches and even stronger interactions between the complementary pairs.

6.3.6 Toehold-mediated strand displacement in solution

A toehold-mediated strand displacement (TMSD) system was designed into the DNA sequences to provide a future option of using a double-stranded (dsDNA) DNA probe to detect a ssDNA target. TMSD is the process by which a ssDNA invader strand binds to an overhang termed a ‘toehold’, in a probe strand in a double-stranded complex and displaces a weaker incumbent strand in a migration process driven by thermodynamics[17]. The motivation behind this design was to increase the selectivity of the system that may be hindered when using probe and target sequences 18 bps long, to ensure room temperature BioFET operation and DNA dissolution and stability. Toehold-exchange systems can be applied to boost the selectivity of the system whilst keeping the DNA sequences sufficiently long[18]. This results section concerns the validation of this TMSD mechanism. Fluorescence was used as a readout, with a fluorophore present on the probe-DNA strand and a quencher present on the weak-DNA strand used to form the double-stranded complex as depicted in Figure 6.20.

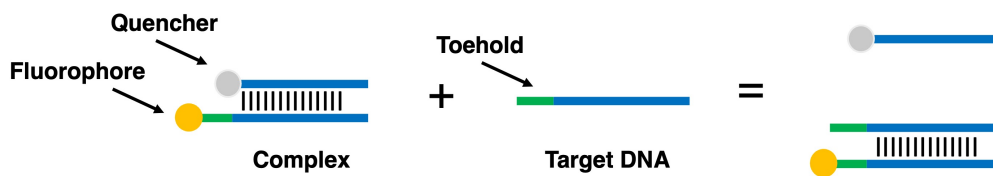


Figure 6.20: Schematic depiction of the toehold-mediated strand displacement mechanism with a fluorophore-quencher system.

In the presence of no target, the fluorophore and quencher remain close in the duplex and no signal should arise. However in the presence of target, the quencher should be displaced with the weak strand, replaced with the target, causing fluorescent signal to appear. Figure 6.21(a) shows that before the addition of any target or mismatch sequences, the positive control well emits the highest intensity (green curve), as expected, whilst the others are relatively low. Upon addition of the sequences, Figure 6.21(b) shows that the target well signal (red curve) increases to almost the positive control signal (green curve), demonstrating successful invasion of the target into the double-stranded complex and the releasing of the quencher. The increased signal of the negative control buffer well (pink curve) above the noise of the other controls is likely a result of interference from the other wells that are exhibiting fluorescence. This suggests that the use of clear-walled plates for this assay can cause some signal contamination between the wells and perhaps could be interfering with the target well, causing an overestimation in signal. This suggestion was corroborated by carrying out the same experiment instead using opaque black-walled well plates as shown in Figure 6.22.

