

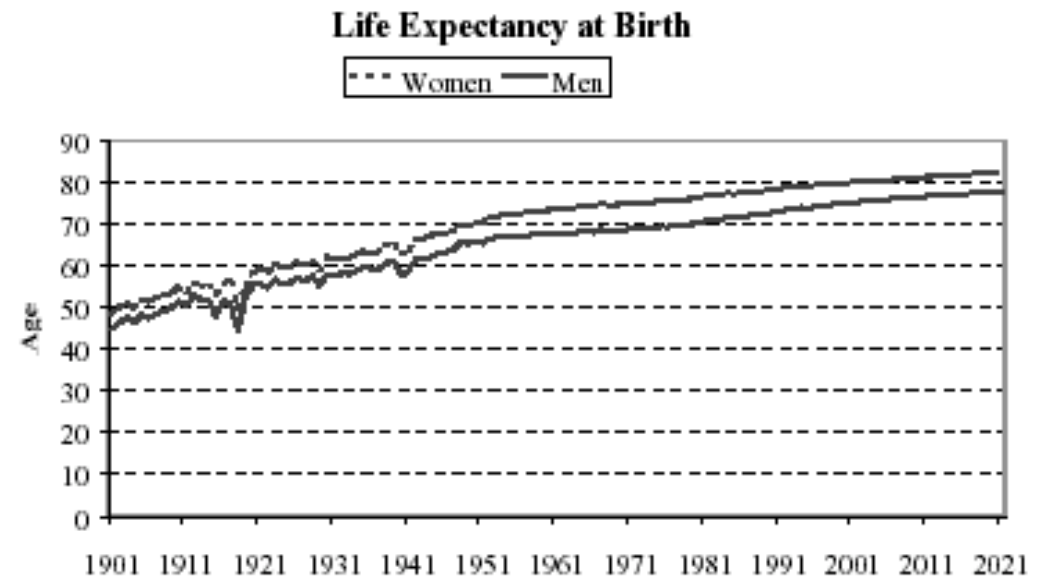
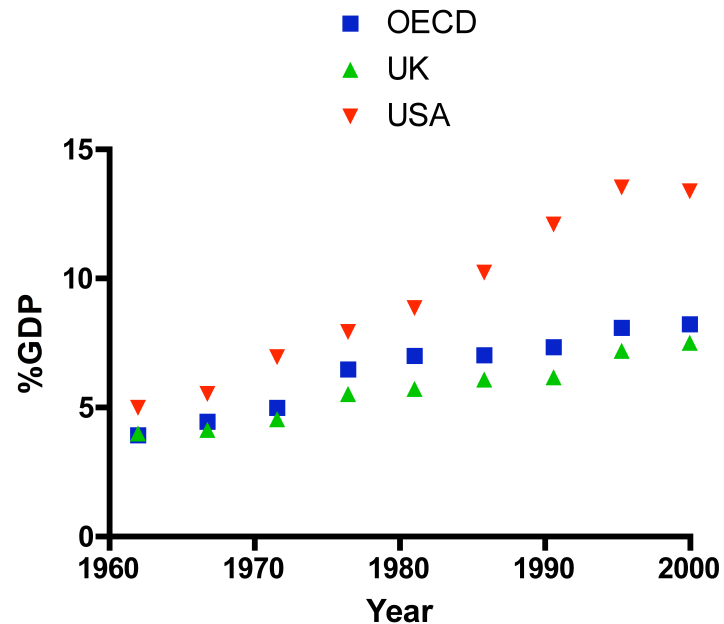
# Molecules to Devices-The Role of Engineering in Next Generation Point of Care Tests

Tony Cass  
Institute of Biomedical Engineering  
Imperial College London

# Outline of Lecture

- The Challenges of Genes and Lifestyles in 21<sup>st</sup> Century Healthcare
- The Role of IVD and PoCT in Healthcare Delivery
- Component Building
  - Aptasensors
  - Microfluidics
  - Nanostructured Surfaces
  - Minimally Invasive Sensing
- Conclusions

# Societal Drivers: Driving Up Healthcare Costs



Victims of Our Own Success

... but it's worse, much worse

Uninformed and Poor Lifestyle Choices



Poor Diet

+



Sedentary  
Lifestyle

=



Obesity ~20% of  
population (UK)

Chronic diseases such as diabetes and heart disease

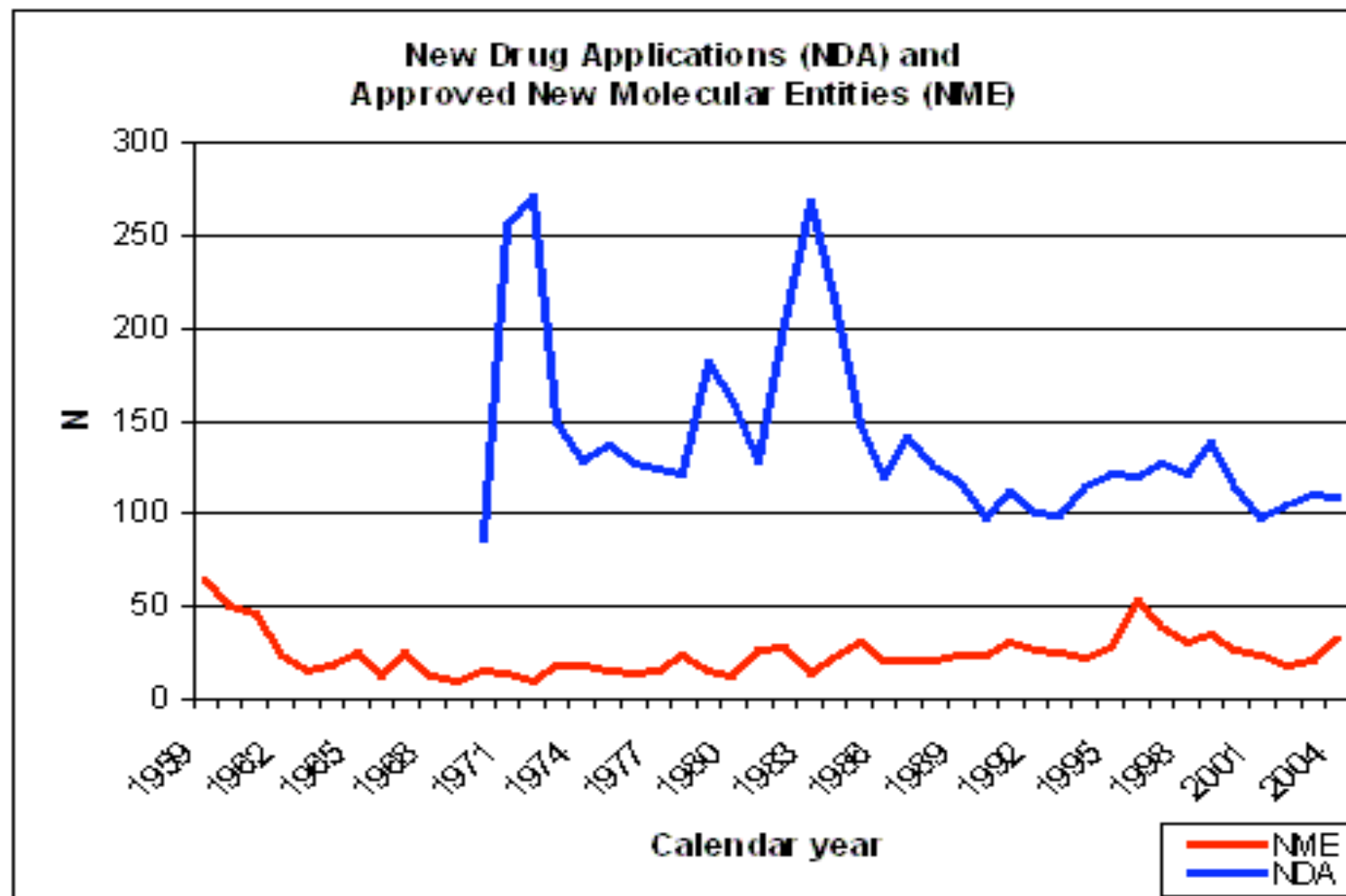


# A Pharmaceutical Solution?

- ✓ Long history of success
- ✓ Global reach
- ✓ Advances in biological research driving innovation



But the Challenges for Therapeutics are substantial



# Biomarkers in Disease Management

Type of Biomarker	Definition
Diagnostic	Differentiates diseased from non-diseased
Burden of Disease	Associated with extent or severity of disease
Prognostic	Predicts onset or progression
Efficacy of intervention	Indicative or predictive of treatment efficacy
Investigative	Not yet meeting criteria for another category

# What About Early (presymptomatic) Detection?

Often neither cost-effective, practical nor ethical for population as a whole (mass screening) unless the test has very high specificity and sensitivity

Look at Risk Factors

Age

Genes

Lifestyle

Self testing as the solution?

Convenience?

Motivation?

Clinical Acceptability?

Interpretation?

# Arguably the Greatest Achievement of Analytical Science in the Past Decade



# Genotyping and Disease Propensities

- The Human genome Project and subsequent developments (HapMap, SNP database) are providing a vast resource for identifying the genetic basis of disease.
- How to use this in delivering improved healthcare?

