LG-APM's for MHC-Peptide Screening

S. Stanley*, I. A. Dodi*#, C.R. Evans*,
S. J. Paston#, R.C. Rees*, C.J. Percival$,
Glen McHale* and M.I Newton*

*School of Biomedical & Natural Sciences, Nottingham Trent University
#Anthony Nolan Research Institute, London
$Earth, Atmospheric & Environmental Sciences, University of Manchester

Email: glen.mchale@ntu.ac.uk

Overview

1. Immune System and Vaccines

2. Layer Guided Acoustic Plate Mode Sensors

3. MHC-Peptide Recognition Element

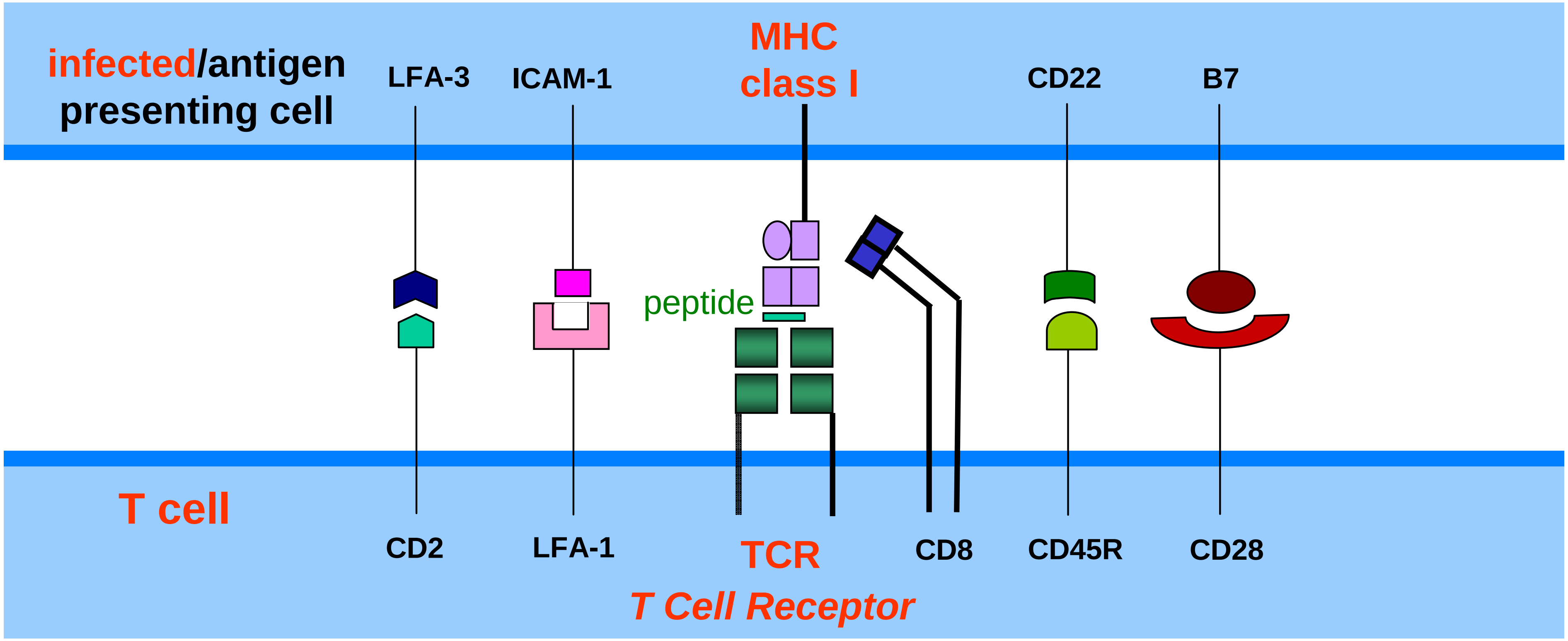
4. Optimising Sensitivity

5. Response to Peptide Binding

Immune System and Vaccines

Peptides and T-Cells

1. Infection/virus broken into peptide fragments and presented on cell surface
2. Cytotoxic T-cells attach to peptides and “read” peptide sequence
3. If foreign, cell is killed by release of a cytotoxic chemical
4. Major histocompatability complex (MHC) antigens are responsible for the expression of peptides on the Infected cell
5. Vaccines introduce peptide to the T-cell – Aim is to find suitable peptides