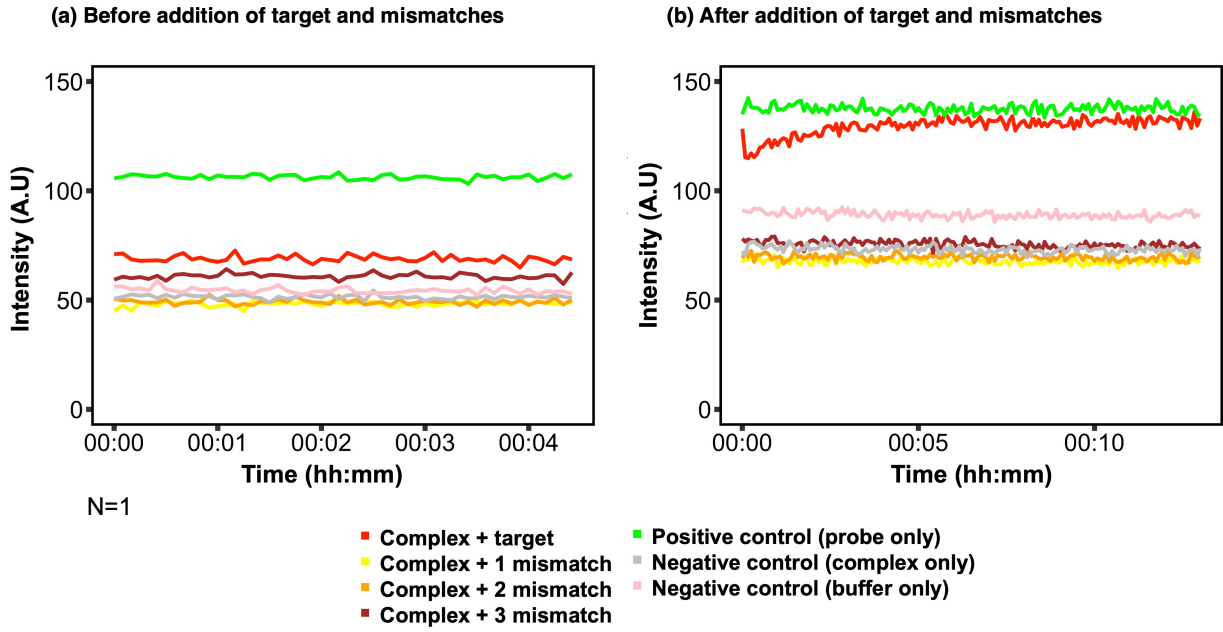


Figure 6.21: Fluorescence intensity measurements of the seven well plates (a) before and (b) after addition of target and mismatch sequences in clear-walled well plates.

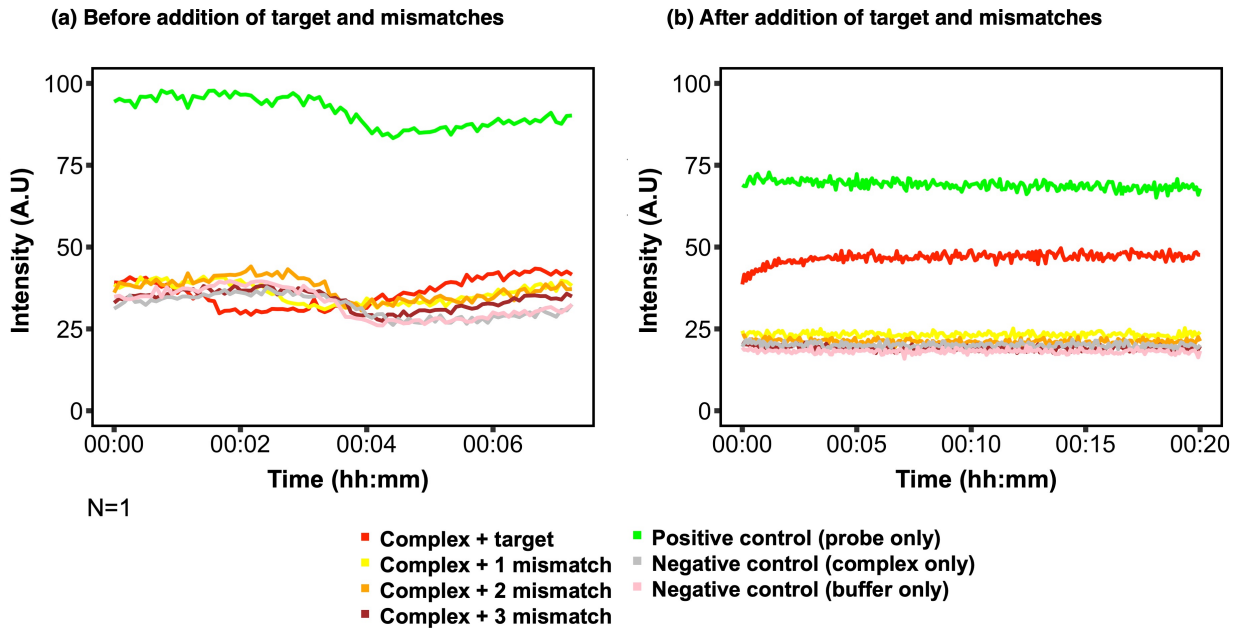


Figure 6.22: Fluorescence intensity measurements of the seven well plates (a) before and (b) after addition of target and mismatch sequences in black-walled well plates.

The data in Figure 6.22(a) demonstrates that before addition of any sequences, only the positive control well (green curve) exhibits a strong signal. Then after addition of the target and mismatches in Figure 6.22(b), the fluorescence signal from only the target well increases (red curve) while the other wells with the mismatch sequences remain low. The lower signal of the target well in this case suggests that this is a more representative

measurement of the fluorescence with the interference from neighbouring wells reduced. The inability of the mismatch sequences to invade the double-stranded complex, displace the quencher on the weak stand cause the fluorophore to fluoresce, demonstrates the selectivity of the system. The results suggest the designed sequences are able to bind in the manner expected, with the probe and target strands able to form a complex through TMSD. Future work is required to explore how this system would translate to the solid-state, with a double-stranded probe bound to a conjugated polymer, however there is a report of a dsDNA probe bound to a solid gold surface in a QCM-D system for TMSD that suggests potential viability[18].