# Point of Care Genotyping

- Fast, Cheap, focused
  - Small numbers of genes/SNP's
  - Disease/therapy specific
  - Time to results-minutes

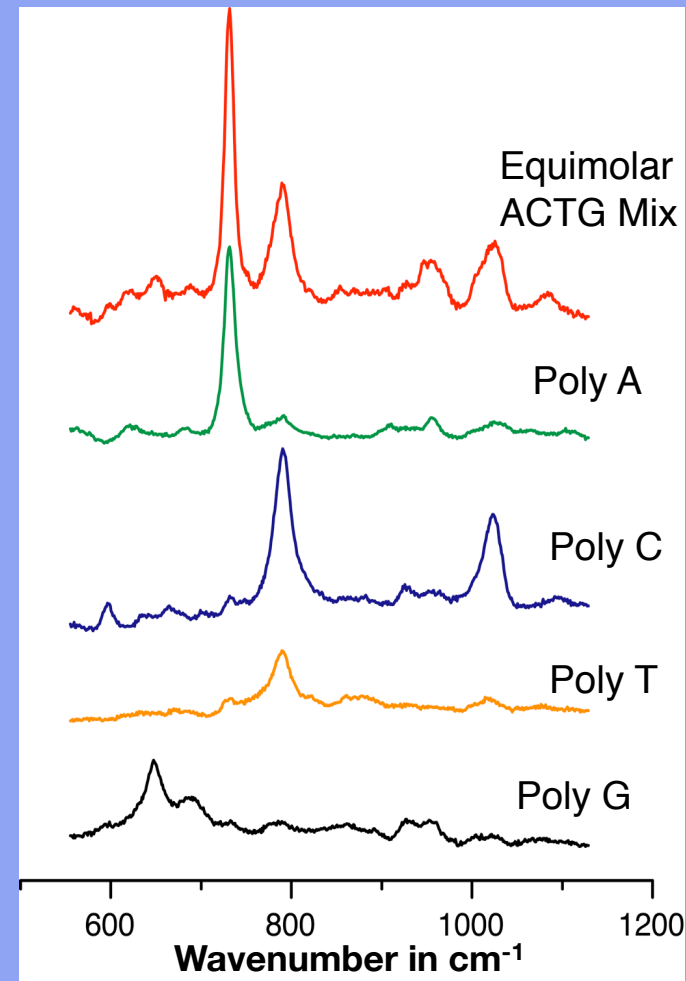
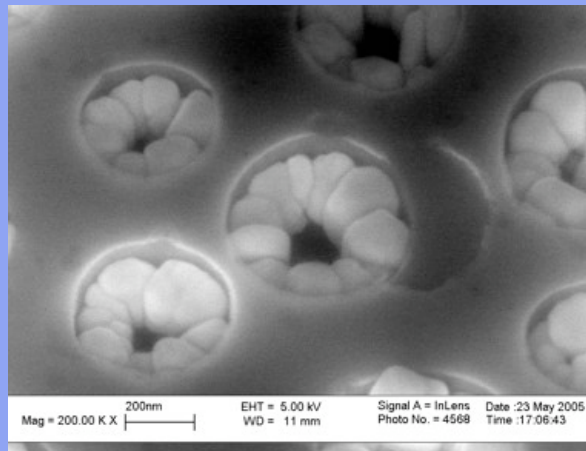
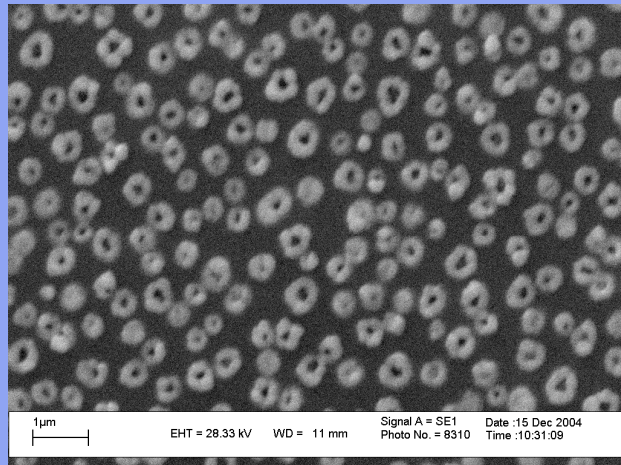


Direct to Consumer Genetic Testing





# Nanoscale properties determine signal generation but device acts as a macroscopic sensor:





# The Point of Care Paradigm

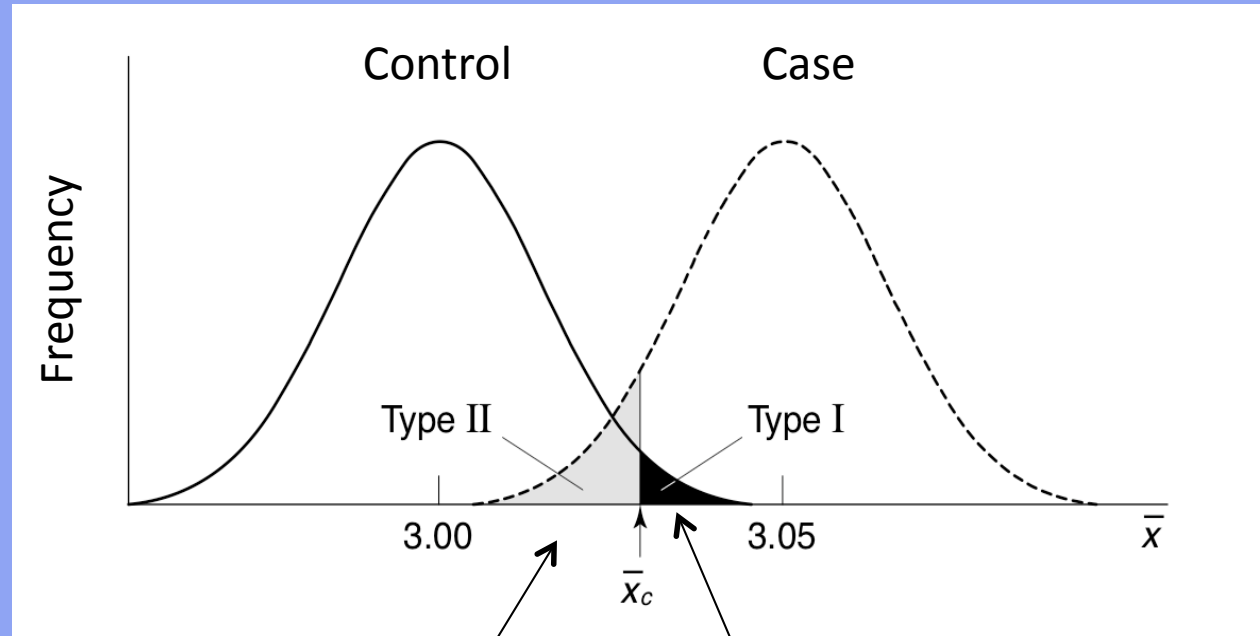
- Take testing from Tertiary Care to primary/pharmacy/home
- Achieve better outcomes through regular testing
- Reduce costs with lower overheads (?)
- Self base-lining: look for change rather than magnitude

Global IVD Market (2008) >\$38bn (USA \$14bn, EU €10bn)

Global PoCT Market (2008) \$6.7bn (USA \$2.4bn)

**BUT** this represents only 1% of total health expenditure

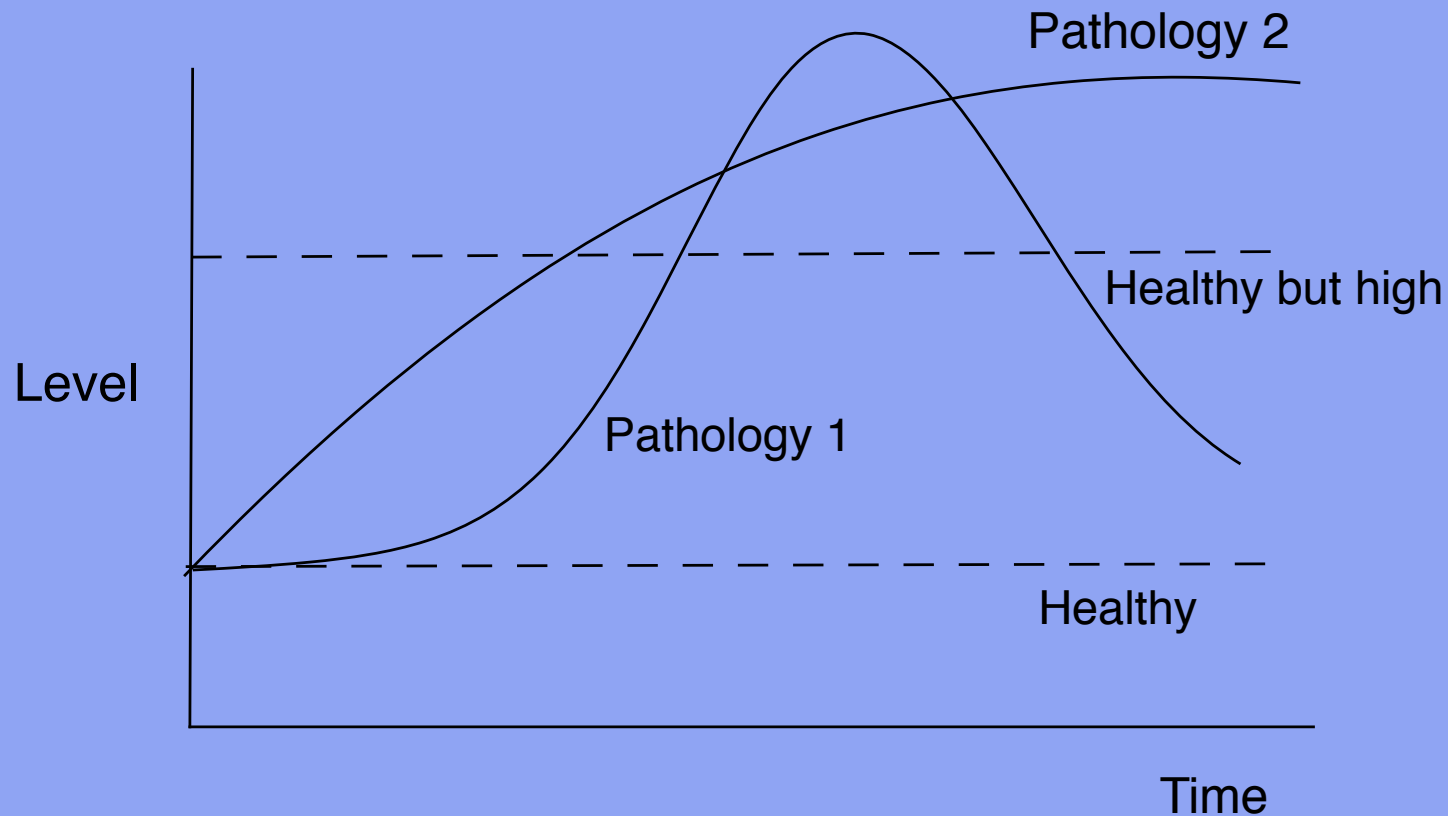
# Populations and Individuals



False Negative

False Positive

# Time Variation in Function and/or Expression of Pathology Related Biomarkers



# Possible Early Application Areas of PoCT

Therapeutic Drug Monitoring & ADR's

Infectious Disease Detection & Progression

Complications in pregnancy (e.g. preeclampsia or obstetric cholestasis)