Peptides and T-Cells

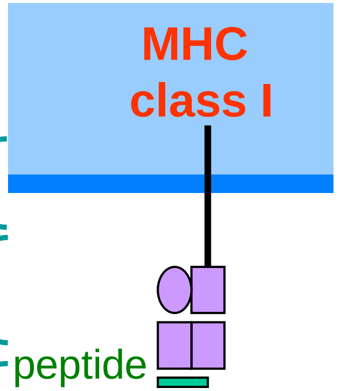
1. Infection/virus broken into peptide fragments and presented on cell surface
2. Cytotoxic T-cells attach to peptides and “read” peptide sequence
3. If foreign, cell is killed by release of a cytotoxic chemical
4. Major histocompatability complex (MHC) antigens are responsible for the expression of peptides on the infected cell
5. Vaccine introduces peptide to the T-cell – Aim is to find suitable peptides

Sensor Strategy

Make this the acoustic wave sensor

Recognition layer is MHC protein

Detect peptide specific binding



Screen for suitable peptides (from the 1000’s that exist) with specificity and strong affinity for the MHC

Current State-of-Art

Cellular peptide-MHC assays

→ yes/no and not real-time

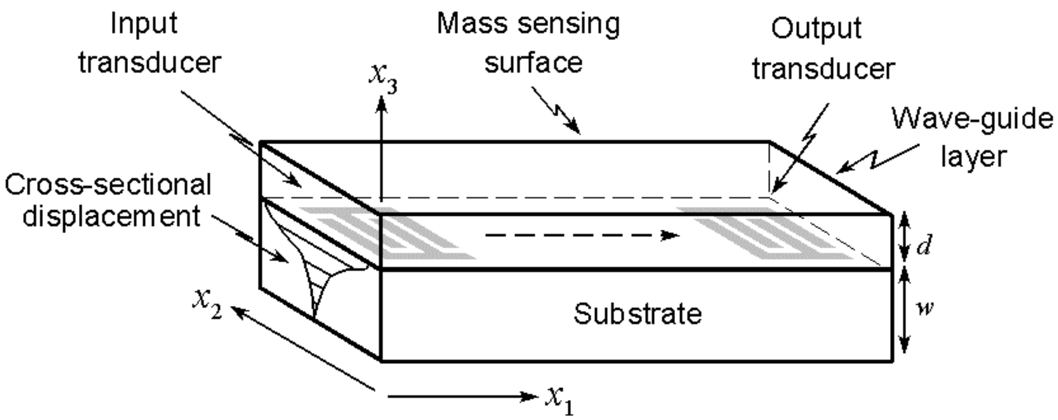
Sensitive, real-time and non-cellular based assay would assist vaccine development

<u>Basic LG-APM Sensor</u>

(*Layer guided acoustic plate modes*)

Love Waves versus SH-APMs

Love Wave

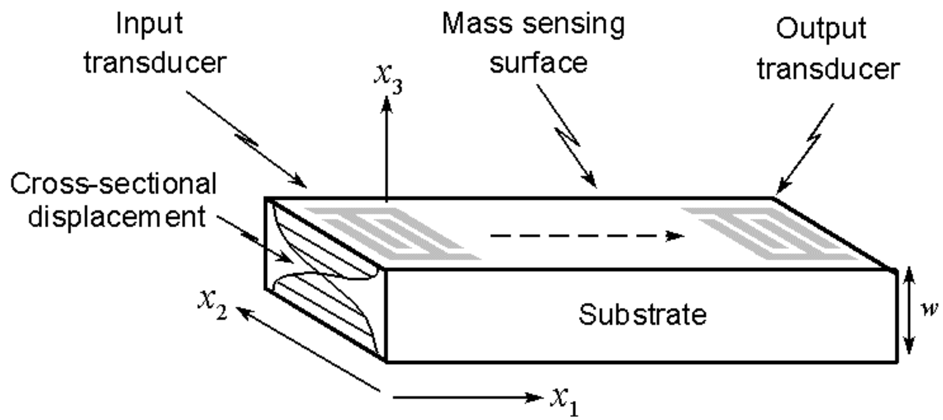


Layer guided SH-SAW with $v_l < v_s$

Surface localised wave

Increased "mass" sensitivity

SH-APM



"QCM with propagation"

