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**Enhancing Sensitivity in the Analysis of  
Small Biomolecules  
by Surface Plasmon Resonance**

A thesis presented in partial fulfilment of the requirements for the degree of

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**MASSEY  
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## Abstract

Highly potent biological micro-pollutants in the aquatic environment can potentially have detrimental effects on marine and human health, but the development of highly sensitive test methods suitable for use in a field environment remains a challenge.

Surface plasmon resonance (SPR) is an optical-electrical phenomenon, which can be applied to the monitoring of surface binding, allowing the measurement of biomolecular interactions in real time, without the use of radioisotope or fluorescent labeling. The technique has wide utility in the application to biological sensing, including quantitative concentration measurements and the qualitative comparison of binding partners.

The central focus of this study was to investigate quantitative techniques and improve sensitivity using various strategies, including the incorporation of linkers into one of the binding partners and exploiting the signal enhancement properties of secondary antibodies and gold nanoparticles. The use of functionalised terthiophene as an alternative scaffold for immobilising the binding partner was investigated. The effect of attaching the binding partner as a protein conjugate compared to its protein-free counterpart was explored.

Presented here is the use of SPR to investigate an estrone-antibody binding system, which has potential application in the analysis of wastewaters. The binding of a number of estrone derivatives was evaluated, with a view to being able to 'tune' the binding system so that the sensitivity range fell within a range suitable for the application. The use of secondary antibodies and gold nanoparticles to enhance the sensitivity further was also examined in the estrone system. The findings were later applied to the development of a highly sensitive test method for the detection of the shellfish toxin, domoic acid. Finally, investigations into an alternative scaffold to which one binding partner was attached to form the recognition element on the

biosensor surface were carried out with a view to creating a generic scaffold for SPR sensor surfaces.

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## Abbreviations

AFM	atomic force microscopy
AP	alkaline phosphatase
BOC	tertiary butoxide protecting group
brs	broad singlet
BSA	bovine serum albumin
CE	counter electrode
CV	cyclic voltammetry
d	doublet
DCC	<i>N,N'</i> -Dicyclohexylcarbodiimide
DCM	dichloromethane
dil.	dilute
DME	1,2-dimethoxyethane
DMF	dimethylformamide
E1	estrone
E1G	estrone glucuronide
E2	estradiol
EDC	N-ethyl-N-(3-dimethylaminopropyl)-carbodiimide (as the hydrochloride)
EDTA	ethylenediamine tetra-acetic acid
EE2	17 $\alpha$ -ethynylestradiol

EI	electron ionisation
ELISA	enzyme-linked immunosorbent assay
equiv.	equivalents
EtOAc	ethyl acetate
eV	electron volts
FC	flow cell
FCC	flash column chromatography
h	hour(s)
HEPES	4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid
Hex	hexane
HOAc	acetic acid
HRMS	high resolution mass spectrometry
HRP	horse radish peroxidase
IFC	integrated fluidics cartridge
LC-MS	liquid chromatography - mass spectrometry
LOD	limit of detection
m	multiplet
mAb	monoclonal antibody
MALDI	matrix assisted laser desorption ionisation
mg	milligrams
ms	milliseconds

MHz	megahertz
Milli-Q water	ultrapure water filtered through a 0.22 micron filter with a resistivity 18.2 MΩ·cm at 25 °C
min	minute
MIP	molecularly-imprinted polymer
mL	milliliters
mmol	millimoles
MS	mass spectroscopy
MW	molecular weight
nBuLi	n-butyllithium
ND	not detected
<i>N</i> -Glu	<i>N</i> -acetylglutamic acid
NHS	<i>N</i> -hydroxysuccinimide
NMR	nuclear magnetic resonance
NSB	non-specific binding
OEG	oligo(ethylene glycol)
OVA	ovalbumin
pAb	polyclonal antibody
PBS	phosphate buffered saline
PBS/T	phosphate buffered saline with Tween
PEG	poly(ethylene glycol)

PES	phenylether sulphone
q	quartet
RE	reference electrode
rt	room temperature
RU	response unit
SAM	self-assembled monolayer
SD	standard deviation
SEM	scanning electron microscopy
SPR	surface plasmon resonance
t	triplet
TBAP	tetrabutylammonium perchlorate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMB	tetramethyl benzidine
TOF	time-of-flight
UV	ultra-violet
WE	working electrode