Effectiveness in treatment of chronic conditions (e.g. osteoarthritis)

Cancer therapy and prognosis

Patient Compliance

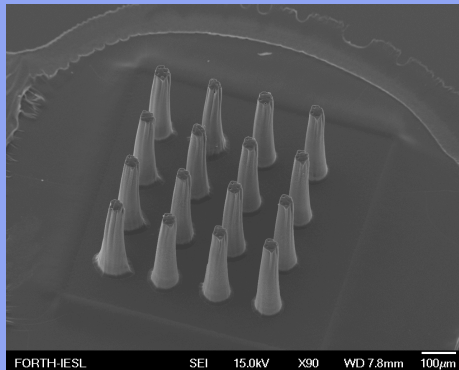
Genotyping

# Technology Needs for Frequent PoCT

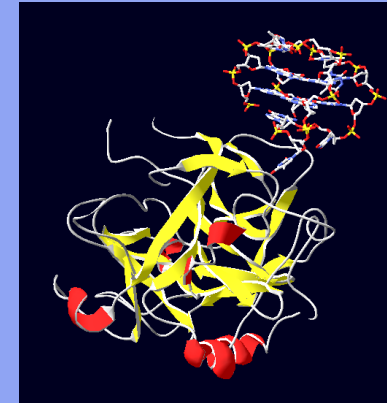
Improved Reagents  
Minimally Invasive Sampling  
Wireless Connectivity  
Decision Support Tools

Samples: Capillary Blood  
Interstitial Fluid  
Urine  
Saliva  
Breath

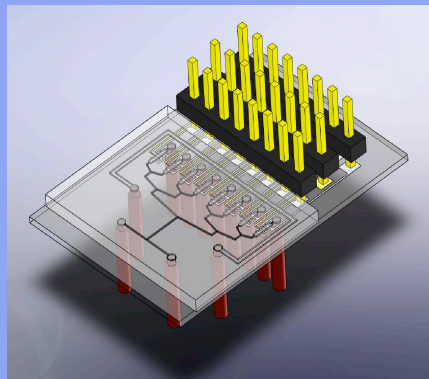
# Component Building



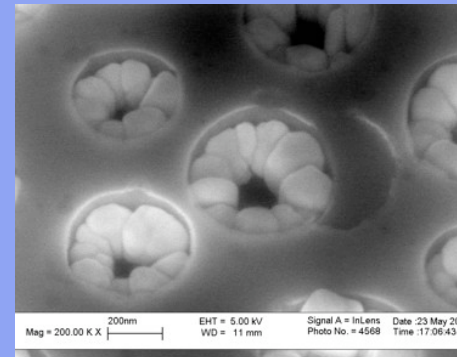
Minimally Invasive Sensing



Aptasensors



Microfluidics



Nanostructured Surfaces

# PoC Reagents: A Wish List\*

Generic Physical and Chemical Properties  
Readily Obtainable  
Traceable  
Stable  
Reproducible  
Controlled affinity and specificity  
Specific chemical modifications  
Can be produced to any target molecule  
Flexible signal transduction schemes  
Small

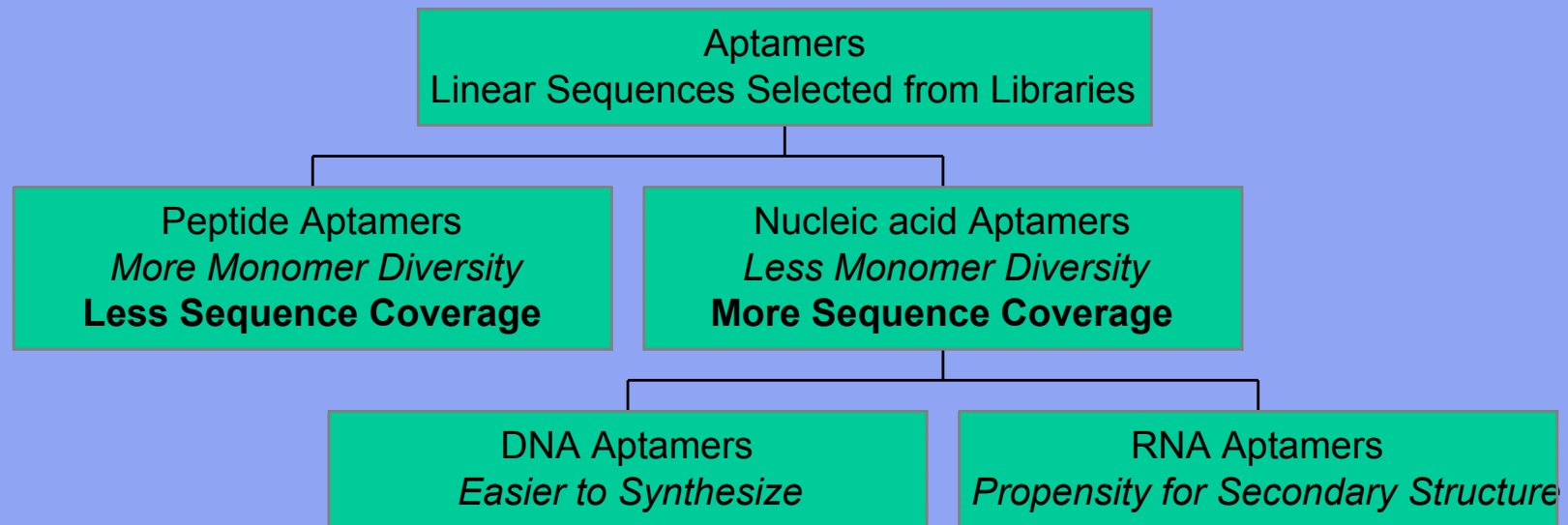
\* Affinity Reagents

# Aptasensors

- Aptamers as molecular recognition elements
- Acquiring aptamers
- Characterizing aptamers-affinity determination by SPR
- From molecular recognition to sensing-electrochemical signal transduction



# Aptamers



In principle (and usually in practise) aptamers can be selected  
*In vitro* against almost any molecular target.

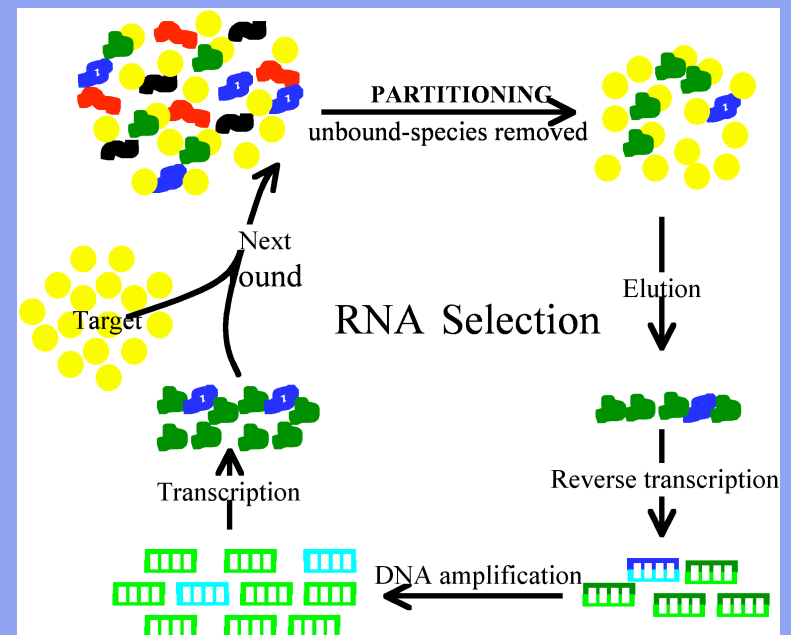
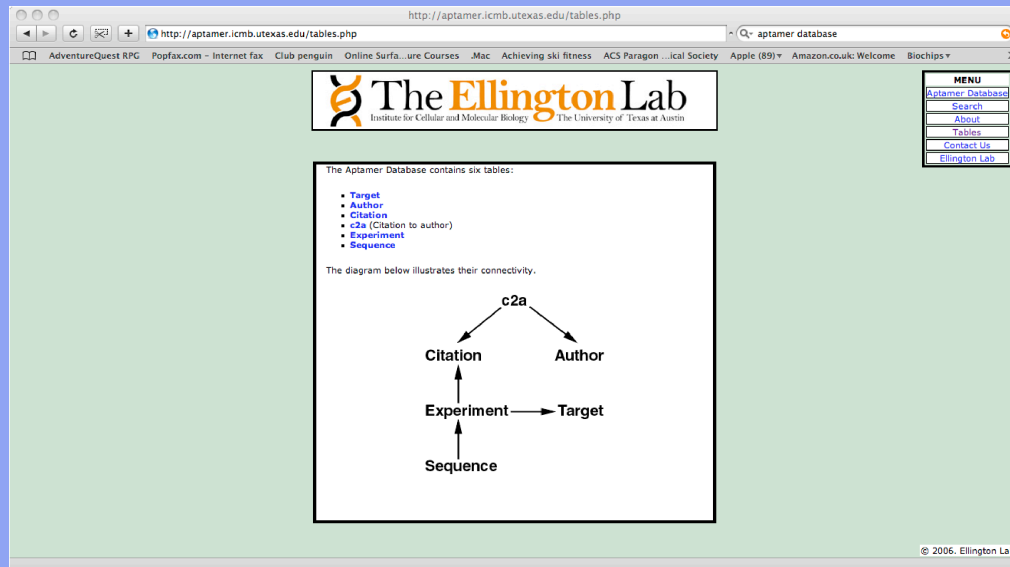
*"You get what you select for"*