Substrate resonance

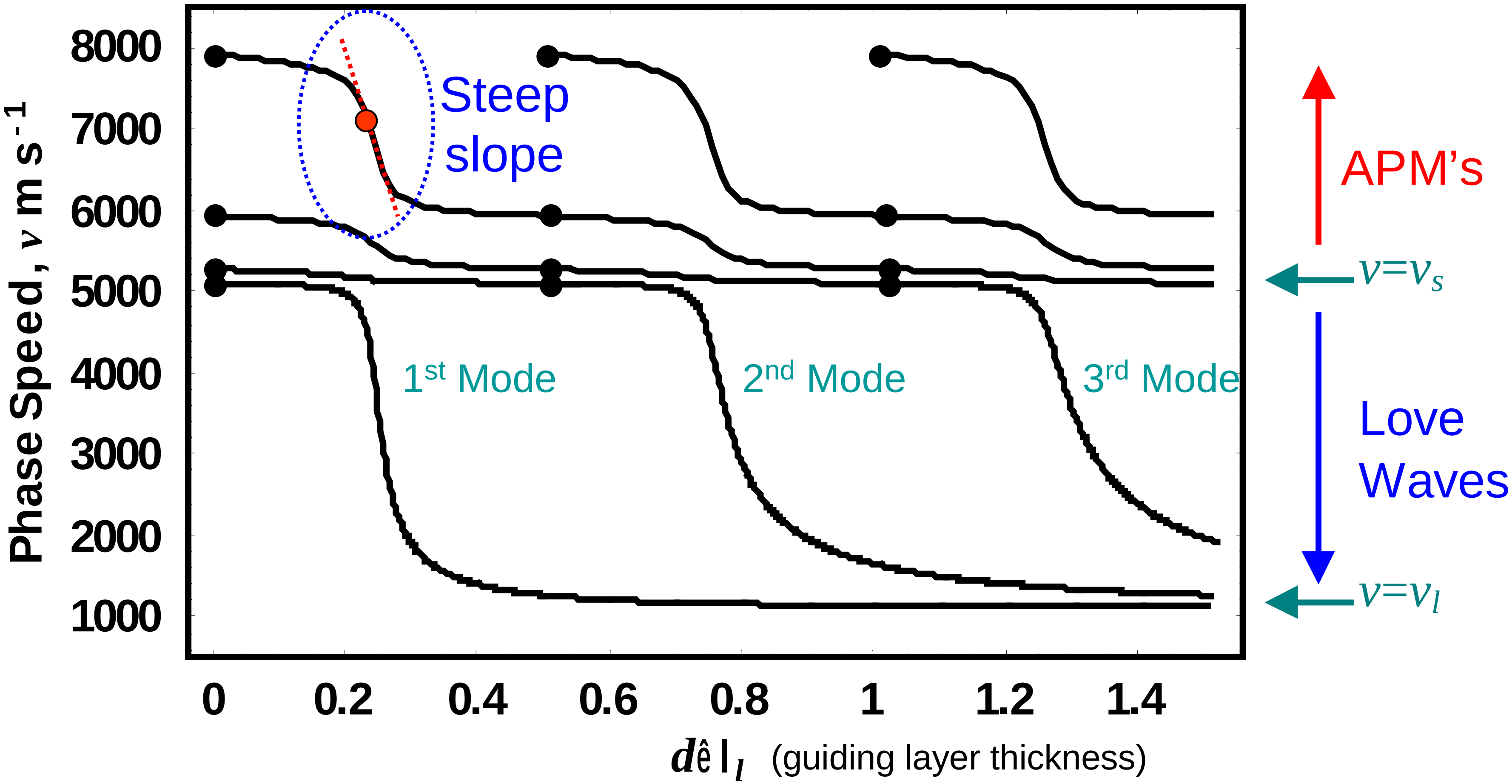
Sensing via both faces

Increased sensitivity versus isolation between sensing face and transduction

Guiding Layer on APM

$\Rightarrow$ ***LG-APM***

Generalized Love Waves - Dispersion Curve



Shear mode in substrate-to-shear mode in layer transition

Increased mass/liquid sensitivity related to slope of dispersion curve

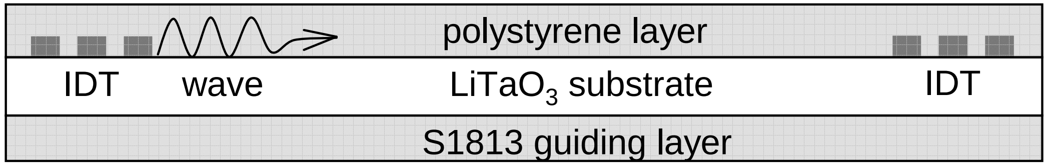