6.4 Conclusions

This chapter reported on a proof-of-concept system for nucleic acid detection with a BioFET platform. The capture probe, target and mismatch sequences were designed using the PIK3CA mutation as a model system which is a prevalent mutation across a range of cancer types, including breast cancer. The electronic detection of ssDNA on selected devices was first demonstrated by immobilising the top half of a chip quadrant with DNA, whilst leaving the bottom unmodified. Significant threshold voltage shifts were observed from the devices functionalised with 10 μM ssDNA, whilst no such shift was observed for the blank devices indicating the selective variation in response induced by local immobilisation.

The electronic detection of 100 nM complementary target ssDNA sequences was validated with the ssDNA-functionalised BioFET. Large positive shifts of the threshold voltage were again observed with no such effect observed in the control devices, however an increase in the variation across the assay steps was observed. This was an expected drawback of using charged nucleic acid probes which intrinsically modulate the electrical activity of the underlying OFET transducer. For this reason, some researchers have opted for peptide-nucleic acid (PNA) probes, which are neutrally charged, synthetic DNA mimics to reduce the electrostatic repulsion between neighbouring DNA strands and minimise the experimental background noise[19] to allow more reliable data comparison.

The utilisation of a high salinity buffer for the reference sample was shown to cause positive threshold voltage shifts from the ssDNA state. The exact origin of this is not clear but suggests biophysical processes altering the surface charge density experienced by the device. Molecular dynamical simulations could be beneficial to determine the best experimental conditions for each assay step. Increasing uncertainty in the extracted threshold voltage values across the assay steps has been demonstrated. As the system becomes more dynamic and disordered from the surface-bound, charged molecules interacting electrostatically and sterically, the device characteristics deviate increasingly from ‘ideal’ behaviour. When this occurs, assumptions used to derive the MOSFET

current-voltage model used to fit the data and extract the response, start to break down and the fits become less accurate. This is a known limitation of the MOSFET equation in organic electronics and this could be improved through the development of better theoretical models which factor in other known mechanisms into the model. However, the lack of suitable model may not be a limiting factor in terms of biosensing, which can also use an empirical interpretation of the device response rather than a mechanistic model. The V_T parameter could be considered for systematic, relative shifts from which we can calibrate the system to a concentration, rather than a ‘real’ physical metric. The queries about the model were also proposed by Zhang *et al.* when evaluating the results from DNA-detecting BioFETs who proposed that nonetheless the V_T device metric remains useful to analyse the sensing response[12].

The effect of the ssDNA immobilisation concentration on the response was first investigated in efforts to optimise the capture probe layer. The greatest threshold voltage shift was observed for the 5 μM chip, with the next greatest for 10 μM and the smallest for 1.25 μM . This indicates electrostatic or steric factors starting to dominate at dense, higher concentrations and simply reduced probe densities at low concentrations. The effect of the target binding efficiency on the ssDNA immobilisation density was also evaluated using the threshold voltage shift parameter and the 5 μM chip exhibited a significantly greater shift relative to the other concentrations, including the 10 μM chip. This provided experimental confirmation of the reports of electrostatic repulsion and steric hindrance becoming increased at higher probe densities which can reduce the binding efficiency.

The non-specific binding in the system was evaluated by exposing pre-reacted ssDNA probes to the sensor surface, essentially passivating all the linkers and stop any attachment of capture probes to the surface. In contrast, a negative shift of the V_T was observed across the assay steps, indicating a lack of negative surface charge accumulation from the ssDNA pre-reacted probe or the ssDNA target sequences, and perhaps experimental degradation as the variation increased across the assay steps.