# Aptamers-Strengths

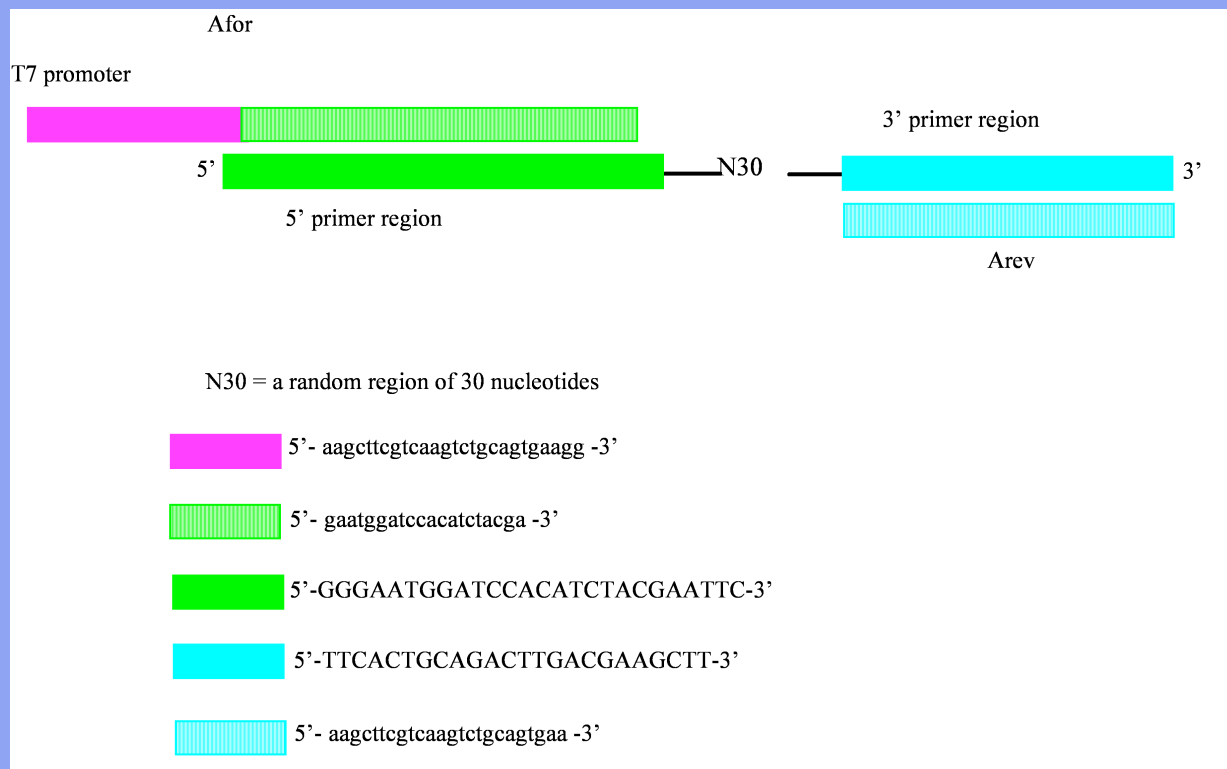
- Well defined at the molecular level
- Available in high quantity and quality via chemical synthesis
- Precision chemical modification
- High stability (with suitable modification)

# Sources of Aptamers

- The literature and the Ellington lab database (<http://aptamer.icmb.utexas.edu/>)
- Selection from libraries



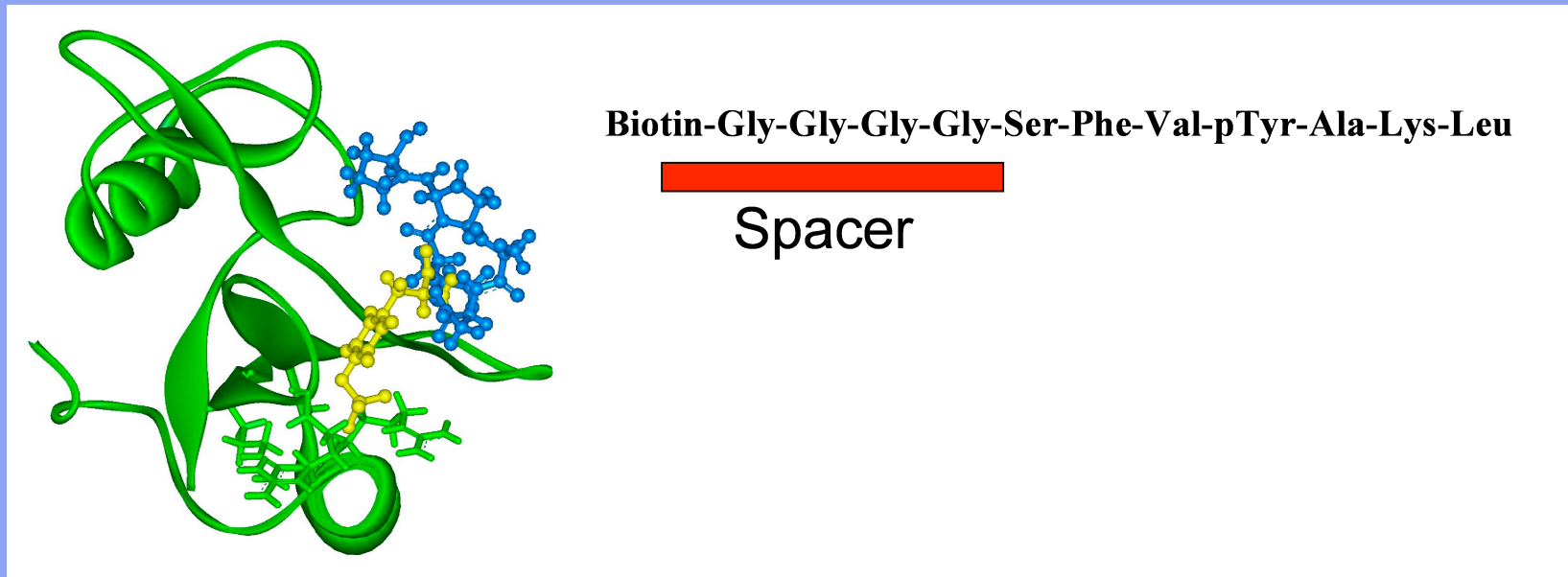
# Library Construction (Ellington Lab)



Pool Size 40µg RNA  
7x10<sup>14</sup> unique sequences  
5-10 Copies per pool

# Phosphotyrosine Peptide Binding Aptamers

A RNA aptamer that mimics SH2 domains

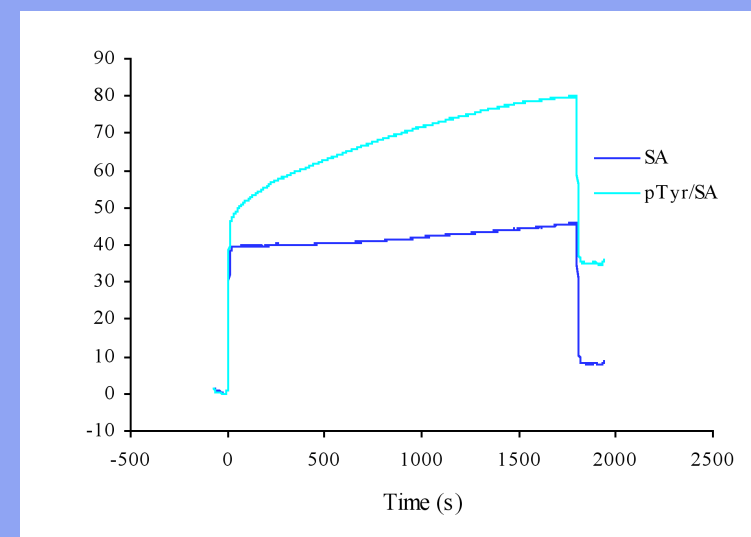


# A pY Peptide Binding Aptamer

## Round 32

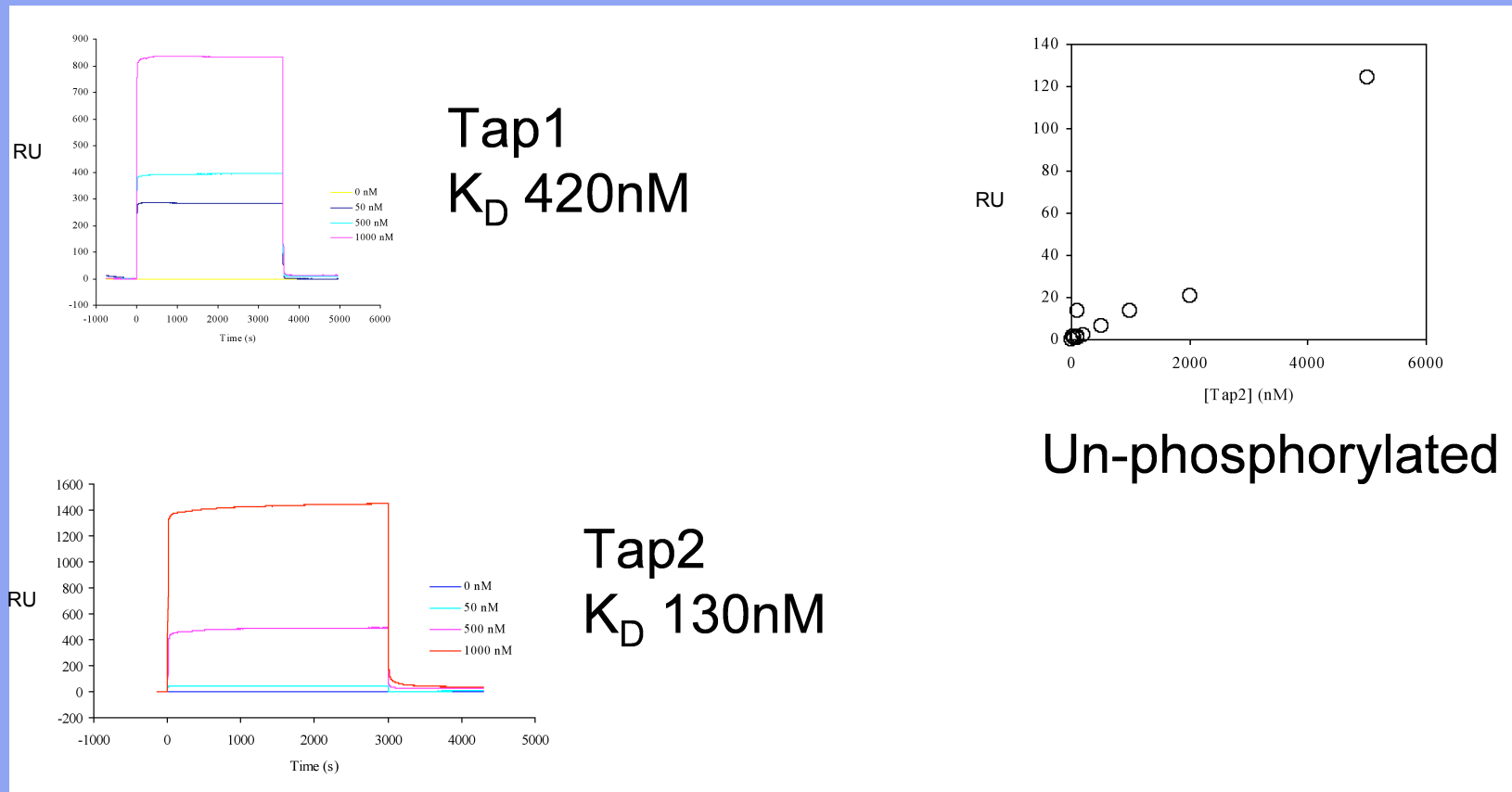
Sequences of 30 randomly selected clones