APM guiding layer thickness, d ,fixes operating point and sensitivity

LG-APM Device Sensitivity

Basic Device

1. 36º rotated Y-cut X propagating $LiTaO_3$ of thickness 540 μm
2. IDTs: Double-double, 100 fingers, width/spacing 20 μm, aperture 3mm
3. Cnt-cnt IDT path length 12 mm

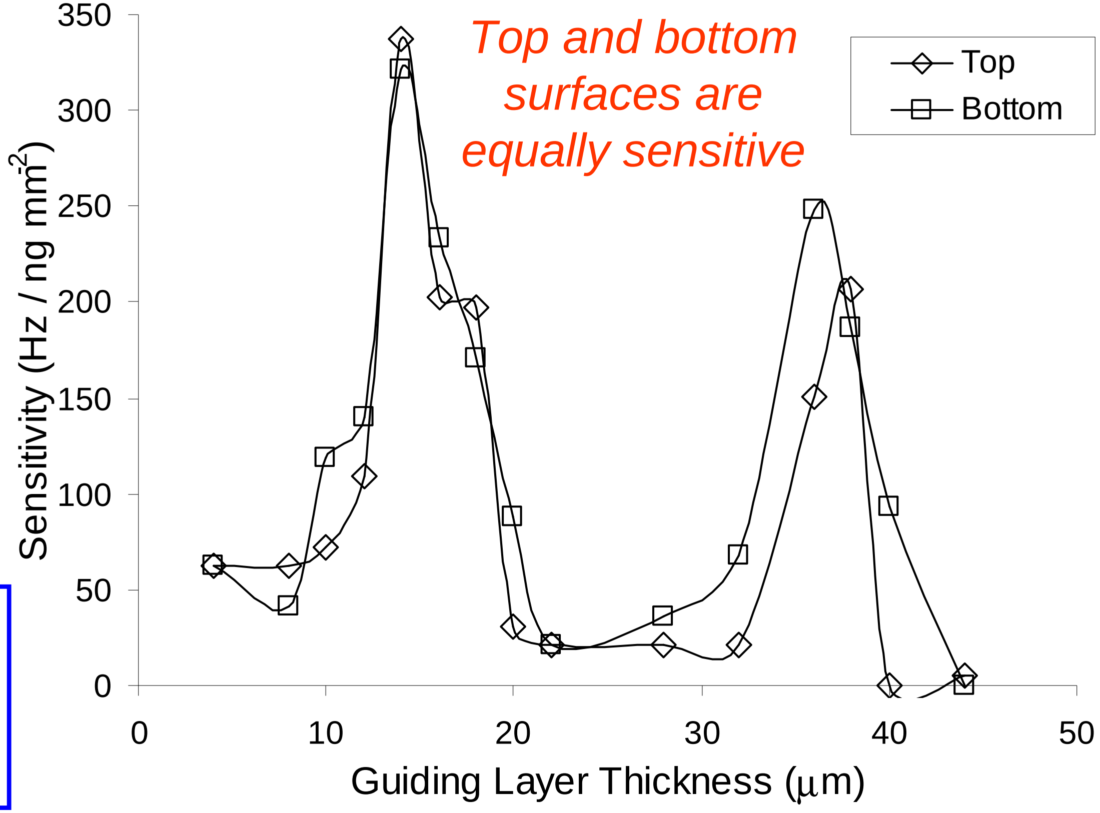
Polystyrene layer to provide coupling for MHC-peptide recognition element



