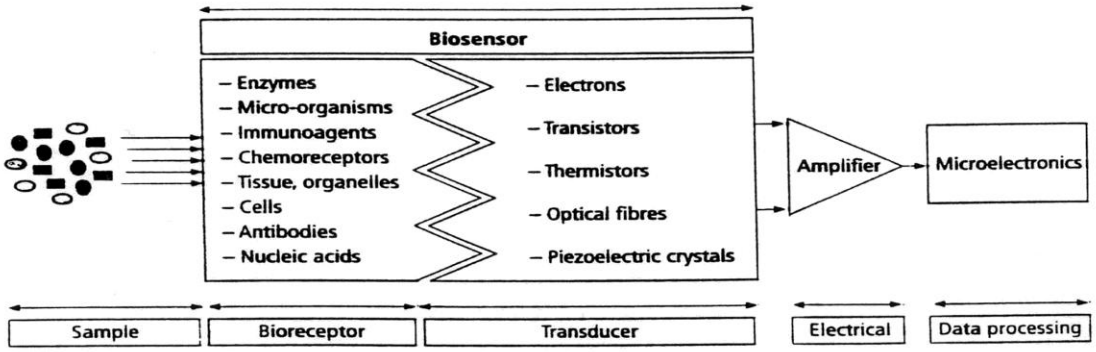
4B6
Solid State Devices and Chemical/Biological Sensors

Biological Sensors

Man Yi Ho
myh20@cam.ac.uk

What is a Biosensor?

"A biosensor is a *bioreceptor* immobolized on a compatible *transducer*."



Biosensors detect interactions between *bioreceptor* (probe) and *unknown species* (target)

Multi-billion $ market

(>70% on glucose sensors!)

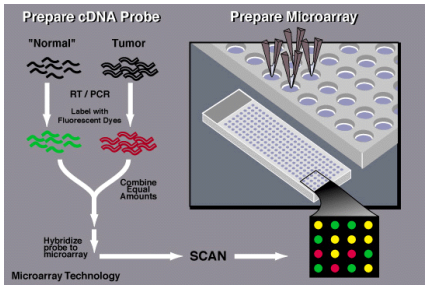
A few of the blood glucose sensors (test strip meters) available at your local pharmacy…

Huge potential in the future, due to:

- **increasing demand on healthcare**
- **decentralizaiton / point of care healthcare system**
- **food safety**
- **environment monitoring**

Successes and Challenges

On the market: Glucose sensor, pregnancy test strip, DNA micro arrays, LFD based other in-*vitro* diagnostic devices (bacteria, antibodies ...)



In research: DNA sensor, protein analysis, in-*vivo* sensors, non-invasive diagnostic sensors. The current focus is on biocompatibility and point-of-care.

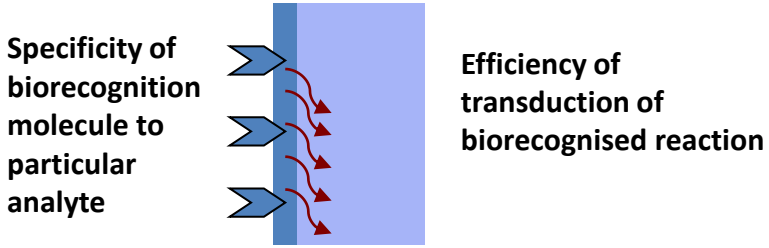
Challenge:

Reliability – as diagnostic deice it has to work with very high reliability.

Stability – bio-receptors are vulnerable to the change of environment.

Biocompatibility – for implantable sensor, coating must be biocompatible.

Point-of-care – portable, disposable, low cost, robust, and easy to operate.



Overview

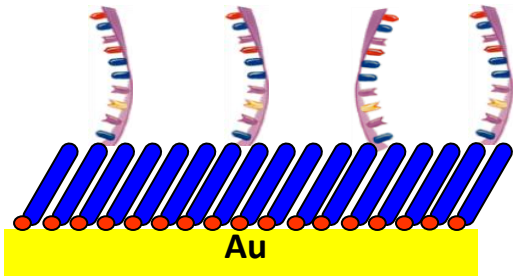
- **DNA detection:**

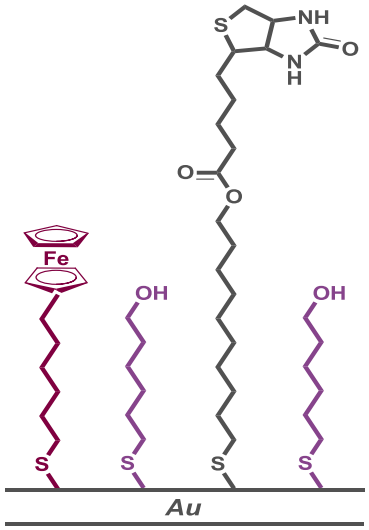
  Platform: PNA Probe for the detection of Avian Influenza Virus
  Methods: Field effect detection
  Electrochemical Impedance Spectroscopy (EIS)

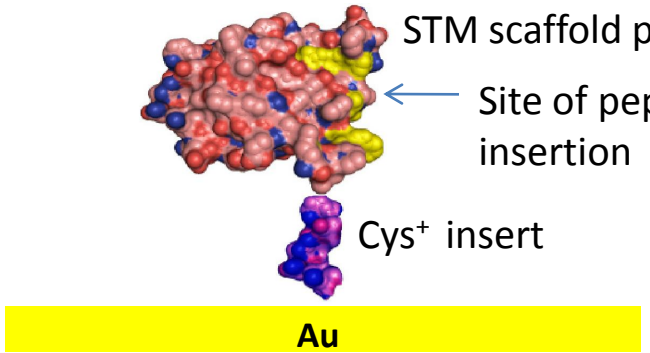
- **Protein detection:**

  I. **Platform: Redox active self-assembled monolayer (SAM)**
  Methods: AC voltammetry

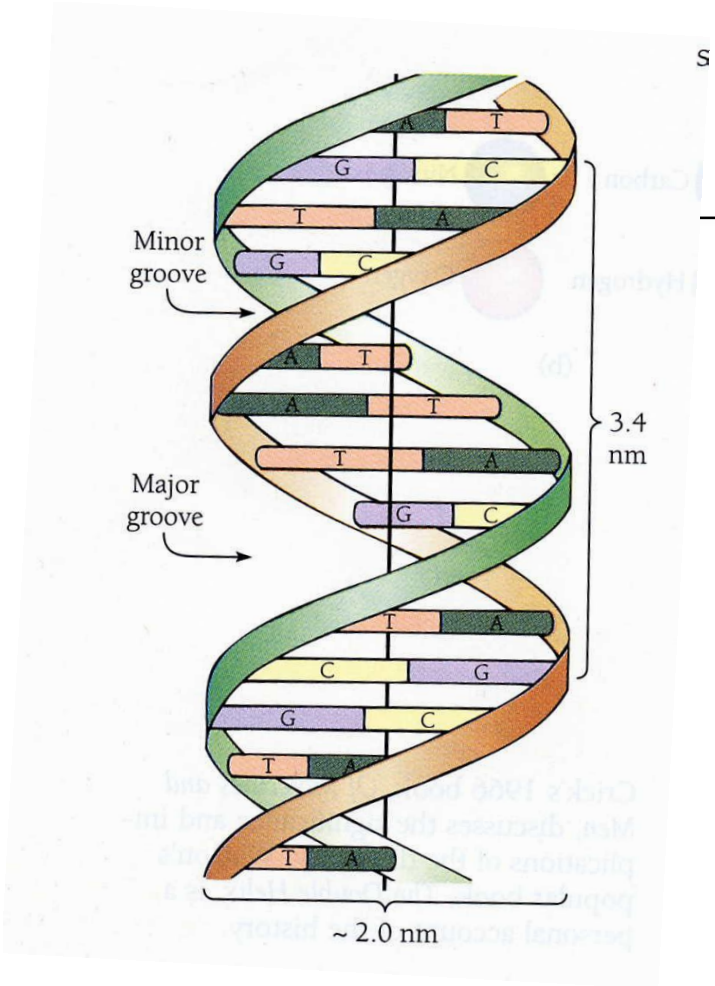
  II. **Platform: Peptide aptamers**
  Methods: Field effect detection - Open circuit potential
  - MOSFET