The response of a 3 bpm sequence was compared to that of the target sequence of the same concentration for an evaluation of the selectivity. A relatively much smaller shift of V_T from the buffer to the 3 bpm sample compared to that observed from the buffer to the target sample indicating fewer hybridisation events. Nucleic acid sequences for the capture probe and target strands were designed with toeholds incorporated, with the future prospect of pivoting to a double-stranded probe and a TMSD mechanism to maximise the selectivity of the system. The binding designed probe and target sequences were verified in solution using a fluorescence assay. The TMSD mechanism was demonstrated through a fluorophore-quencher system on the probe and weak strand and the results demonstrated a high selectivity with the system able to differentiate a 1 bpm from the target.

References

- [1] GLOBOCAN 2020: New Global Cancer Data — UICC, <https://www.uicc.org/news/globocan-2020-new-global-cancer-data> (visited on 10/06/2022).
- [2] S. J. Greco, *Breast Cancer : Targets and Therapy* **2019**, *11*, 111–113.
- [3] L. S. Pessoa, M. Heringer, V. P. Ferrer, *Critical Reviews in Oncology/Hematology* **2020**, *155*, 103109.
- [4] F. Cheng, L. Su, C. Qian, *Oncotarget* **2016**, *7*, 48832–48841.
- [5] F. Yang, J. Tang, Z. Zhao, C. Zhao, Y. Xiang, *Reproductive Biology and Endocrinology* **2021**, *19*, 178.
- [6] M. Elazezy, S. A. Joosse, *Computational and Structural Biotechnology Journal* **2018**, *16*, 370–378.
- [7] R. C. Coombes, K. Page, R. Salari, R. K. Hastings, A. Armstrong, S. Ahmed, S. Ali, S. Cleator, L. Kenny, J. Stebbing, M. Rutherford, H. Sethi, A. Boydell, R. Swenerton, D. Fernandez-Garcia, K. L. T. Gleason, K. Goddard, D. S. Guttery, Z. J. Assaf, H.-T. Wu, P. Natarajan, D. A. Moore, L. Primrose, S. Dashner, A. S. Tin, M. Balcioglu, R. Srinivasan, S. V. Shchegrova, A. Olson, D. Hafez, P. Billings, A. Aleshin, F. Rehman, B. J. Toghill, A. Hills, M. C. Louie, C.-H. J. Lin, B. G. Zimmermann, J. A. Shaw, *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research* **2019**, *25*, 4255–4263.
- [8] O. Martínez-Sáez, N. Chic, T. Pascual, B. Adamo, M. Vidal, B. González-Farré, E. Sanfeliu, F. Schettini, B. Conte, F. Brasó-Maristany, A. Rodríguez, D. Martínez, P. Galván, A. B. Rodríguez, A. Martinez, M. Muñoz, A. Prat, *Breast Cancer Research* **2020**, *22*, 45.
- [9] F. Yan, S. M. Mok, J. Yu, H. L. W. Chan, M. Yang, *Biosensors and Bioelectronics*, Selected Papers from the Tenth World Congress on Biosensors Shanghai, China, May 14–16, 2008 **2009**, *24*, 1241–1245.
- [10] M. Demelas, S. Lai, G. Casula, E. Scavetta, M. Barbaro, A. Bonfiglio, *Sensors and Actuators B: Chemical* **2012**, *171–172*, 198–203.
- [11] S. Lai, M. Demelas, G. Casula, P. Cosseddu, M. Barbaro, A. Bonfiglio, *Advanced Materials* **2013**, *25*, 103–107.

-
- [12] Q. Zhang, V. Subramanian, *Biosensors and Bioelectronics*, Chem and Biosensing Transistors: from materials to systems **2007**, *22*, 3182–3187.
- [13] W. Kaiser, U. Rant, *Journal of the American Chemical Society* **2010**, *132*, 7935–7945.
- [14] N. T. Boileau, O. A. Melville, B. Mirka, R. Cranston, B. H. Lessard, *RSC Advances* **2019**, *9*, 2133–2142.
- [15] A. Jayaraman, C. K. Hall, J. Genzer, *The Journal of Chemical Physics* **2007**, *127*, 144912.
- [16] A. N. Rao, D. W. Grainger, *Biomaterials Science* **2014**, *2*, 436–471.
- [17] T. Mayer, L. Oesinghaus, F. C. Simmel, *Journal of the American Chemical Society* **2022**, DOI 10.1021/jacs.2c11208.
- [18] D. Wang, W. Tang, X. Wu, X. Wang, G. Chen, Q. Chen, N. Li, F. Liu, *Analytical Chemistry* **2012**, *84*, 7008–7014.
- [19] J. Tao, W. Sun, L. Lu, *Biosensors and Bioelectronics* **2022**, *216*, 114667.