Aptamer		Clone
Tap1	Atgtggaaagctccgaacagcctctatgaa	1 (10)
Tap2	Cgtgtgggtgccatattcaattgattggaa	4 (10)
	Aatgtggaattgtcaatctcttgtga	17 (2)
	Atgtgggaagctcatcgttttcgtactg	22 (2)
	Tggacaagctttcagtcacaggtcataccg	2
	Atcatgtgtaagcttttaactcctgctca	6
	Aagggggaattgcctcgtcttgcca	9
	Ttgtgggggtttcgatcacgtgctgcggg	10
	Atgtggaaatgcttaactgctgctgctata	13
	Tgcagtacccagtgggtccttagataaggg	23

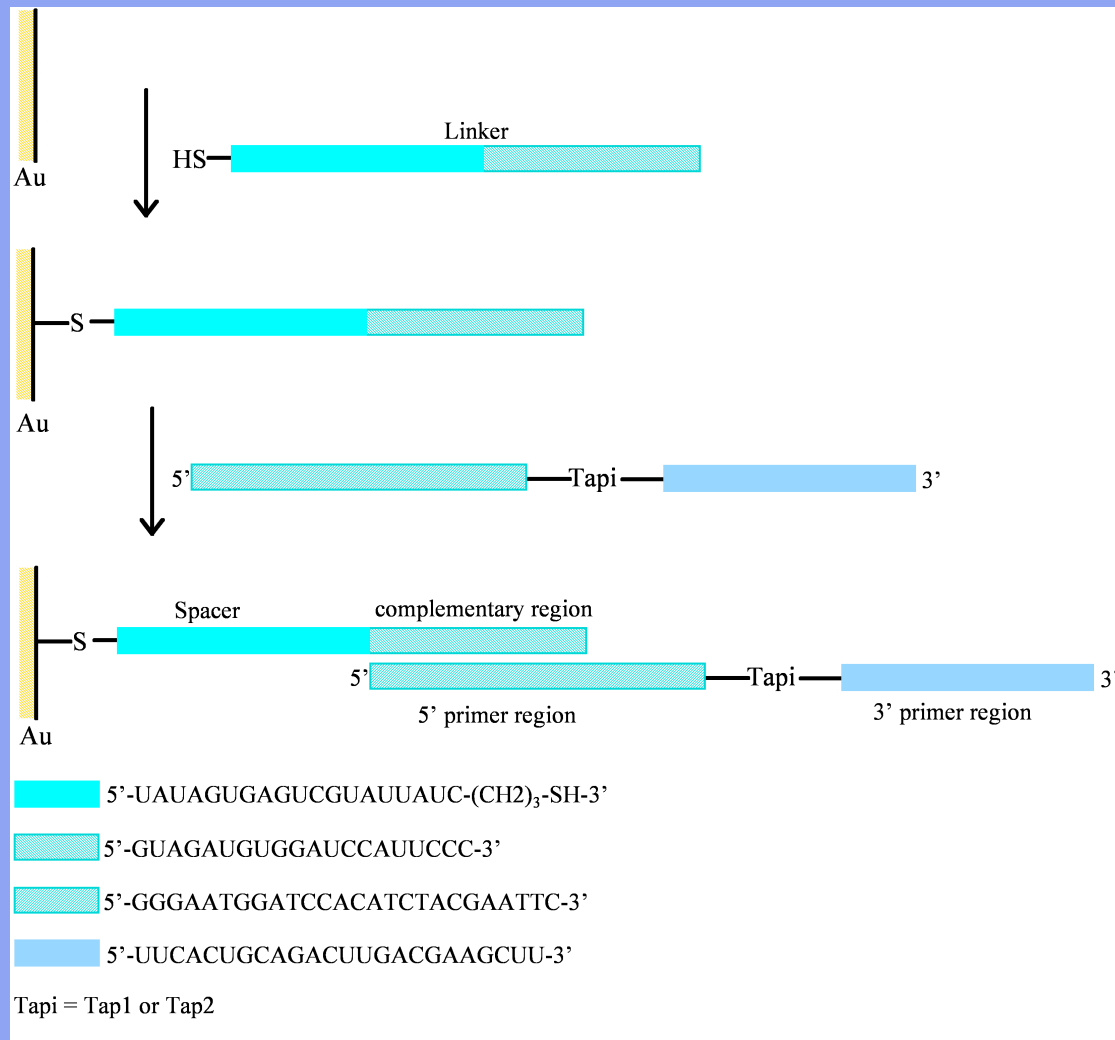


SPR data

# SPR Binding Data for Tap1 and Tap2

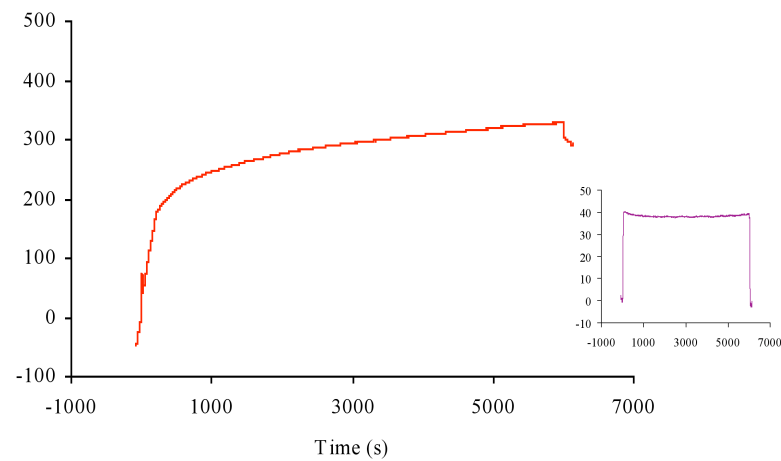


# Inverse Binding Data-Immobilisation Strategy

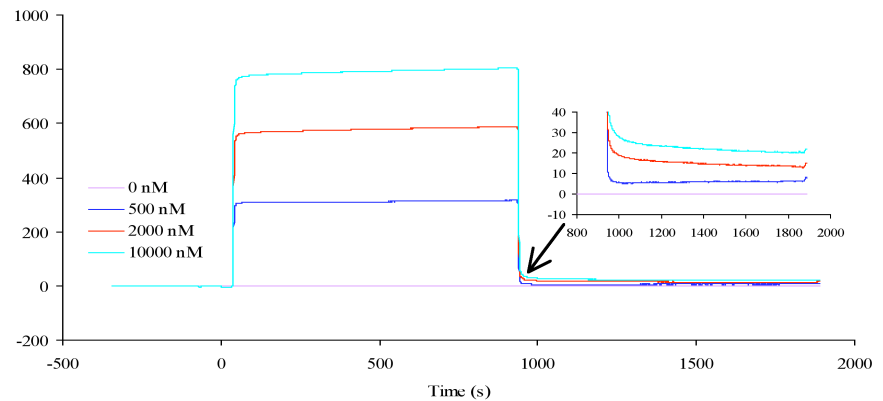




# SPR Data

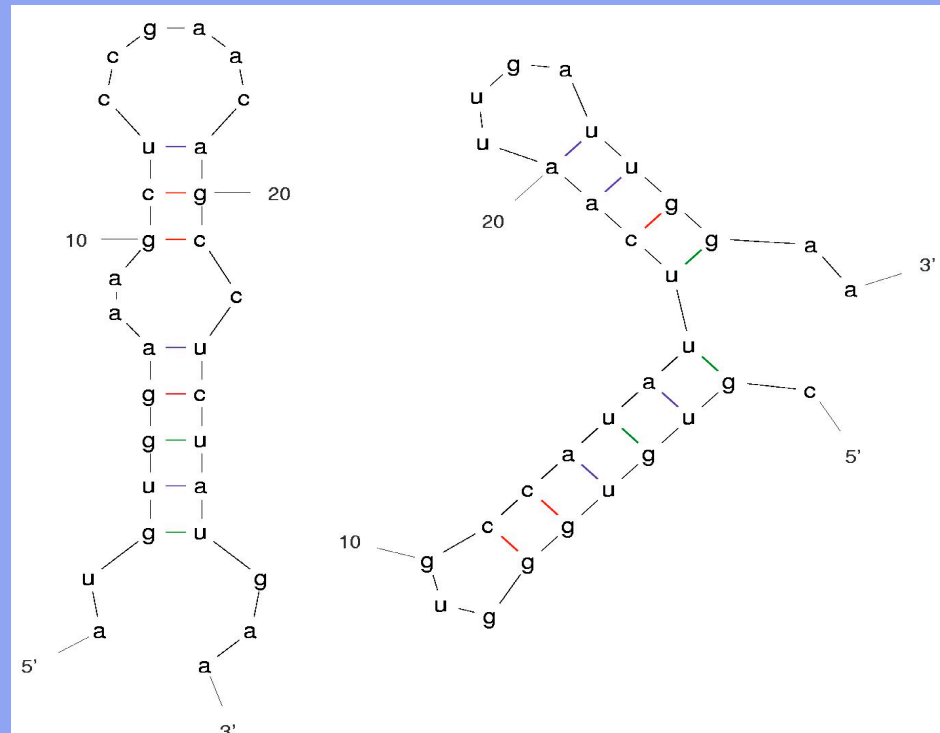


TaL Immobilisation



pY Peptide binding to Tap1  