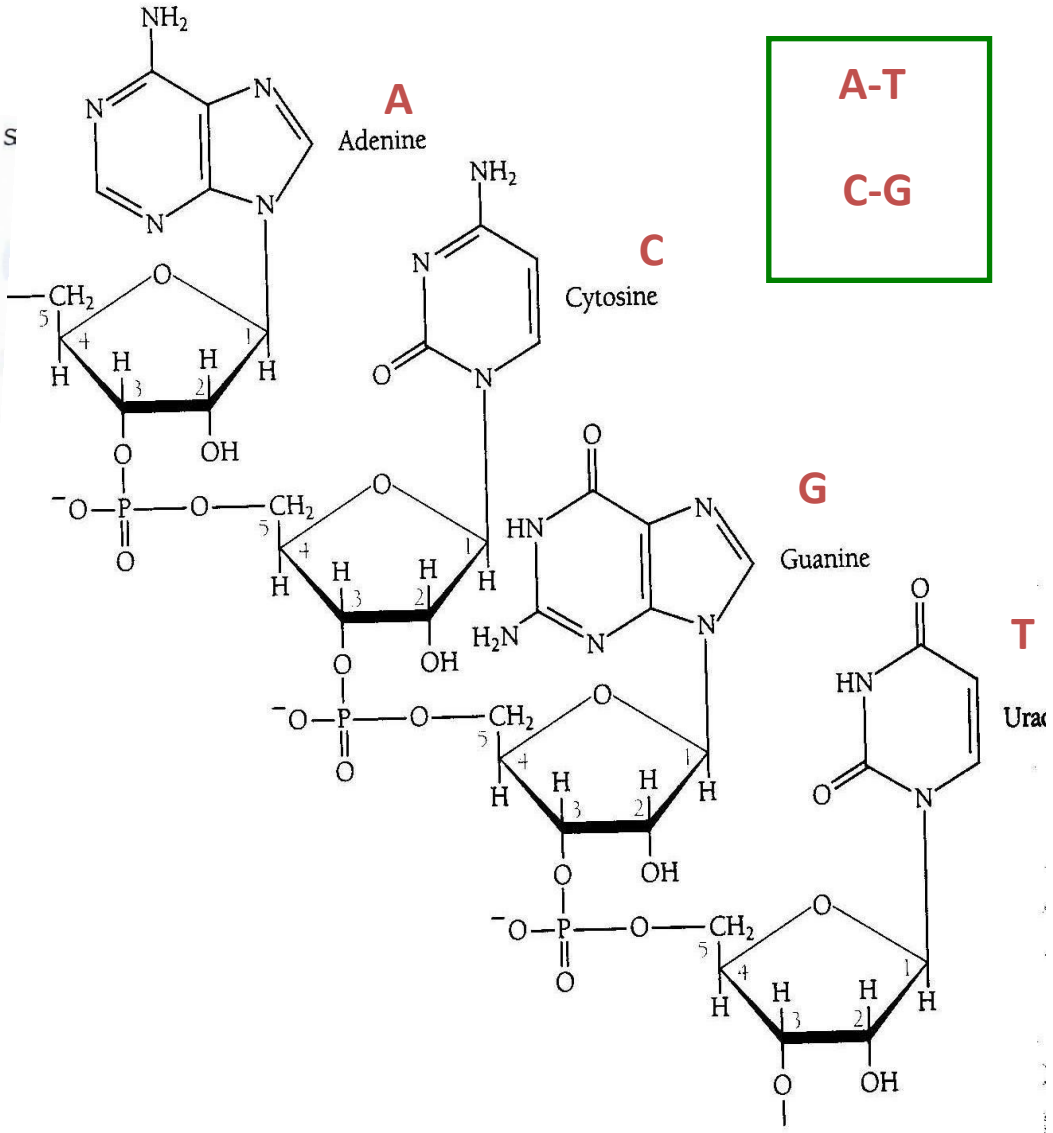




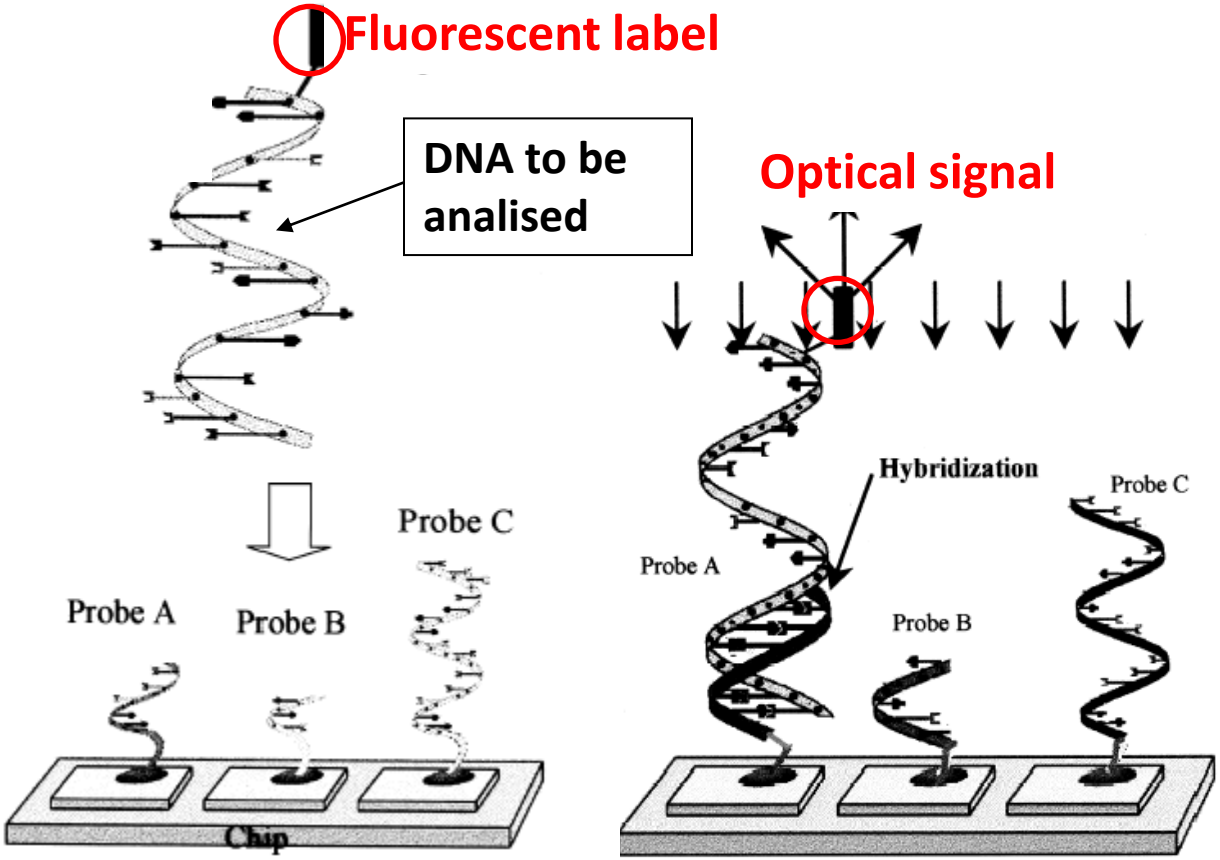


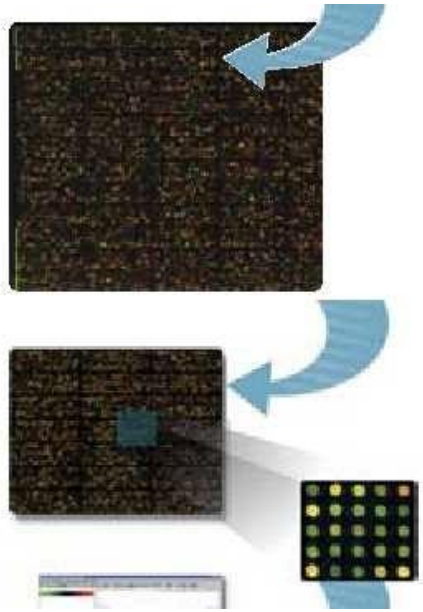
DNA Structure and Binding





Conventional Fluorescent Detection





Disadvantages:
Fluorescent labels are expensive
Fluorescence emission is weak
Expensive optical equipment and signal processing

DNA Detection

Motivation of DNA detection

Label-free Detection platform :

Mixed self-assembled monolayer (SAM) with DNA probe

Method:

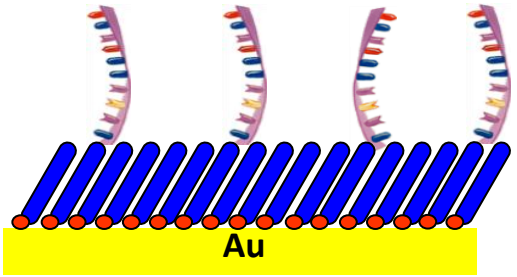
Field effect detection

Electrochemical Impedance Spectroscopy (EIS)