Chapter 7

Summary and outlook

This chapter summarises the main conclusions from this thesis and discusses potential next steps for future work and the prospects for the field.

Chapter 3 Organic field-effect transistors

With the aim of having control over device structures and materials to optimise electrical performances, microfabrication of OFETs was carried out in-house, resulting in the successful formation of device arrays. The best performing device yielded a hole mobility μ , threshold voltage V_T and ON/OFF ratio of $4.4 \times 10^{-3} \text{ cm}^2\text{V}^{-1}\text{s}^{-1}$, +1.6 V and 119 respectively. The challenge was low device yields, likely from unsatisfactory dielectric properties, demonstrating that careful engineering of the dielectric-semiconductor interface to minimise defects and interfacial roughness is essential[1]. The inclusion of the PVDF-based fluoropolymer dielectric in the architecture was unable to facilitate the desired low operating voltage, presumably due to the aforementioned dielectric defects or due to it possessing a lower dielectric constant than originally assumed. However, the inclusion of a high- k dielectric material remains a priority to reduce device power consumption and facilitate real-time response measurements without triggering water electrolysis[2]. This also has implications in wearable electronics, for which high voltage operation is not applicable, due to the current limitations of battery capacity and safety concerns[3]. The application of inorganic metal-oxides such as Al_2O_3 could be an effective alternative for low-power OFETs due to their relatively high k values[4]. Al_2O_3 dielectrics can also be prepared by solution-processable methods and have demonstrated excellent uniformity which benefits the morphology of the deposited semiconductor layer and aides charge transport[5].

The performance benefits of applying silane SAM treatments to SiO_2 substrates was demonstrated through reductions in hysteresis and increased drain current I_D . However silane coupling agents are prone to aggregation and can form inhomogeneous

surfaces[6]. This is particularly detrimental for OFETs where inhomogeneous surface morphologies at this interface are critical. The liquid-phase SAM deposition approach was utilised in this project due to the simplicity of the method however it can be difficult to control the amount of water infiltrating the system[7]. Exploration of silane SAM deposition protocols are necessary to find robust methods that form high quality homogeneous layers to reduce the variation within and across device arrays. Solvent-free vapour-based SAM deposition has been proposed as a more controllable alternative for SAM deposition[8].

In terms of the classic OFET figures of merit, devices fabricated via automatic dispensing exhibited inferior performance over spin coated substrates, with a mobility two orders of magnitude lower. Inferior morphological properties of printed layers can negatively affect molecular orientation and charge transport at the critical interface where the conducting channel forms, leading to increase charge trapping[9]. Nonetheless, this method was chosen for the BioFET development because such high-speed automatic systems have the potential to scale, an essential for commercialisation. Improved printing protocols are required to optimise the quality of the deposited thin films and reduce inter-device variations, however there are many parameters to consider. Ink viscosity and ink surface tension and substrate surface energy will effect the ejection and deposition of droplets. To form very precise, homogeneous, patterned arrays is relatively complex, requiring knowledge of fluid flow, solvent evaporation processes and surface tension properties[10]. One strategy adopted to control this process involved using droplets of an antisolvent with the semiconducting ink component to trigger controlled formation of the thin films[11]. To realise fully-printed solution-processed devices would require the careful consideration of all interlayer compatibilities in terms of solvents and processing temperatures[9].

Among the molecular additives that were incorporated into semiconducting inks to improve device performance, overall the 10 wt.% F4TCNQ formulation demonstrated the most stabilising effect in terms of reduced intra-device and inter-device variation in the ON/OFF ratio. However, the inter-chip variation that occurs with printed substrates means this is not yet conclusive. Across all additives, the hysteresis remained at the same level as the control devices, indicating persisting charge trapping behaviour. Further intervention is likely required to curb environmental degradation when devices are fabricated and operated in ambient conditions. One method could be through utilisation of organic semiconductor-insulator ink blends[12]. With an appropriate molecular weight and the right processing conditions, the incorporation of an insulating polymer in the formulation can trigger a vertical phase-separated thin film. This insulating layer can act as a ‘passivation layer’ reducing trap states at the interface and improving air stability[13].