 $K_D$  700nM

# Mfold Predictions



Tap1

Tap2

# Lysozyme Diagnostics

Non-specific antibacterial protein

Serum, urine and saliva levels in the  $\mu\text{M}$  to nM range

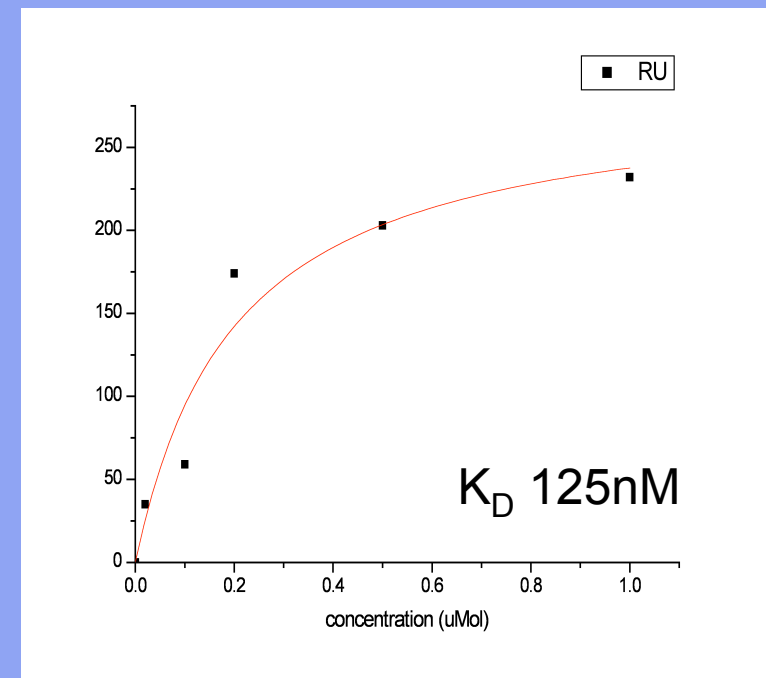
Maybe useful in the diagnosis of TB and HIV

Elevated urine levels in kidney disease and leukemia

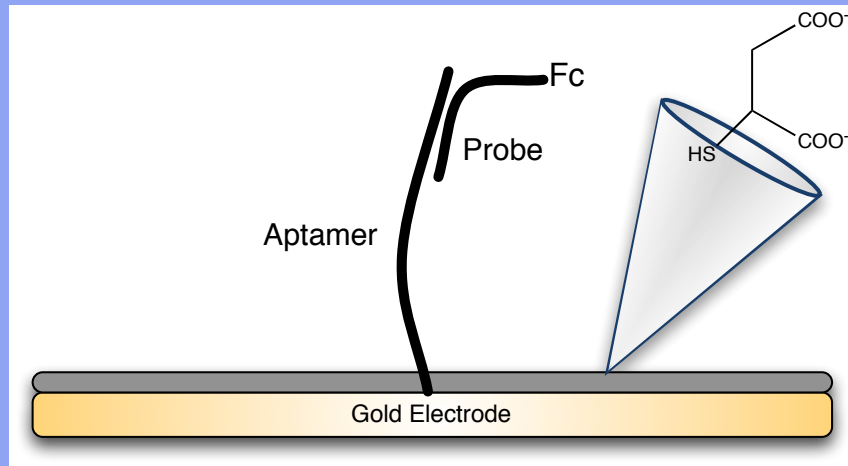
A DNA aptamer that binds lysozyme

Originally selected by Ellington group as an RNA aptamer.

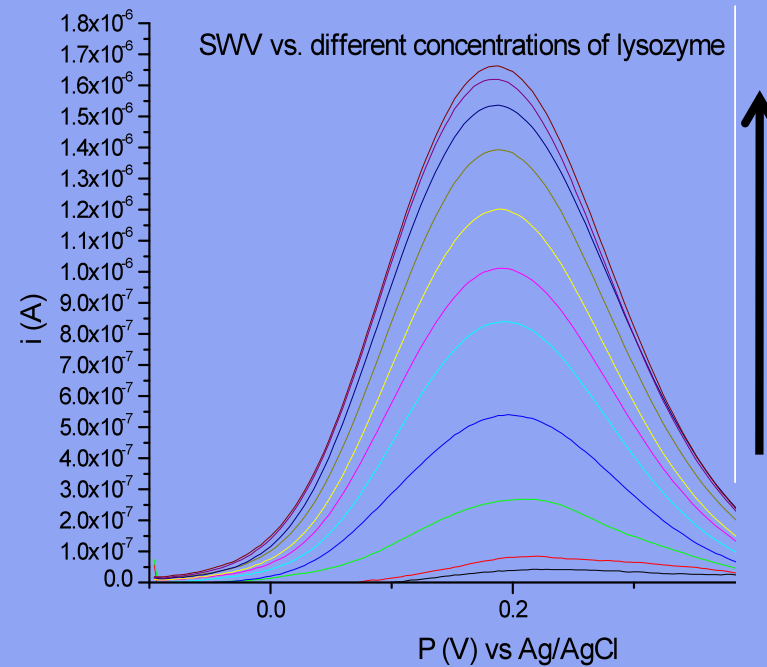
DNA sequence synthesised and described for electrochemical (impedance) sensing by Wang group



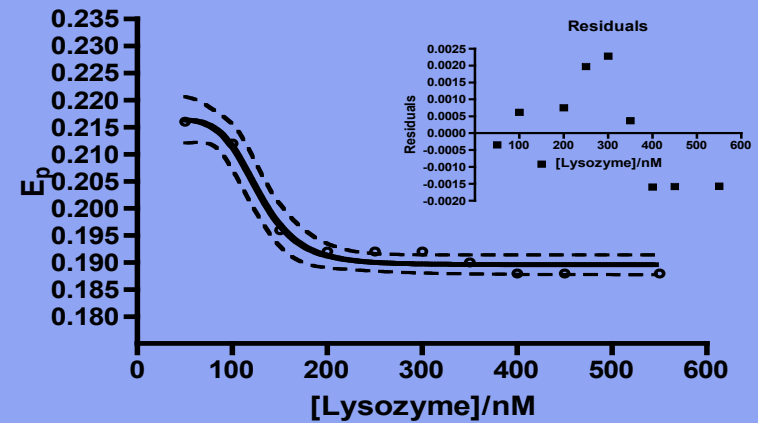
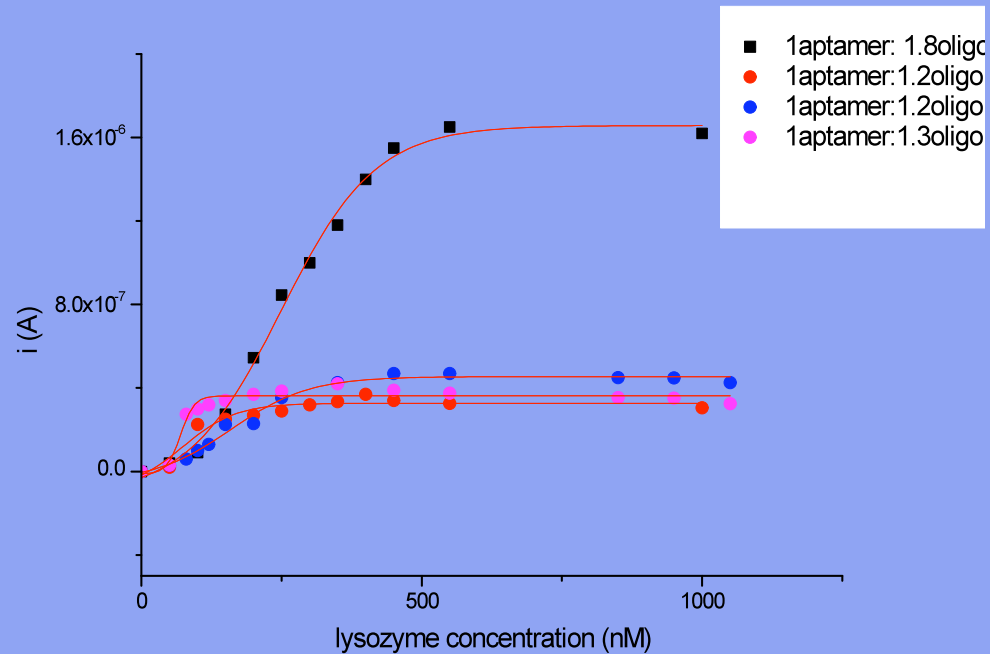
# 1<sup>st</sup> Generation Assembly