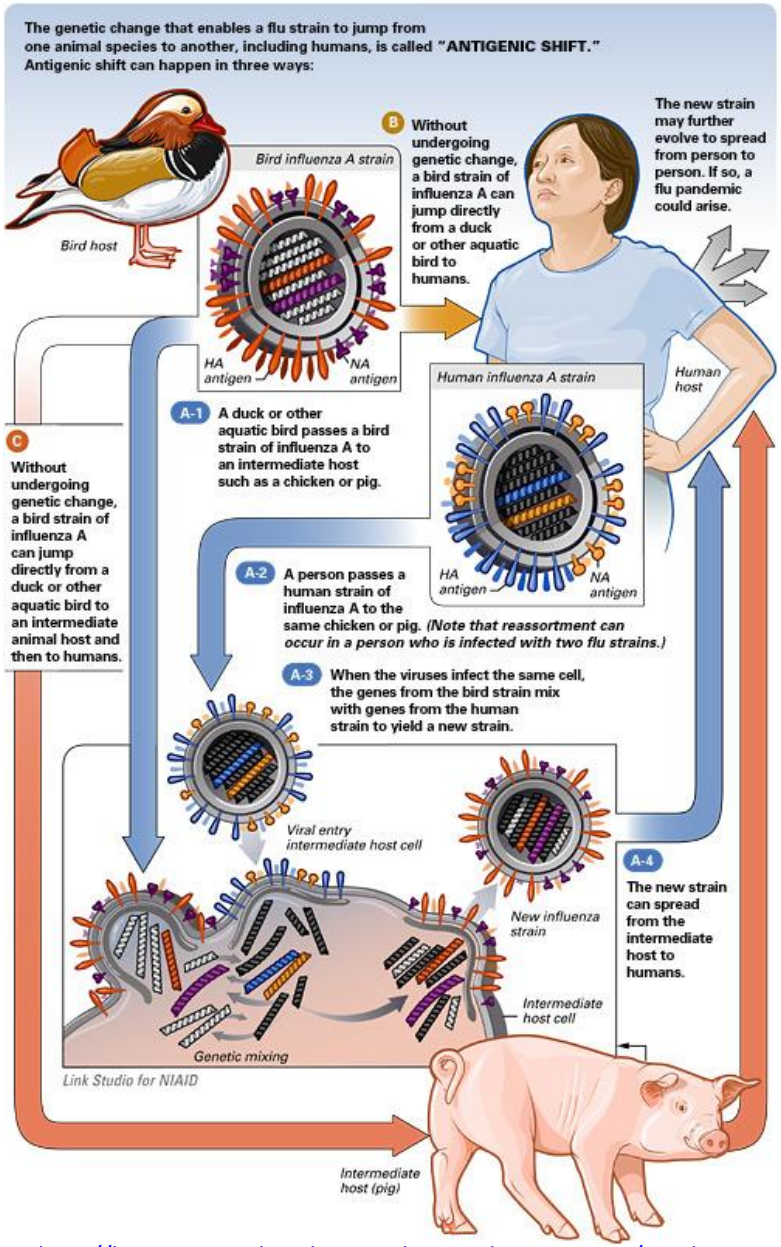
Peptide nucleic acid (PNA) probes:

Optimization of PNA mixed SAM

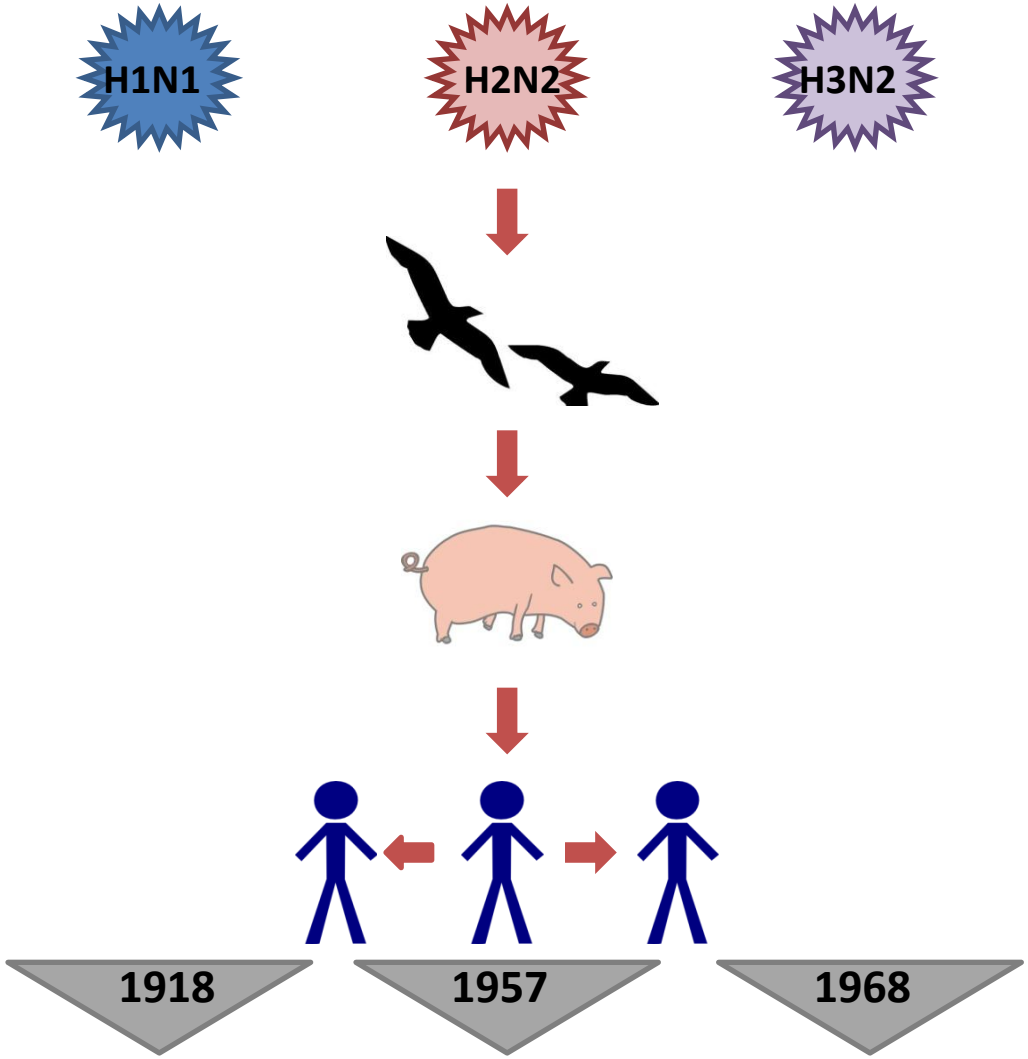
DNA probes vs PNA probes



Past Pandemics in the Last Century



http://beginnersguidetotheapocalypse.wikispaces.com/Pandemic



Spanish Flu
50 Millions

Asian Flu
2 Millions

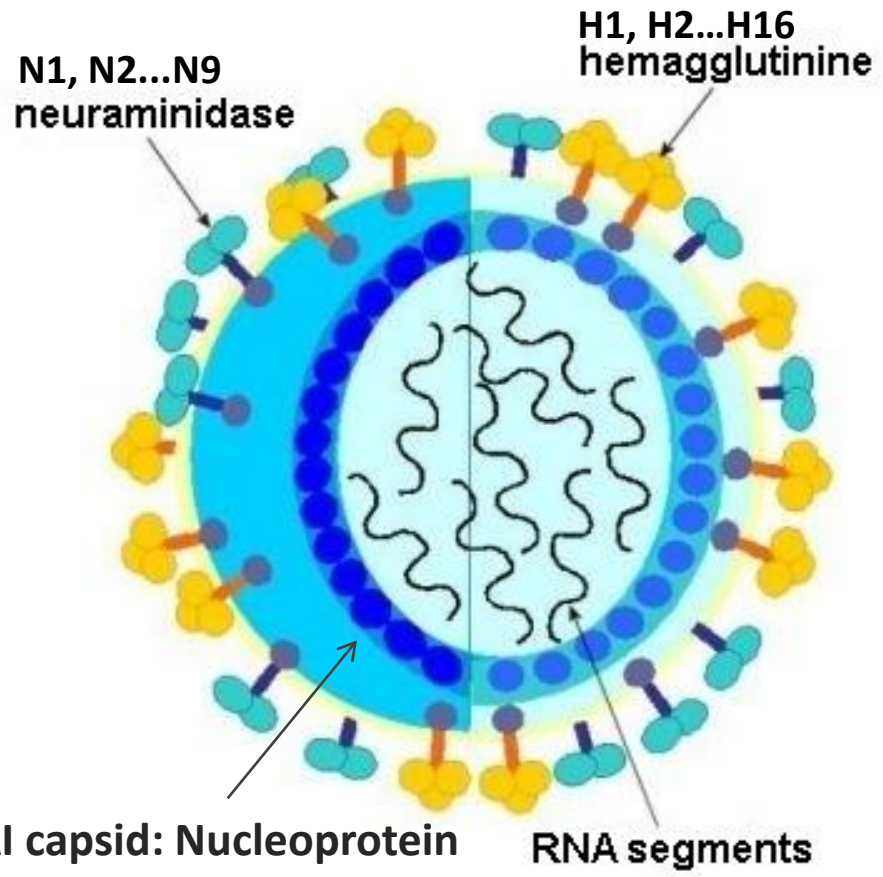
Hong Kong Flu
1 Millions

Reservoirs of the avian influenza virus

- Wild birds are the natural reservoirs of the AI virus
- Little clinical signs in most infections
- Migratory waterfowl are believed to be responsible for the spread of avian influenza virus

Avian Influenza Virus

Neuraminidase and Hemagglutinine determines the subtype of the virus



Matrix gene (M gene)

Encode M-protein
Conserved among all 16 H-types
Generic AI detection

Subtyping (H1, H2..)