Chapter 4 Surface functionalisation of conjugated polymers

Conjugated polymers were modified with reactive azide handles through a nucleophilic aromatic substitution reaction which was verified through a blue shift in the absorption maximum λ_{max} and NMR. This was used to successfully immobilise PEG, biotin and nucleic acid sequences on conjugated polymer thin films through the SPAAC reaction and a combination of surface analysis and microscopic techniques were used for the validation. Pre-reacted DBCO conjugate molecules were employed to validate that the immobilisation occurs through the selective click reaction rather than non-specific adsorption to the surface. Towards BioFET development, this method has both the advantages of simplicity and controllability, characteristics lacking from the most common strategies used to biofunctionalise organic semiconductors for sensing[14]. Though, reducing the reaction time would increase the efficiency of the functionalisation process considerably, as the DBCO- N_3 reaction occurs on the timescale of hours. Changing the click handles to the trans-cyclooctene-tetrazine (TCO-Tz) system would bring the reaction on a timescale of minutes, however these are relatively larger molecules than azides or normal alkynes which may alter the intrinsic properties of target molecules[15].

The importance of receptor density on target binding efficiency was demonstrated using fluorescence microscopy where too dense biotin probe layers reduced the binding efficiency of fluorophore-tagged streptavidin molecules providing useful information on how probe densities may effect BioFET responses. Additionally, PEG coatings on conjugated polymer thin films suggested potential effectiveness in antifouling applications, by resulting in less non-specific adsorption of fluorophore-tagged streptavidin molecules compared to the control. However, for effective protein resistance chain properties would require optimisation[16]. One approach utilised mixture of longer (5K) and shorter (2K) PEG chains for complete antifouling characteristics against both high molecular weight and low molecular weight proteins[17].

XPS was demonstrated to be a particularly effective system to verify DNA immobilisation on organic semiconductor thin films with the phosphorus elemental scan providing the strongest evidence of nucleic acid groups on the surface. A further step would be to measure the elemental spatial distributions of the surface to construct high resolution images of the surface immobilised DNA and guide optimisation[18]. AFM measurements were considered as another method to validate DNA immobilisation. Images of DNA molecules on substrates have been achieved with a high enough resolution to measure the cross-section[19]; imaging can provide information on orientation and/or damages and the capability for measurements in fluid could provide useful comparisons between the properties of surface-immobilised DNA in the wet and dry state.

Chapter 5 Streptavidin-targeting BioFET

An organic BioFET prototype integrated with biotin receptors was successfully applied to streptavidin sensing. The density of the biotin probe layer was shown to have a substantial effect on the response. Too high densities of biotin probes were shown to severely reduce target binding efficiency and the resulting signal response, in corroboration with the fluorescence microscopy data presented in the previous chapter. This is an effect of steric crowding and consistent with reports[20], and there will be a surface density threshold, above which binding sites become increasingly inaccessible for the target.

The selectivity of the sensing mechanism was assessed by measuring the response from exposure to non-specific BSA molecules. Non-specific interactions were also investigated by measuring the response for a sensor functionalised with PEG in response to the streptavidin target. The relative lack of response measured in both control assays demonstrated the selectivity of the response to streptavidin-biotin complex formation, rather than non-specific adsorption of the target or interfering molecules. The low response of the control assays also indicate low levels of nonspecific binding in the system without significant intervention such as substrate passivation with blocking groups[21].

The effect of the conjugated polymer deposition method on the BioFET sensing response was compared. Device bearing printed organic semiconductor layers resulted in a reduction of the LOD by an order of magnitude compared to spin coated layers. The benefits of organic semiconductor patterning are known to reduce leakage current and cross-talk between OFET devices which could be providing the increased signal of the printed BioFET device[22]. Additionally, the increased response could be an effect of more concentrated target binding on the device areas with patterned probes.

Logistic regression was demonstrated to be a suitable model to fit the obtained dose-response data and LOD values in the fM range were obtained. BioFET sensing would benefit from the development standardised protocols for the response extraction and LOD calculations to help compare reports and guide experimental decisions.

The biotin-streptavidin is a practical system for a biosensor proof-of-concept due to the relative inexpensiveness and wide availability of reagents. However, extensive optimisation of this system was avoided due to the stark differences with the eventual DNA platform and the lack of clinical relevancy.