S1813 photoresist layer to optimise sensitivity

Optimising Sensitivity

1. Chose 47 MHz plate mode
2. At each guiding layer thickness use Au coating with thickness from 0 to 400 nm to assess sensitivity
3. Optimum guiding layer thickness was found to be 14 μm



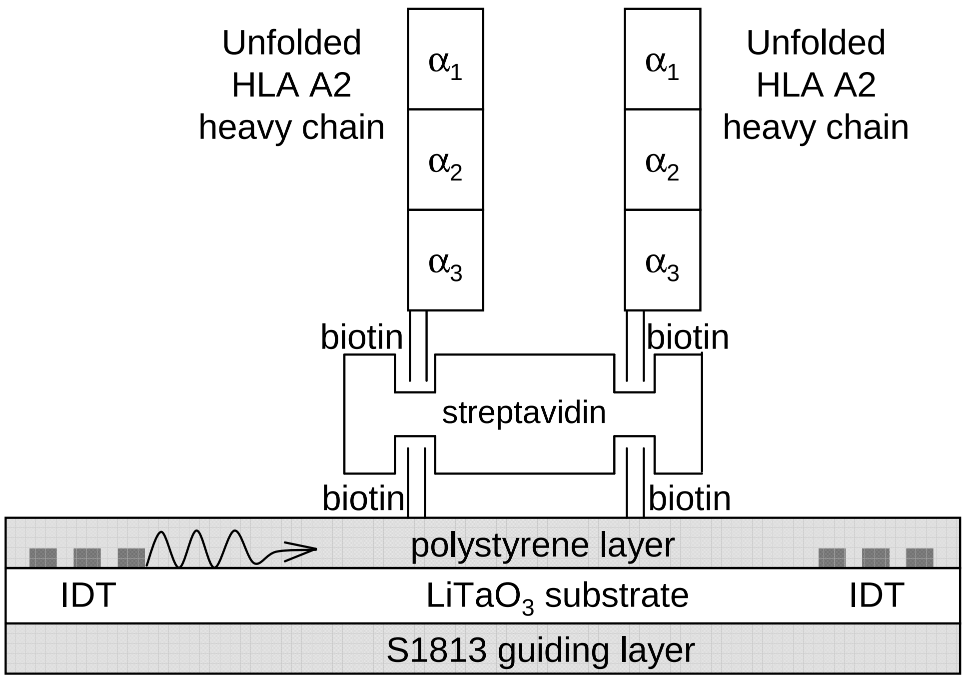
Estimated mass sensitivity for 14 μm S1813 guiding layer is:

321 Hz/(ng mm^{-2})

The Recognition Element

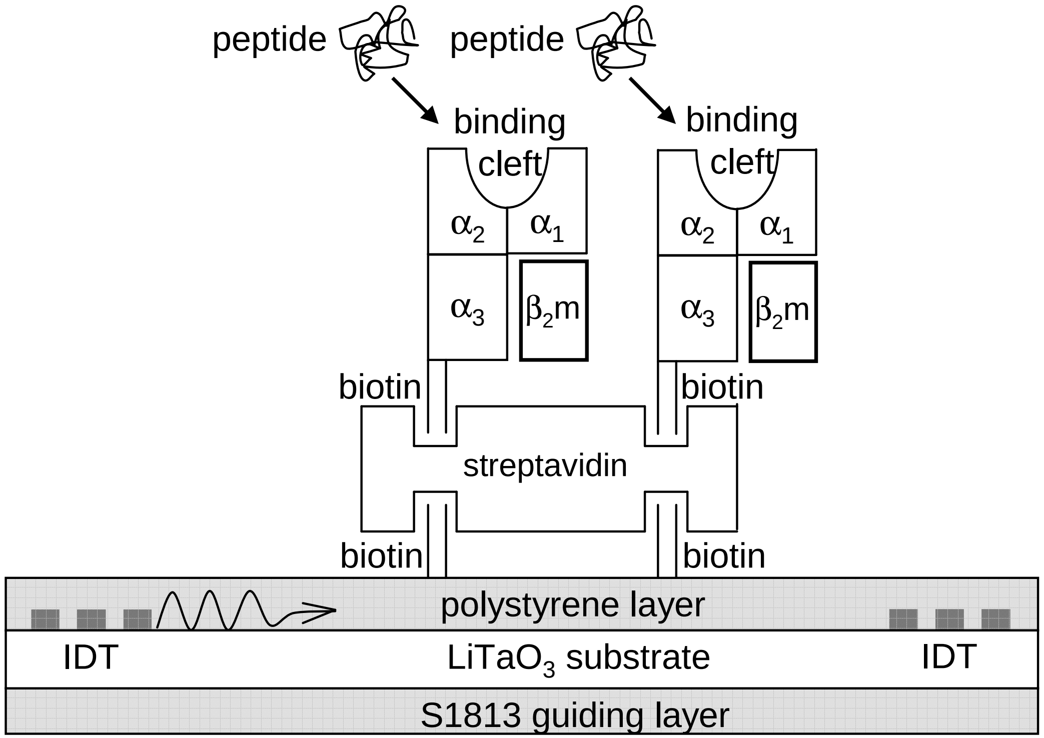
Formation of Recognition Element

Unfolded State



1. 2 µm polystyrene & 14 µm of S1813
2. Ozone exposure of polystyrene; photobiotin acetate in 80:20 water ethanol overnight; UV
3. Flow cell with premixed (2:1 cocktail) of Streptavidin/HLA-A2 heavy chain
4. System is in unfolded state

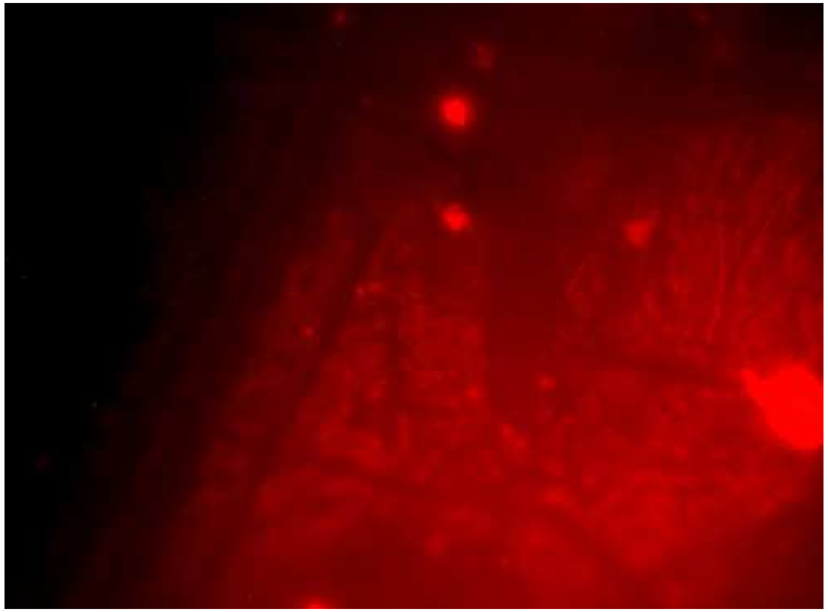
Peptide Binding Cleft



1. β_2-microglobulin introduced via flow cell
2. β_2m binds and causes partial folding of the HLA-A2
3. Forms a peptide specific binding cleft
4. Peptide binding completes final conformation with all components more rigidly bound