DNA Detection

Motivation of DNA detection

Detection platform :

Mixed self-assembled monolayer (SAM) with DNA probe

Method:

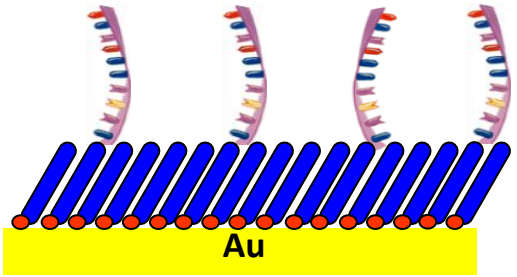
Field effect detection

Electrochemical Impedance Spectroscopy (EIS)

Peptide nucleic acid (PNA) probes:

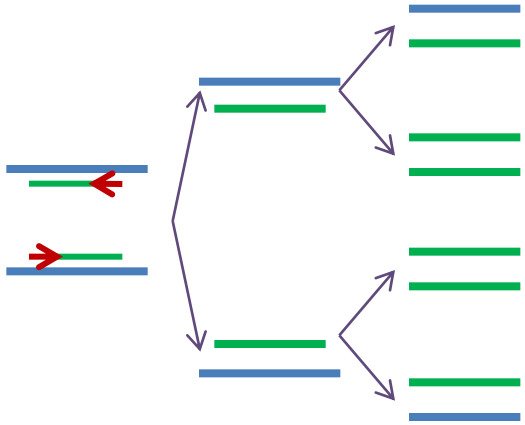
Optimization of PNA mixed SAM

DNA probes vs PNA probes



Label-free Detection of Avian Influenza Virus

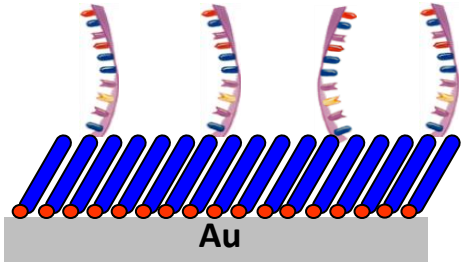
Sample pretreatment



Reverse Transcription Polymerase Chain Reaction (RT-PCR)

asymmetric RT-PCR

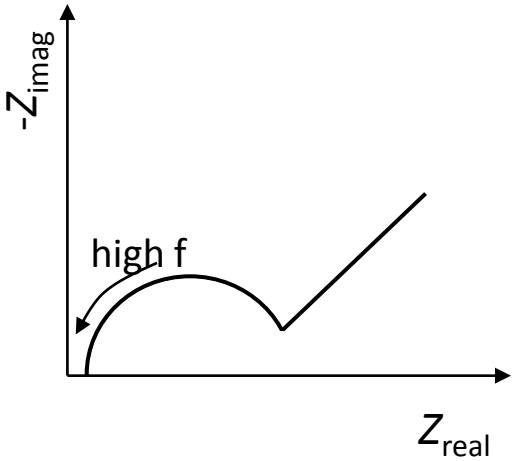
Detection platform



Self-assembled monolayer (SAM) with DNA or PNA probe:

optimization of probe density

Signal transduction



FET or Electrochemical impedance spectroscopy:

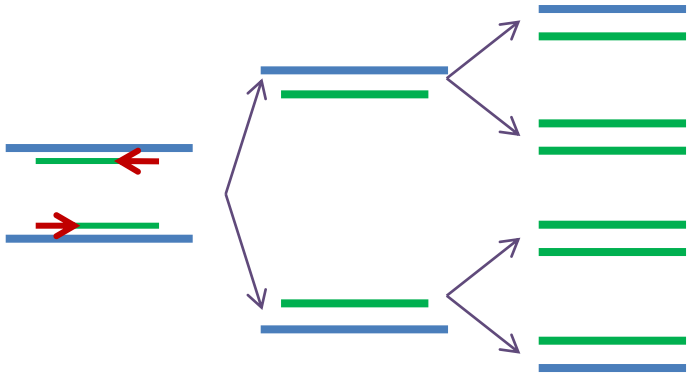
optimization of measurement buffer ionic strength

Sample Pretreatment: Reverse Transcription Polymerase Chain Reaction (RT-PCR)

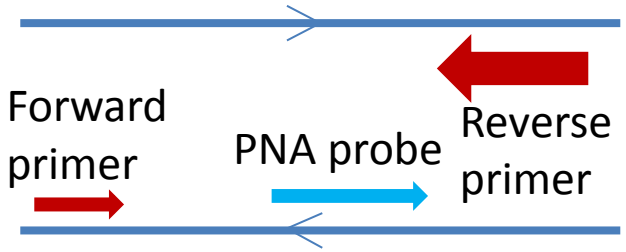
PCR: Amplify a few copies of DNA to several orders of magnitude, generating millions copies of a particular DNA sequence (example)

RT-PCR: An RNA strand is first reverse transcribed into its complement DNA (cDNA) using the enzyme – reverse transcriptase, and the resulting cDNA is amplified with PCR

RT-PCR for double stranded DNA (dsDNA) production

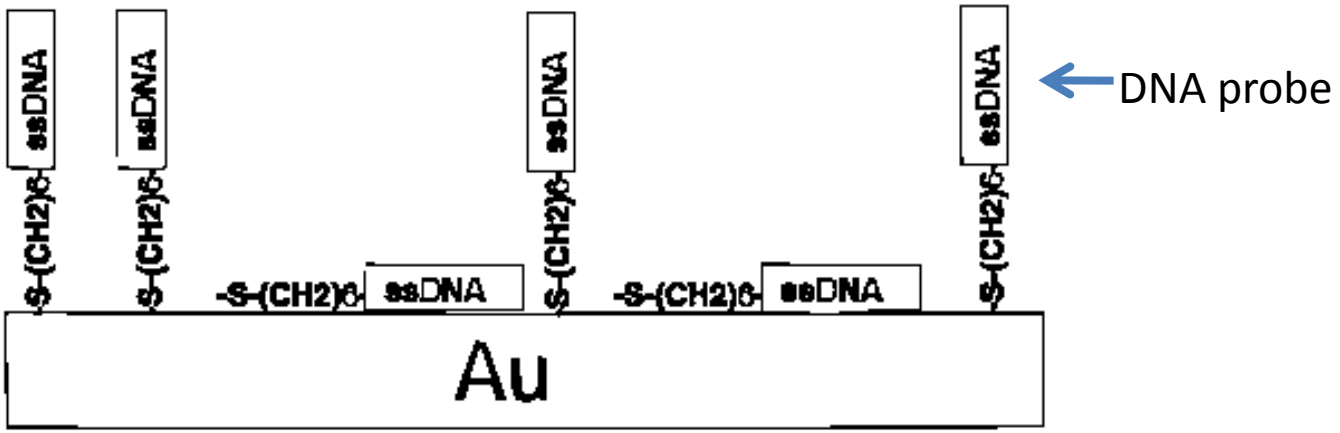


Asymmetric RT-PCR for single stranded DNA (ssDNA) production

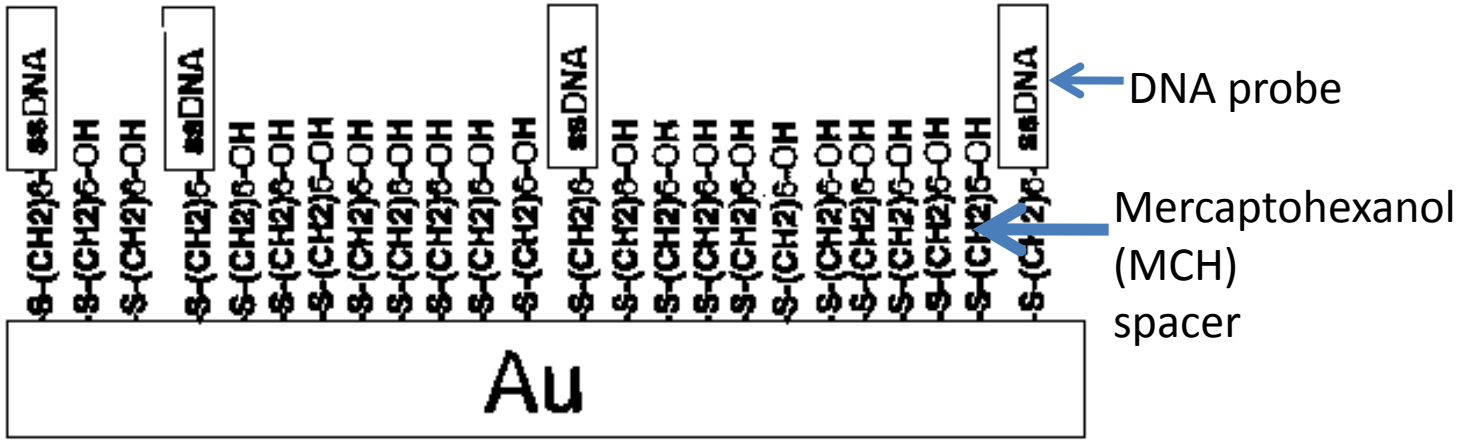


Detection Platform: Mixed Self-assembled Monolayer (SAM)