Chapter 6 ctDNA-targeting BioFET

A PIK3CA mutation model for breast cancer diagnosis was employed in the prototype BioFET system for DNA sensing. Employing the automatic dispensing system, ssDNA probes were deposited to pre-defined areas of the OFET array, leaving control devices for determination of non-specific interactions. Electronic detection of ssDNA was demonstrated through measuring shifts of the threshold voltage in agreement with prior reports. The magnitude of the shift was shown to depend on the immobilised density of ssDNA with the 5 μ M concentration showing the greatest shift from -24.9 V to -18.4 V. Printing the ssDNA probes to pre-defined locations was a step taken towards multiplexing, a requirement for the next generation of biosensors, where multiple analytes can be detected simultaneously[23][24]. This would require controlled immobilisation of probes to specific device areas as demonstrated here.

Electronic detection of 100 nM of the complementary target was demonstrated with positive shifts of the threshold voltage. The magnitude of the target response was shown to depend on the immobilised density of ssDNA, illustrating the phenomenon of too high probe densities reducing target binding efficiency. This effect is more significant than in the streptavidin-biotin system, as nucleic acids are charged, and thus experience electrostatic repulsion in addition to steric hindrance between the probe strands[25]. As mentioned, PEG groups can be immobilised in a controlled manner with the biofunctionalisation method reported here and has been reported as an effective candidate for anti-fouling coatings[26]. Furthermore, co-immobilisation of the ssDNA and PEG groups could allow for a simple, single-step assembly procedure where the surface density of DNA is controlled by the varying levels of PEG molecules and could improve the orientation of DNA chains tethered to surfaces for more efficient hybridisation with the target[27].

Further work would involve pushing the sensitivity of the system to detect lower target concentrations in the clinically relevant range (low pg/mL) and the construction of a calibration curve for LOD calculations. However, variations in the sensor response remains a challenge to control. The optimisation of sensor performances would greatly benefit from molecular dynamic simulations to model the behaviours of DNA immobilised on surfaces in different experimental conditions such as the buffer ionic strength and probe density. One study simulated the distribution of probe DNA molecules on BioFETs and the effect on the signal response to calculate the lower bound of probe coverage for a sufficient signal-to-noise ratio[28]. Another study simulated buffer conditions in BioFET measurements and reported significant variations in the sensitivity depending on the chemical composition[29]. Both studies highlight the need for well-controlled experimental environments to achieve reliable BioFET performances for DNA detection and the need for

more fundamental understanding of biophysical processes in these systems for biosensor development.

Outlook

In summary, this thesis demonstrated a proof-of-concept system for biosensor development utilising a highly modular surface biofunctionalisation strategy. For real-world applications, the critical requirement of high stability under complex biosensing conditions remains an ongoing engineering challenge. There are many sources of variation in the response occurring simultaneously that must eventually be decoupled for effective optimisation. A holistic approach with regards to biosensor development should include factors such as: (i) rational organic semiconductor design to optimise intermolecular structures and packing, (ii) improved deposition protocols for semiconducting polymer inks for better morphology of deposited films (iii) tuned device geometries and interface engineering to maximise device performances, (iv) investigation into immobilisation conditions for effective probe receptor layers, (v) systematic study into sensing mechanisms and (vi) increased standardisation in response analysis protocols. Advancement in these areas to facilitate real-life application in ctDNA detection could disrupt the state-of-the-art of breast cancer diagnostics, by providing the capability of detecting prevalent mutations in a point-of-care setting. This would facilitate easier monitoring of disease status and response to treatment whilst improving the patient experience considerably.