Designed as a displacement assay



# Dose-Response is Sigmoidal

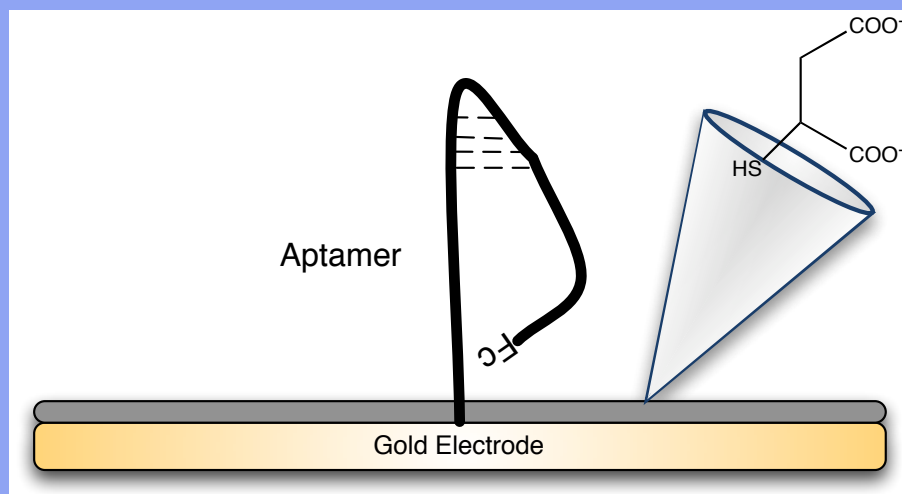


$\Delta E_p$  20mV

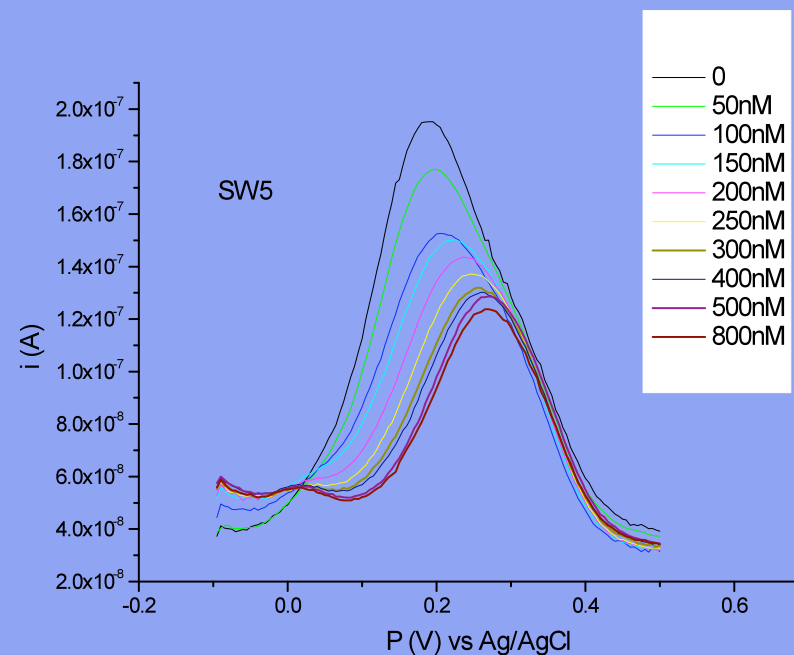
$K_d$  130nM (SPR 125nM)

Cooperative surface restructuring?

## 2<sup>nd</sup> Generation Beacon Assembly



Lysozyme binding disrupts beacon structure

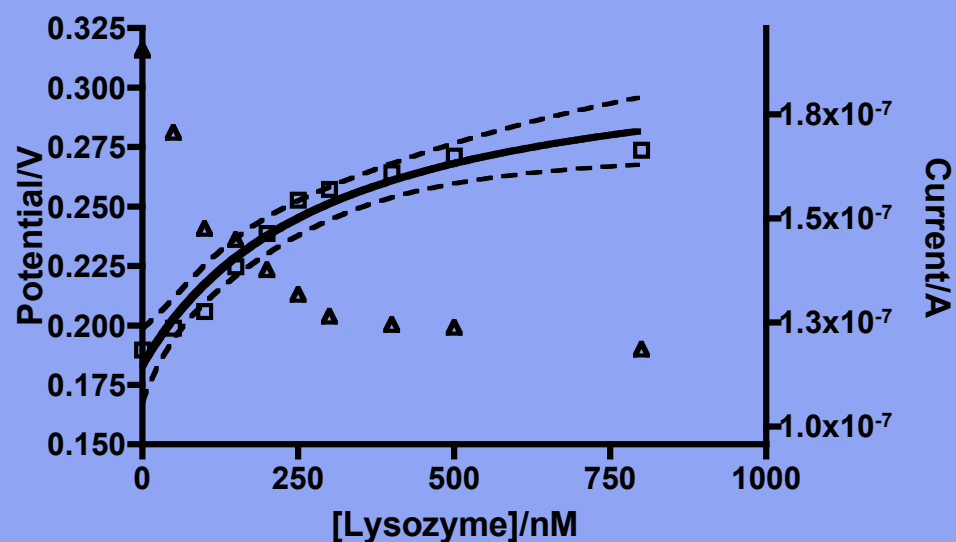


Notes:

1. Current decreases with increasing [Lysozyme]
2. Potential shifts +ve

Fc moves away from surface

# Beacon Dose Response Curves



□ Potential

▲ Current

$K_d = 290 \text{ nM}$

Higher  $K_d$  expected as Lysozyme binds competitively with internal hydrogen bonds

fig 2.1

20

40

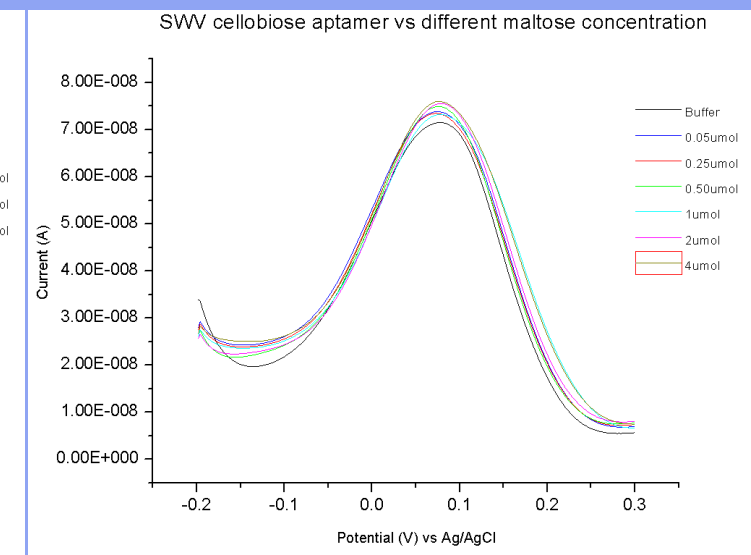
Redox Label

$C_6H_{12}$

S

Gold Electrode Platform

Electrochemically labeled aptamer for detection of cellobiose





# Minimally Invasive Measurement Tools for ISF

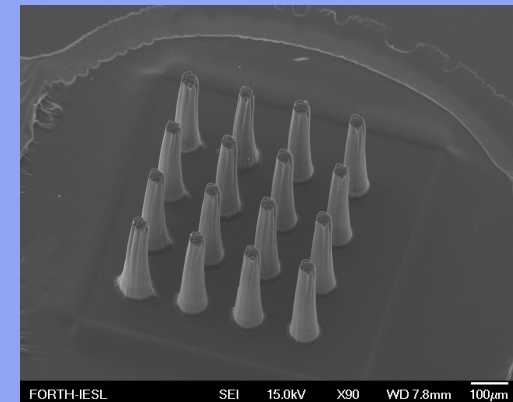
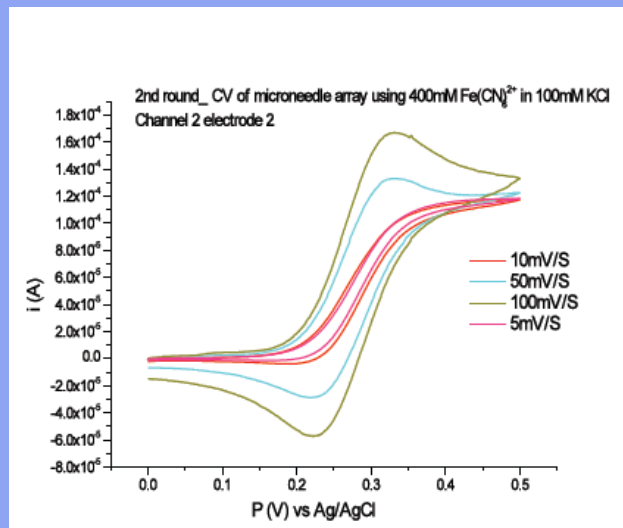
Attractions of ISF: 'Painless' access

Cell free

Drawbacks:

Potential lag with blood levels

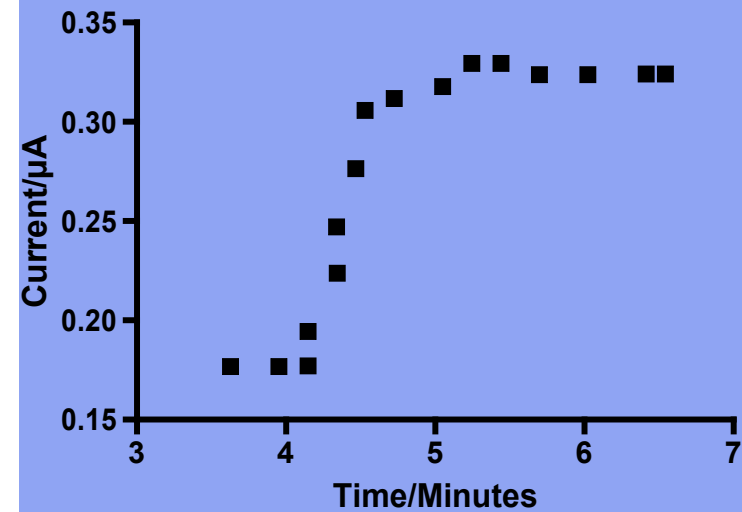
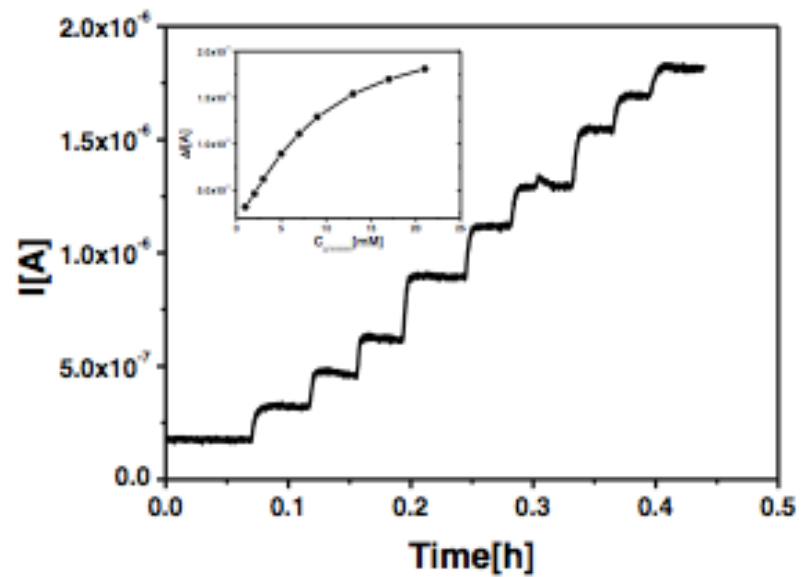
Less validated