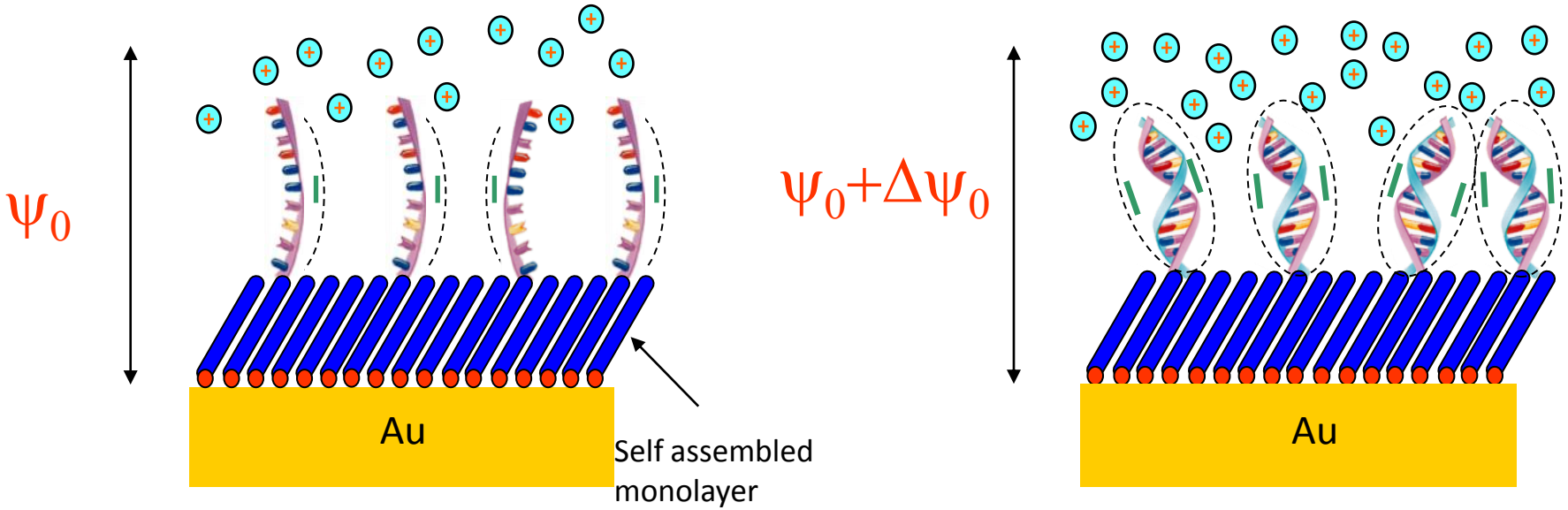
DNA probe covalent immobilization on Au electrode



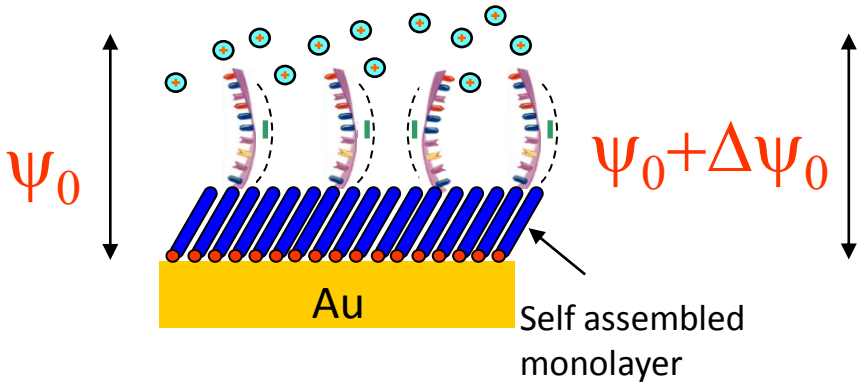
Mixed DNA-Mercaptohexanol SAM

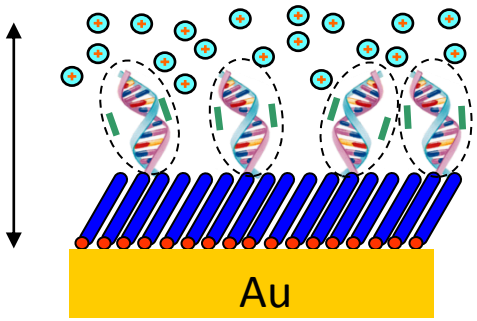


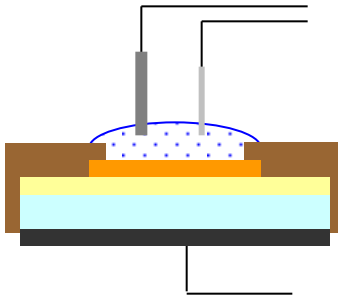
Detection Platform: Mixed Self-assembled Monolayer (SAM)