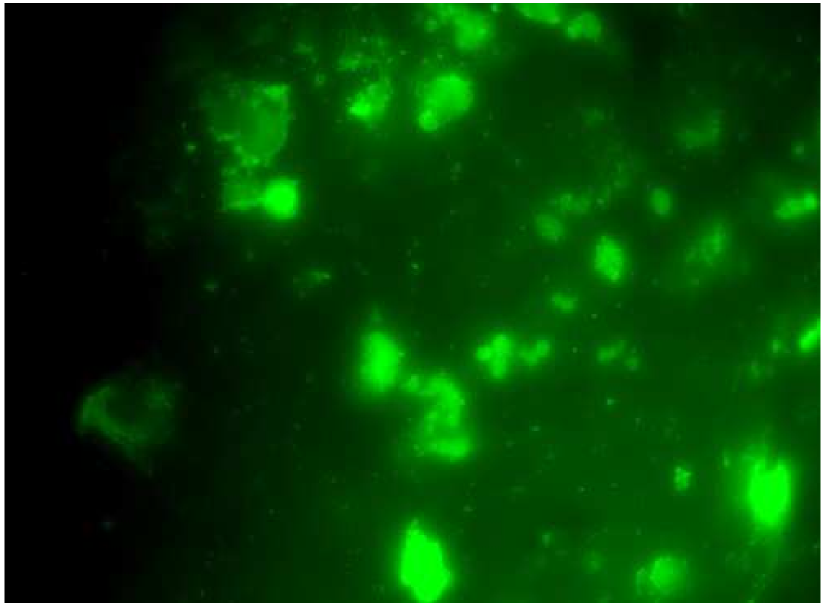
Confocal Fluorescence Microscopy

Biotin-Streptavidin



1. HLA-A2 site on streptavidin replaced by a fluorescent molecule (streptavidin –pe)
2. Streptavidin-pe on photobiotin fluoresced
3. Confirms that streptavidin binds to the immoblised photobiotin

HLA Surface



1. Polystyrene/ photobiotin/ streptavidin/HLA/ and fluorescent marker
2. Confirms that HLA is in place
3. In separate experiments acoustic phase change accompanied biotin deposition

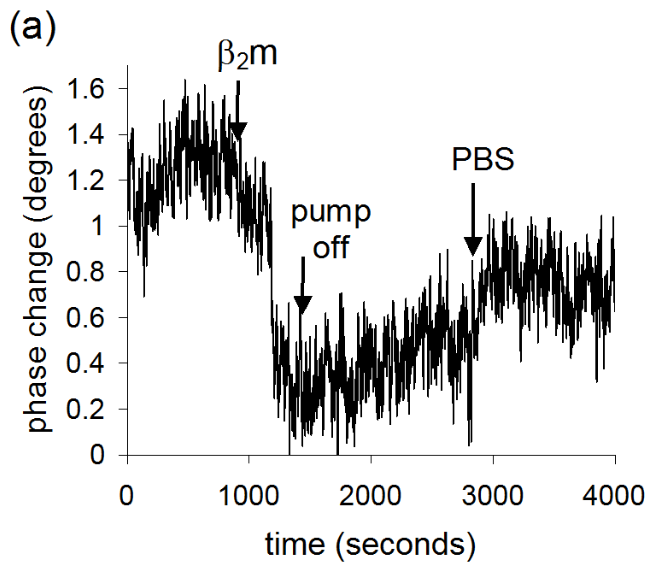
Binding Experiments

Addition of β_2m and Peptide

Experimental Sequence

1. Device prepared with photobiotin
2. Flow cell with network analyzer for phase measurements
3. Streptavidin and HLA-A2 heavy chain introduced, pump paused (30 min), pump restarted with buffer.
4. Introduce β_2m (small protein MW~11.5 kDa)