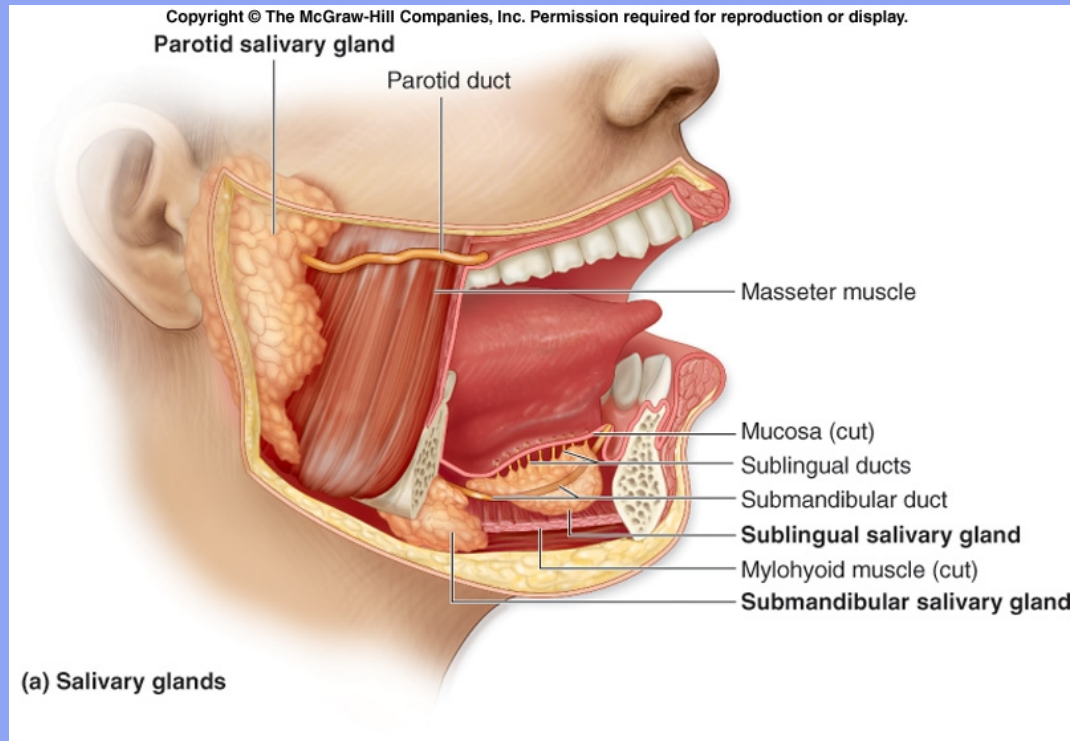
Microspike Electrodes

# *In vitro* Glucose Sensing

“Classical” polymer/mediator/enzyme system



# Salivary Diagnostics



Accessible

Already established for drugs  
& antibody tests and genotyping

Drug levels represent 'free'  
Concentration in serum

Variable Composition so best suited  
to threshold measurements

Easy to collect

Not discrete

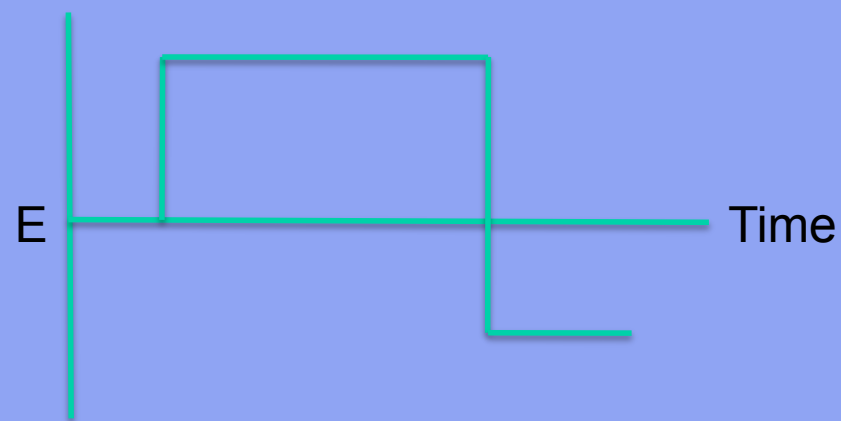
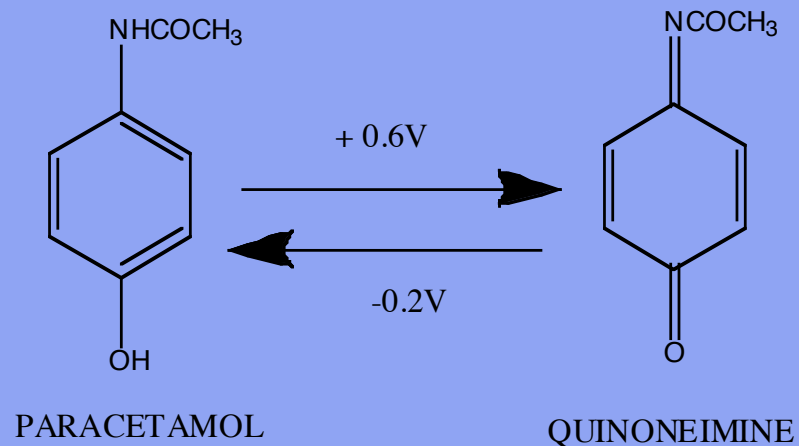
Sample often requires filtration or  
centrifugation



# Therapeutic Drug Monitoring

Paracetamol (acetaminophen) overdose:

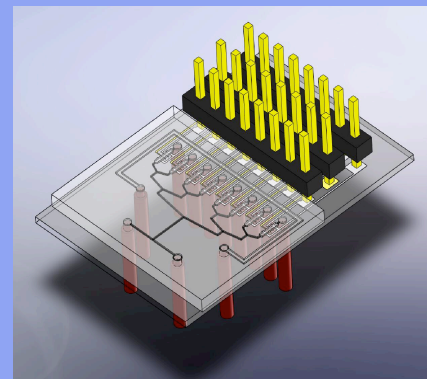
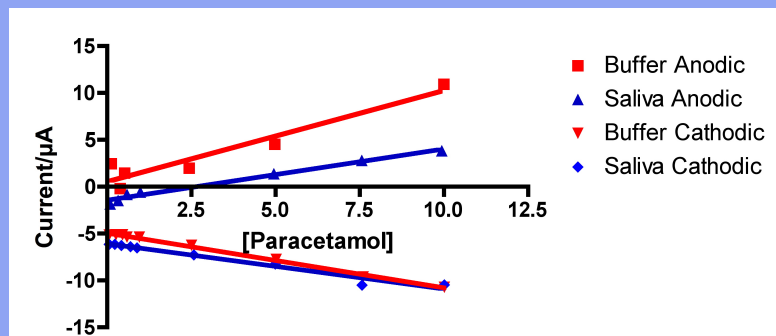
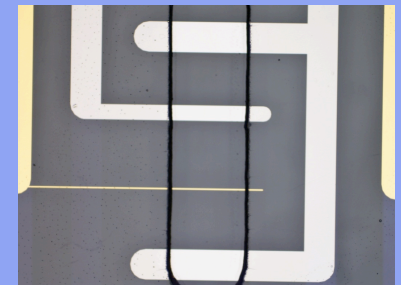
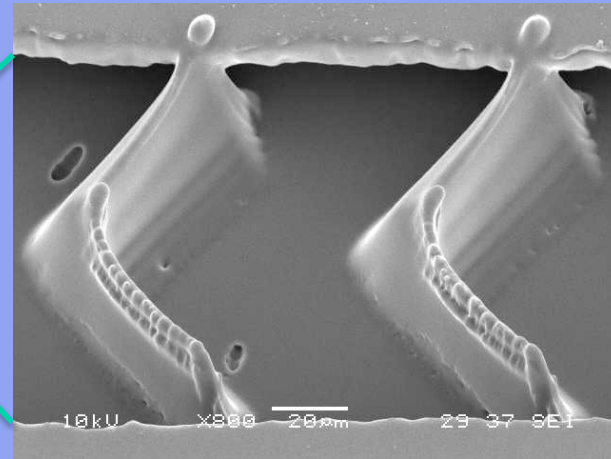
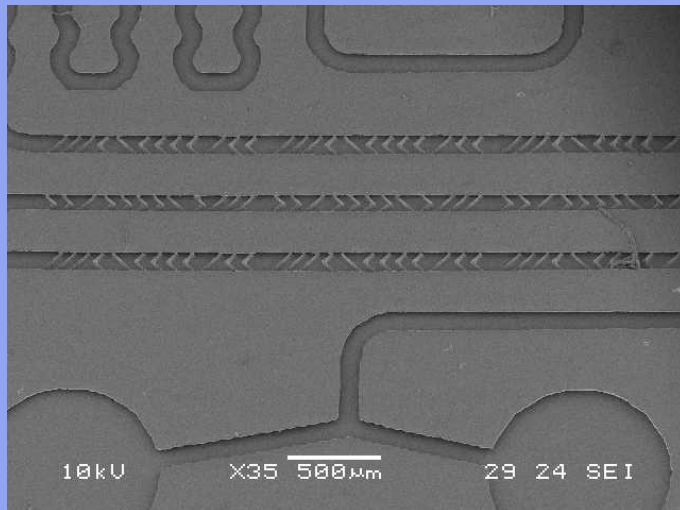
Clinical decision- to give antidote or not. Currently based on threshold (1.2mM)  
Clearance rate may be better



Double potential Step  
Chronocoulometry

# Chip Design

8 Electrochemical Cells each with 3 Electrodes  
Automated "On chip" Dilution Series using Chevron Mixers  
Saliva back pressure comparable to water



# Conclusions

- Many convergent trends in this area ('Biofusion' Bio+Nano+Informatics)
- Current developments are piecemeal
- Ultimately it won't be technology but patient/clinician acceptance/willingness to pay that determines take up

# Acknowledgements

- Group

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