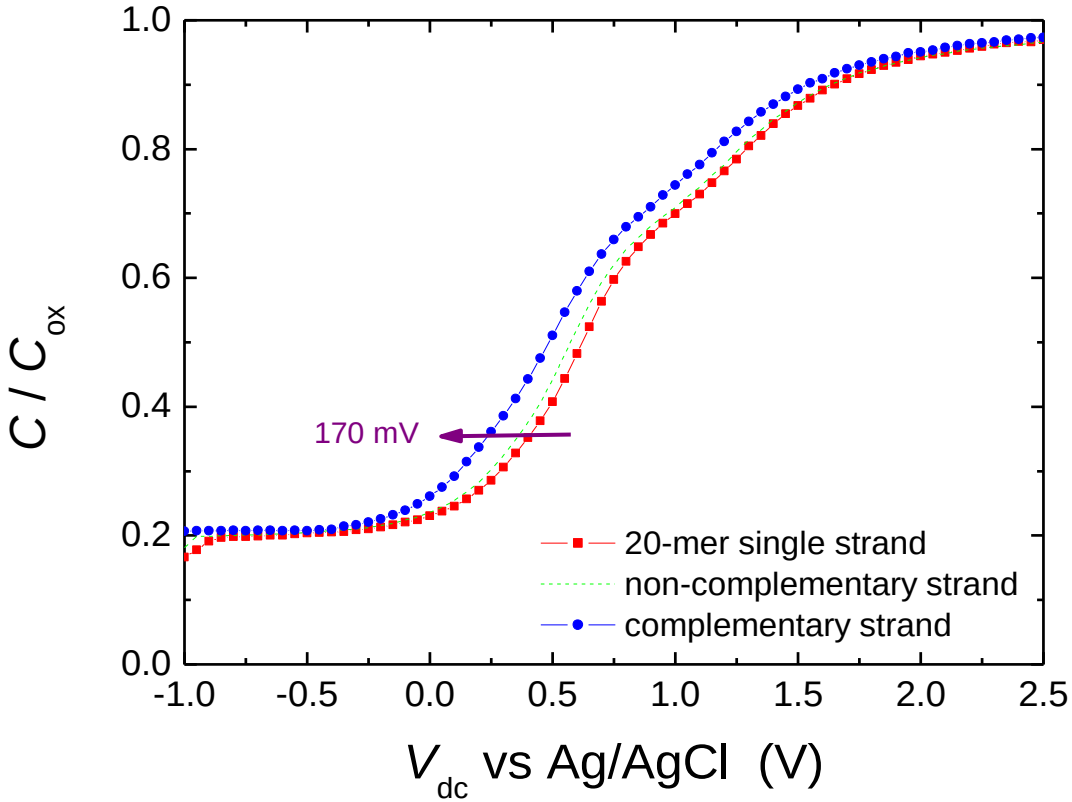


Method: Potentiometric Detection – FET Detection





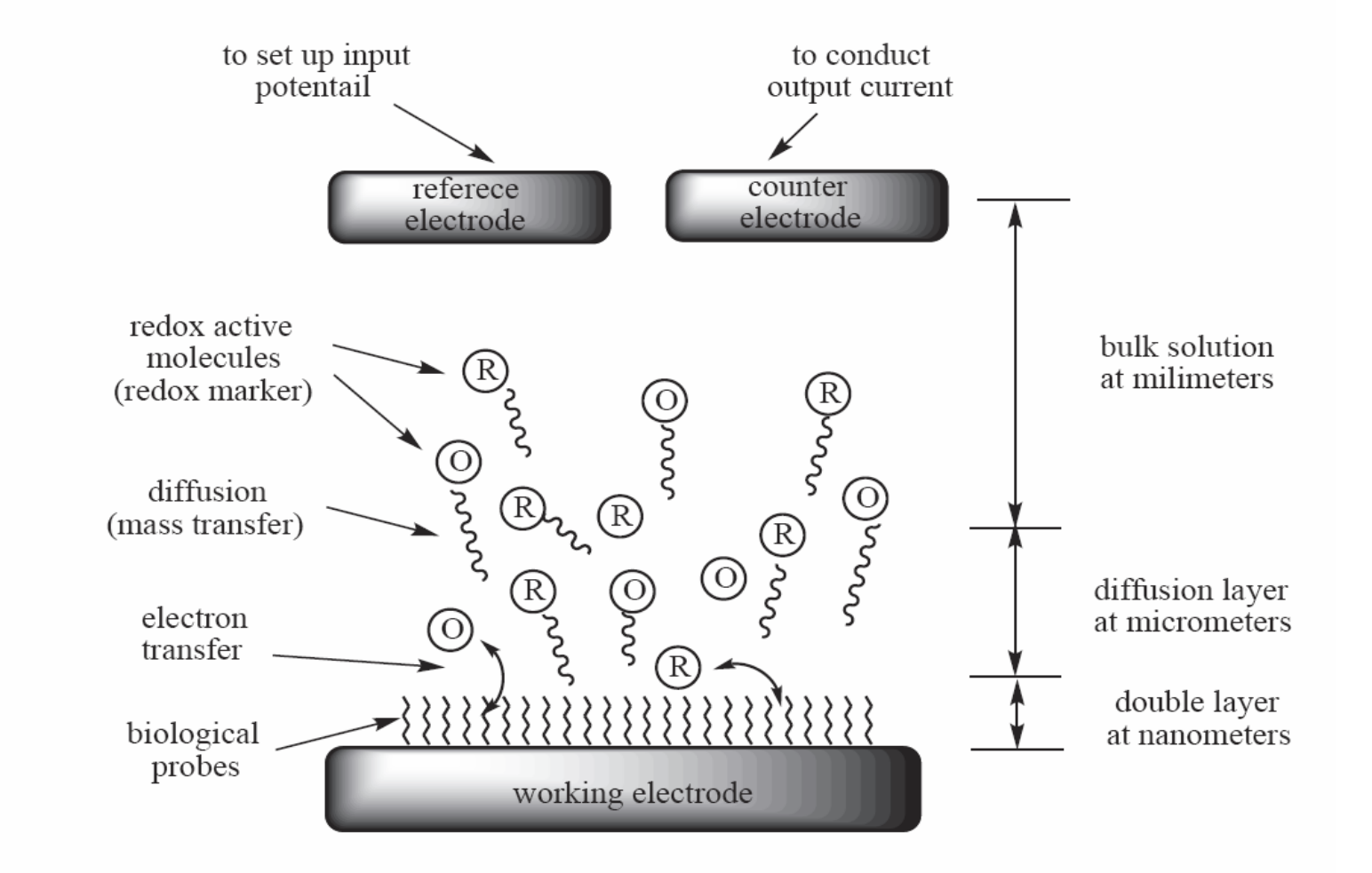




DNA Sensor – MOS Diode

d_{SiO2} = 500 Å
f = 1 kHz
V_{ac} = 20 mV
50 mM PBS pH 7.0

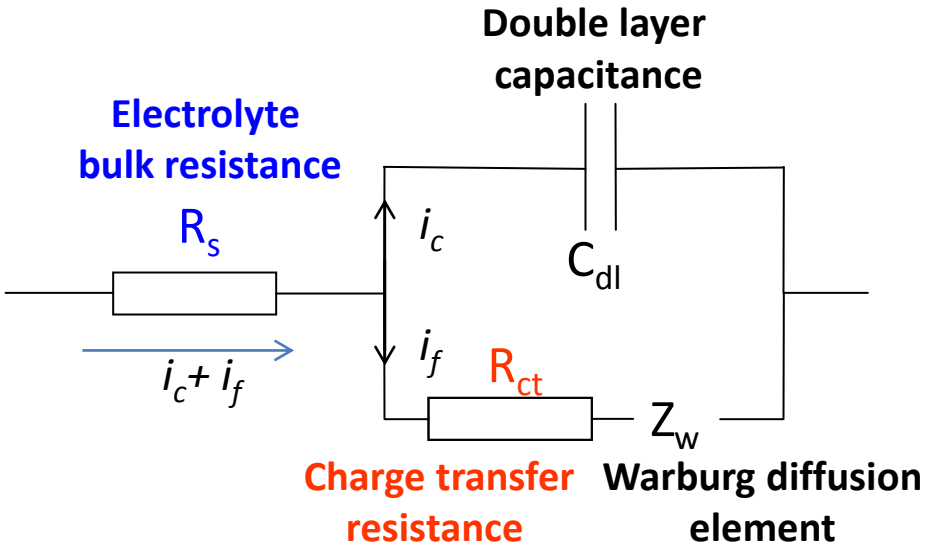
A typical electrochemical cell

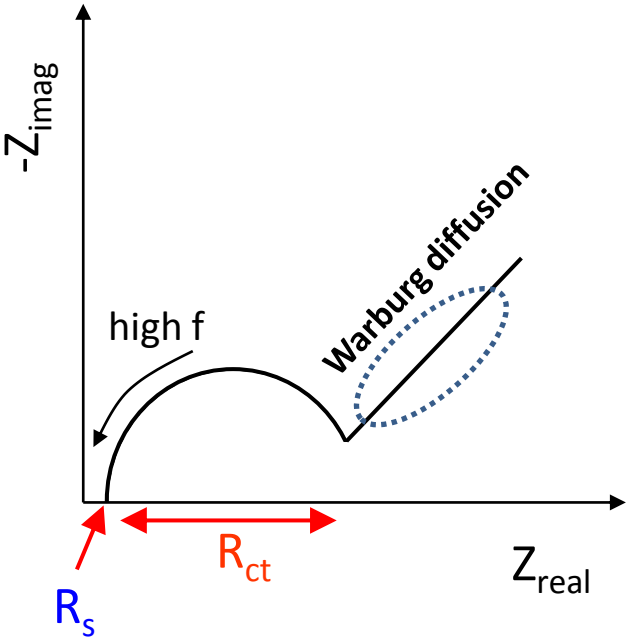


Electrochemical Impedance Spectroscopy

- Electrolyte contains redox couple: $[Fe(CN)_6]^{3-/4-}$
- Measure ac current in phase and out of phase

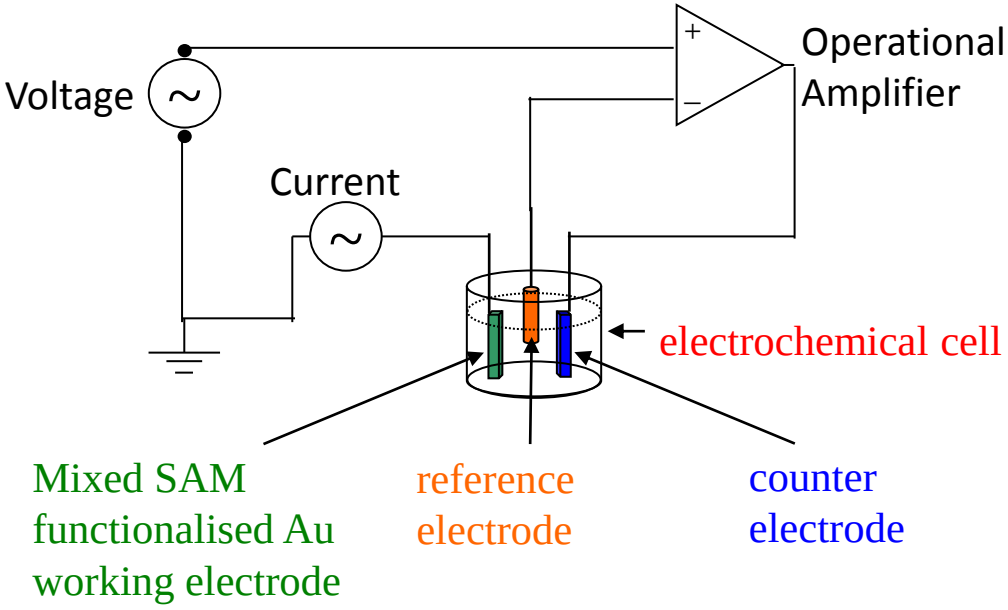
  → real and imaginary parts of the impedance : $Z(\omega) = Z_{real} + j\, Z_{imag}$
- Scan frequency of measurement **ω**
- Fit electrical circuit model