References

- [1] X. Sun, C.-a. Di, Y. Liu, *Journal of Materials Chemistry* **2010**, *20*, 2599–2611.
- [2] S. Lai, M. Barbaro, A. Bonfiglio, *Sensors and Actuators B: Chemical* **2016**, *233*, 314–319.
- [3] X. Ren, Z. Lu, X. Zhang, S. Grigorian, W. Deng, J. Jie, *ACS Materials Letters* **2022**, *4*, Publisher: American Chemical Society, 1531–1546.
- [4] Y. Su, C. Wang, W. Xie, F. Xie, J. Chen, N. Zhao, J. Xu, *ACS Applied Materials & Interfaces* **2011**, *3*, Publisher: American Chemical Society, 4662–4667.
- [5] Q.-J. Sun, T. Li, W. Wu, S. Venkatesh, X.-H. Zhao, Z.-X. Xu, V. A. L. Roy, *ACS Applied Electronic Materials* **2019**, *1*, Publisher: American Chemical Society, 711–717.
- [6] R. M. Diebold, D. R. Clarke, *Langmuir* **2012**, *28*, Publisher: American Chemical Society, 15513–15520.
- [7] M. Singh, N. Kaur, E. Comini, *Journal of Materials Chemistry C* **2020**, *8*, 3938–3955.
- [8] J. K. Ahn, S. J. Oh, H. Park, Y. Song, S. J. Kwon, H.-B. Shin, *AIP Advances* **2020**, *10*, Publisher: American Institute of Physics, 045213.
- [9] D. Kim, S. H. Lee, S. Jeong, J. Moon, *Electrochemical and Solid-State Letters* **2009**, *12*, Publisher: IOP Publishing, H195.
- [10] Y. Diao, L. Shaw, Z. Bao, S. C. B. Mannsfeld, *Energy & Environmental Science* **2014**, *7*, Publisher: Royal Society of Chemistry, 2145–2159.
- [11] H. Minemawari, T. Yamada, H. Matsui, J. Tsutsumi, S. Haas, R. Chiba, R. Kumai, T. Hasegawa, *Nature* **2011**, *475*, Number: 7356 Publisher: Nature Publishing Group, 364–367.
- [12] Y.-J. Kwon, Y. D. Park, W. H. Lee, *Materials* **2016**, *9*, 650.
- [13] Y. Chen, W. Deng, X. Zhang, M. Wang, J. Jie, *Journal of Physics D: Applied Physics* **2021**, *55*, Publisher: IOP Publishing, 053001.
- [14] L. Torsi, M. Magliulo, K. Manoli, G. Palazzo, *Chemical Society Reviews* **2013**, *42*, 8612–8628.

-
- [15] E. Kim, H. Koo, *Chemical Science* **2019**, *10*, 7835–7851.
- [16] S. R. Benhabbour, H. Sheardown, A. Adronov, *Macromolecules* **2008**, *41*, Publisher: American Chemical Society, 4817–4823.
- [17] K. Uchida, Y. Hoshino, A. Tamura, **2007**, *2*, 6.
- [18] N. Graf, T. Gross, T. Wirth, W. Weigel, W. E. S. Unger, *Analytical and Bioanalytical Chemistry* **2009**, *393*, 1907–1912.
- [19] V. Hornáková, J. Příbyl, P. Skládal, *Monatshefte für Chemie - Chemical Monthly* **2016**, *147*, 865–871.
- [20] L. M. Bonanno, L. A. DeLouise, *Langmuir* **2007**, *23*, Publisher: American Chemical Society, 5817–5823.
- [21] C. Suspène, B. Piro, S. Reisberg, M.-C. Pham, H. Toss, M. Berggren, A. Yassar, G. Horowitz, *Journal of Materials Chemistry B* **2013**, *1*, 2090–2097.
- [22] C. Martínez-Domingo, S. Conti, A. de la Escosura-Muñiz, L. Terés, A. Merkoçi, E. Ramon, *Organic Electronics* **2020**, *84*, 105794.
- [23] S. Cheng, S. Hideshima, S. Kuroiwa, T. Nakanishi, T. Osaka, *Sensors and Actuators B: Chemical* **2015**, *212*, 329–334.
- [24] A.-M. Pappa, V. F. Curto, M. Braendlein, X. Strakosas, M. J. Donahue, M. Fiocchi, G. G. Malliaras, R. M. Owens, *Advanced Healthcare Materials* **2016**, *5*, 2295–2302.
- [25] A. W. Peterson, R. J. Heaton, R. M. Georgiadis, *Nucleic Acids Research* **2001**, *29*, 5163–5168.
- [26] C. Boozer, S. Chen, S. Jiang, *Langmuir* **2006**, *22*, 4694–4698.
- [27] Y. Nagasaki, *Polymer Journal* **2011**, *43*, Number: 12 Publisher: Nature Publishing Group, 949–958.
- [28] M. W. Shinwari, M. F. Shinwari, M. J. Deen, P. R. Selvaganapathy, *Sensors and Actuators B: Chemical* **2011**, *160*, 441–447.
- [29] M. W. Shinwari, M. J. Deen, *Sensors and Actuators B: Chemical* **2012**, *171–172*, 463–468.