   $\Rightarrow$ 1^o fall in acoustic phase

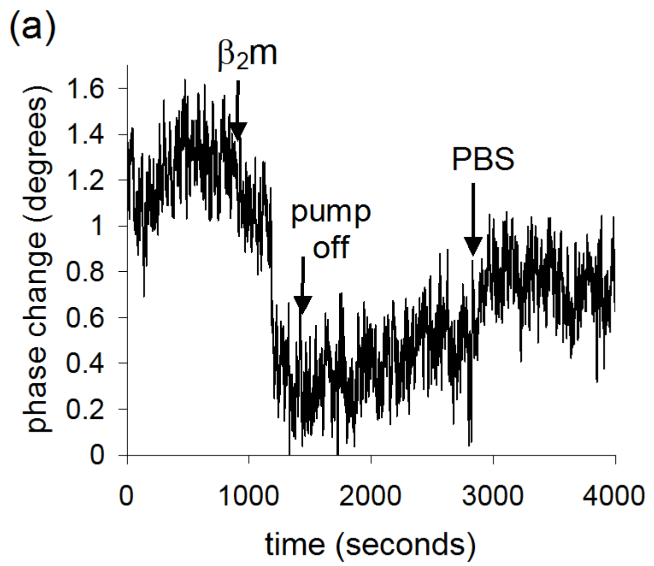


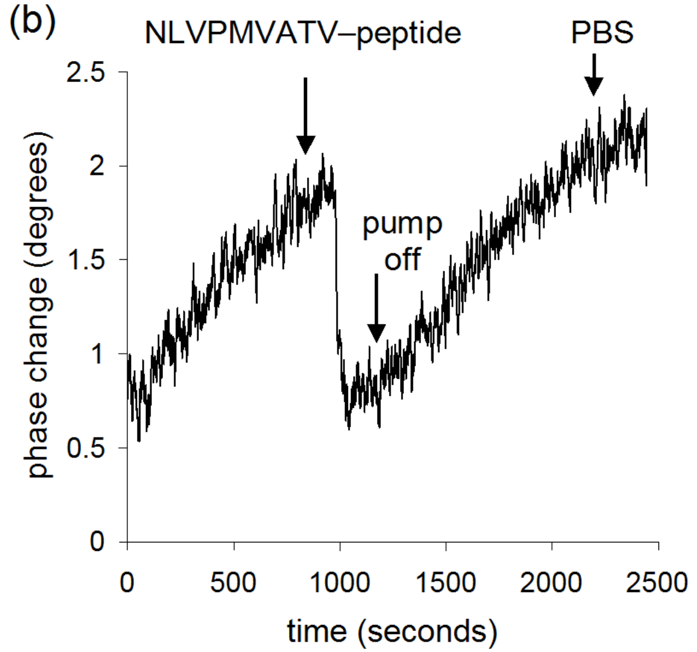
Addition of β_2m and Peptide

Experimental Sequence

1. Device prepared with photobiotin
2. Flow cell with network analyzer for phase measurements
3. Streptavidin and HLA-A2 heavy chain introduced, pump paused (30 min), pump restarted with buffer.
4. Introduce β_2m (small protein MW~11.5 kDa)
   ⇒ 1º fall in acoustic phase
5. Introduce CNV–peptide (very small protein MW~0.95 kDa) linked to immune deficient patients with Leukemia and HIV
   ⇒ 1º fall in acoustic phase
6. Repeated steps 1-5, but using a TPH peptide epitope known to bind only weakly with MHC
   ⇒ no change in acoustic phase

Peptide (class) specific binding is detected





Binding Sensitivity

Mass Sensitivity Estimates

1. Measured sensitivity 321 Hz/(ng mm^{-2}) $\Rightarrow$ phase sensitivity ~ 0.1^{o}/ng mm^{-2}
2. Assume full monolayer of streptavidin
3. $MW_{Streptavidin}$=60 kDa, molecular Xtal with diameter 84 Å $\Rightarrow$ 2.08 ng mm^{-2}
4. MW_{HLA}=45 kDa, average 2 HLA per streptavidin $\Rightarrow$ 3.12 ng mm^{-2}
5. $MW_{\beta 2m}$=11.5 kDa, average 2 HLA per streptavidin $\Rightarrow$ 0.8 ng mm^{-2}
6. $MW_{peptide}$=0.95 kDa, 1 peptide per β_2m $\Rightarrow$ 0.07 ng mm^{-2}

Mass Expectations

Expected mass induced phase change for β_2m is 0.08^{o}

Expected mass induced phase change for peptide is 0.007^{o} (x10 less than β_2m)

Observations

Order of magnitude greater response (~1^{o}) is observed for β_2m

Peptide response (~1^{o}) is similar to that observed for β_2m

Only other known change is conformational folding

Conclusions

1. Layer Guided Acoustic Plate Mode Device

    Higher sensitivity at lower frequencies due to guiding layer

    Separated bio-recognition layer from guiding/sensitivity layer

2. MHC-Peptide Recognition Element

    Proof of principle for acoustic wave approach

    Real-time assessment of protein-protein/ protein-peptide binding

3. Vaccine Screening Potential

    Increased sensitivity possible by higher frequency operation

    Possible parallel operation using an array approach

Acknowledgements
UK EPSRC
Anthony Nolan Trust
NTU

EPSRC
Engineering and Physical Sciences Research Council

NOTTINGHAM TRENT UNIVERSITY