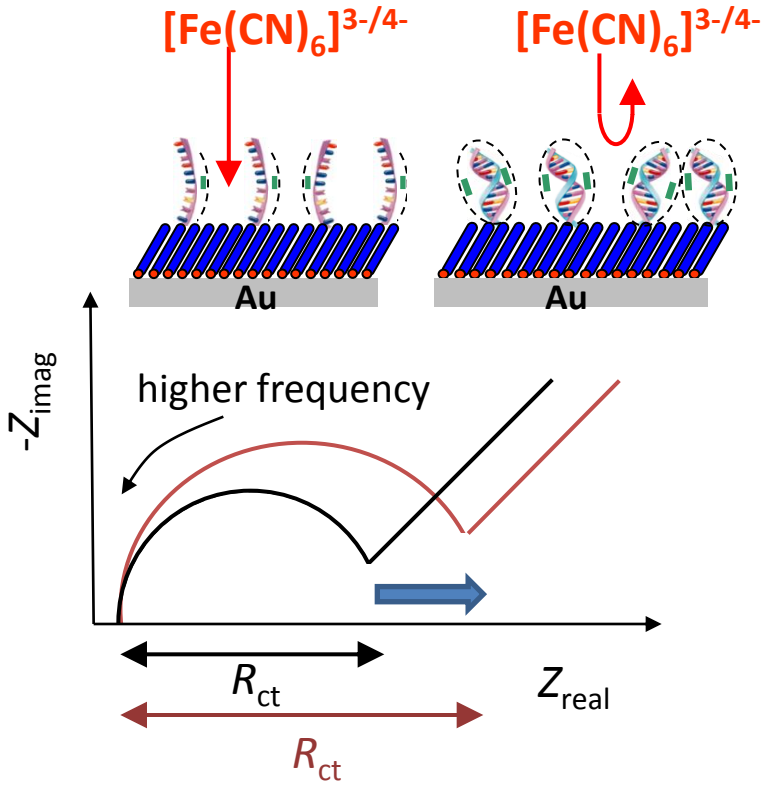




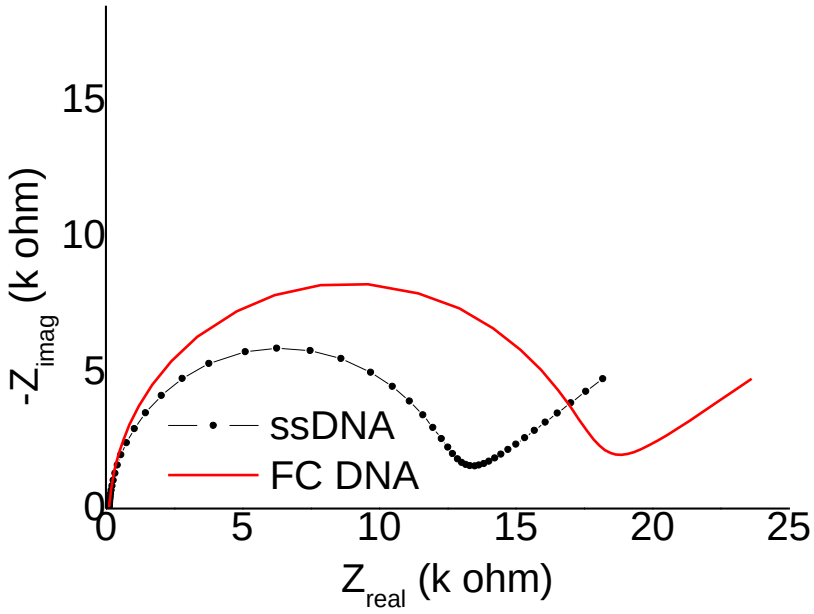
Method: Electrochemical Impedance Spectroscopy (EIS)

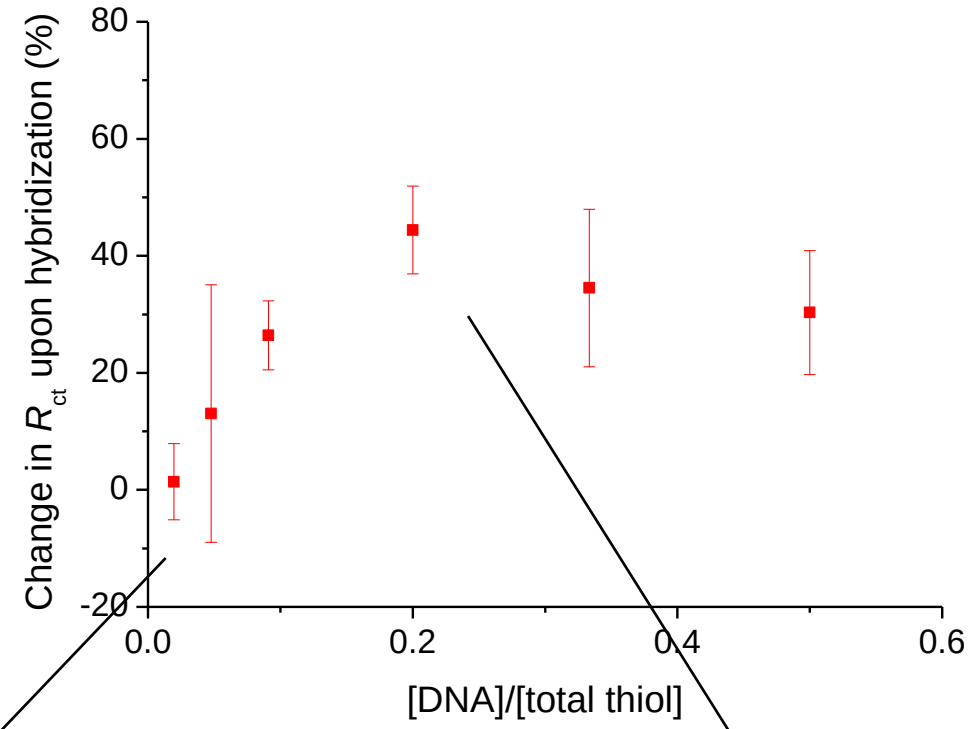
- Relies on detecting the intrinsic negative charge of the DNA
- Electrolyte contains negatively charged redox couple **$[Fe(CN)_6]^{3-/4-}$**
- Superimpose small ac voltage on formal potential of redox couple
- By measuring the ac current, the real and imaginary parts of the impedance as a function of frequency (10kHz to 1Hz) are extracted
- By fitting electrical model, the charge transfer resistance **Rct,** is obtained

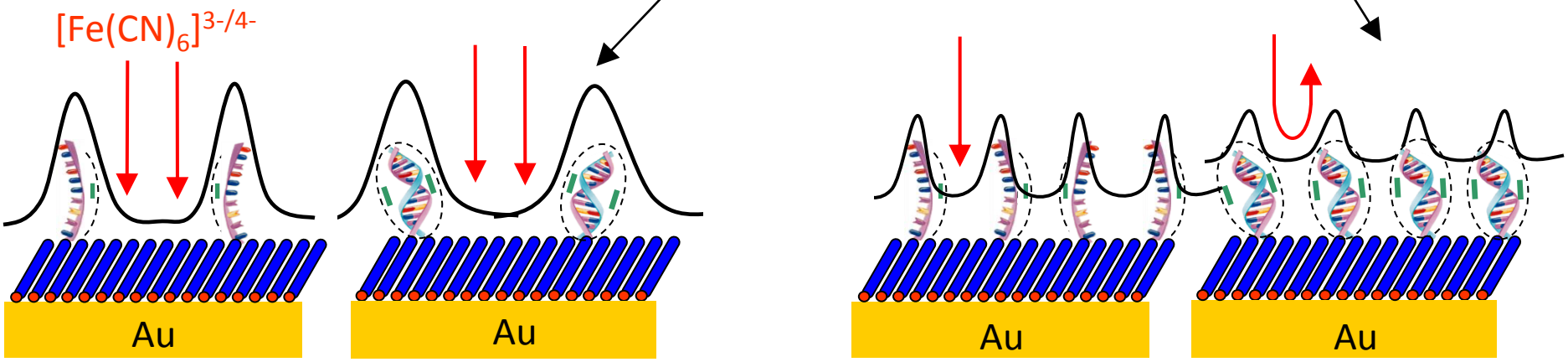




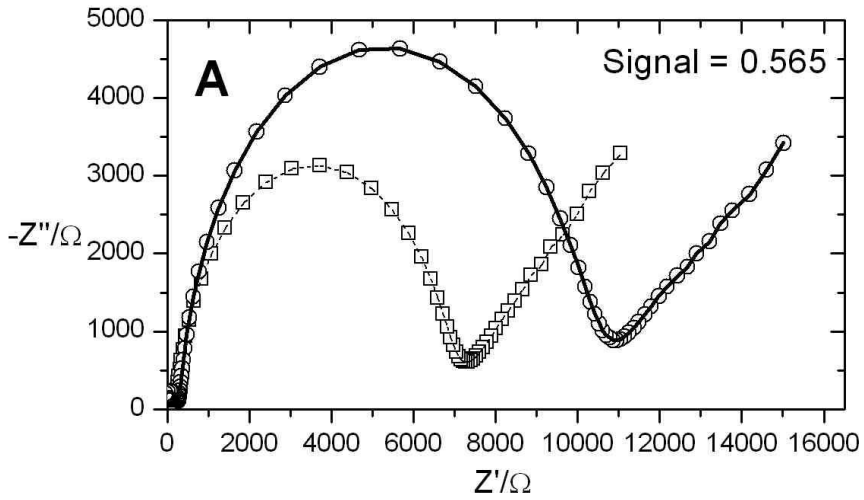
DNA Probe – Optimization of Probe Density





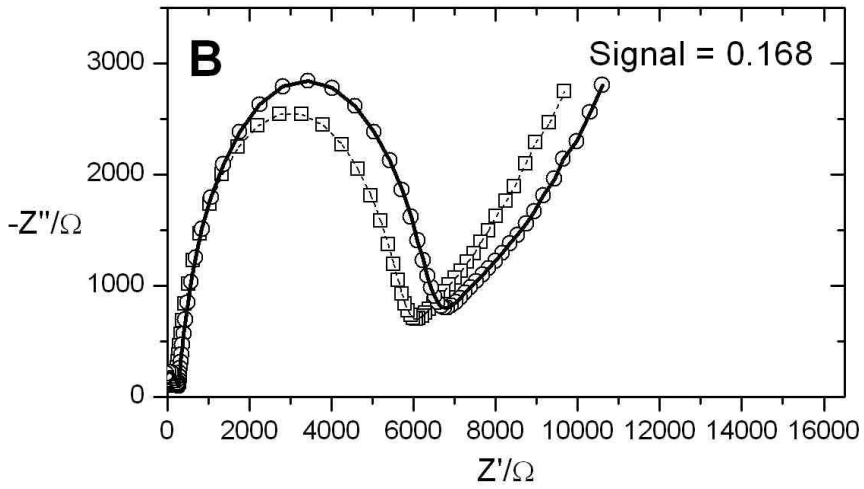


EIS detection of a gene sequence cMA20 characteristic of avian influenza matrix protein H5N1



MA20 probes □

Hybridization with complementary target cMA20 ○



MA20 probes □

Treatment with non-complementary target cHA23 ○

A Kukol, P Li, P Estrela, P K Ferrigno, and P Migliorato
Analytical Biochem., 374(2008) 143-153

Impedance Simulation

- Potential distribution described by the Poisson-Boltzmann equation:

$$\nabla^2\phi = -\frac{\rho}{\varepsilon} \qquad \phi(x,y,z) \ \text{electric potential}$$

$$\text{where } \rho = \text{charge} = \sum_i e_0 z_i n_i$$

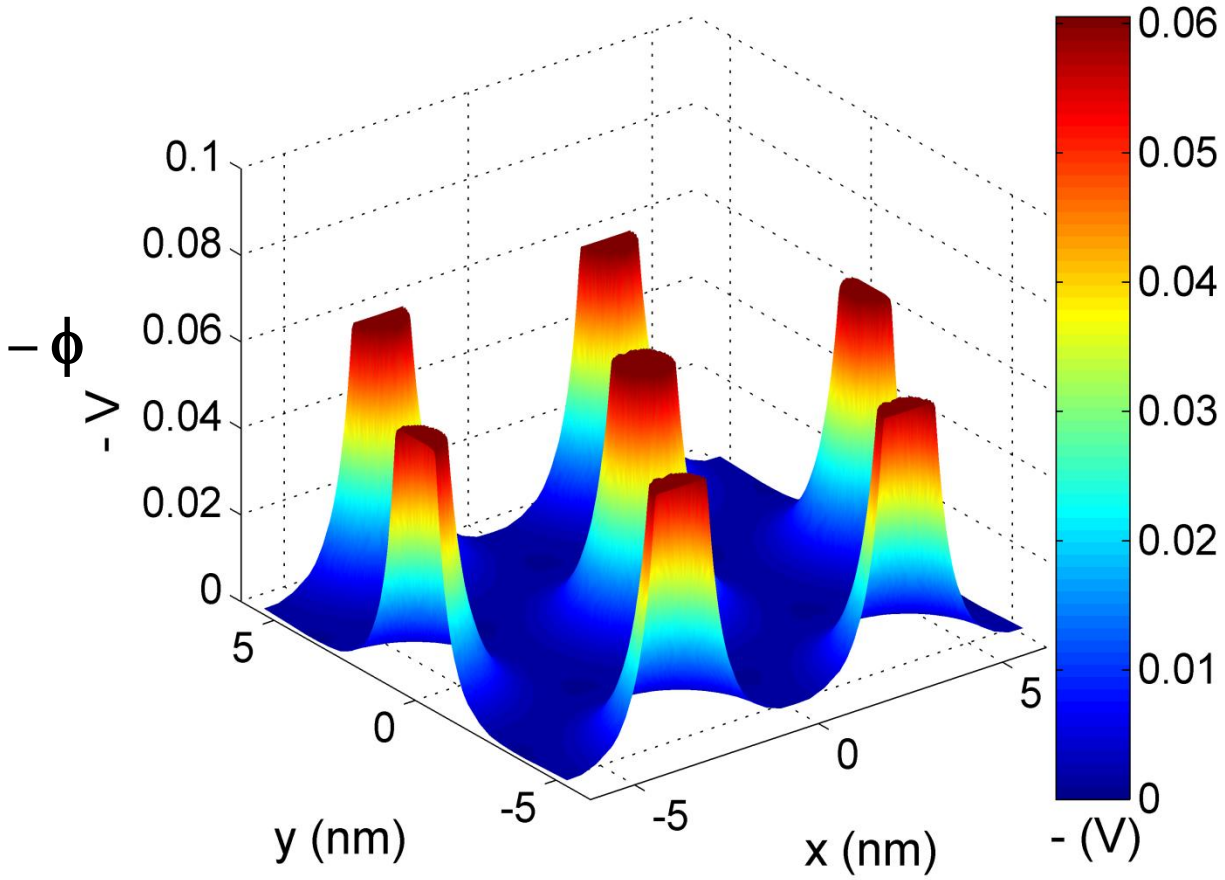
$$n_i = \text{concentration of ionic species} = n_{0i} \exp\left(-\frac{e_0 z_i \phi}{kT}\right)$$

- Use finite element method in FemLAB to solve in 3D

S Keighley et al. to be published

Simulation of the Electrochemical Double Layer Potential

ssDNA [3×10^{12} cm $^{-2}$]
Electrolyte concentration: 100mM PB



DNA Detection

Motivation of DNA detection

Detection platform :

Mixed self-assembled monolayer (SAM) with DNA probe

Method:

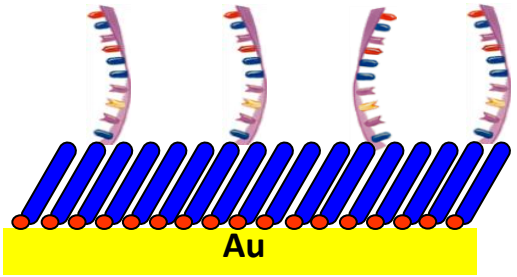
Field effect detection

Electrochemical Impedance Spectroscopy (EIS)

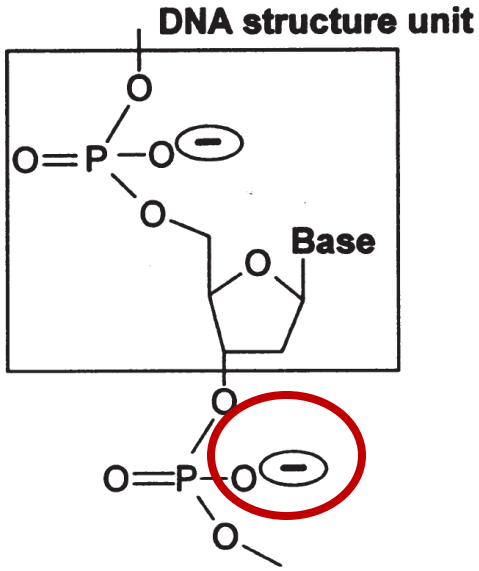
Peptide nucleic acid (PNA) probes:

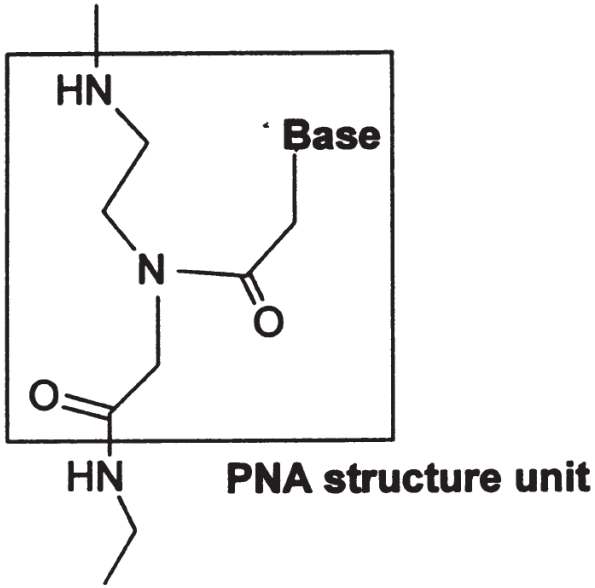
Optimization of PNA mixed SAM

DNA probes vs PNA probes



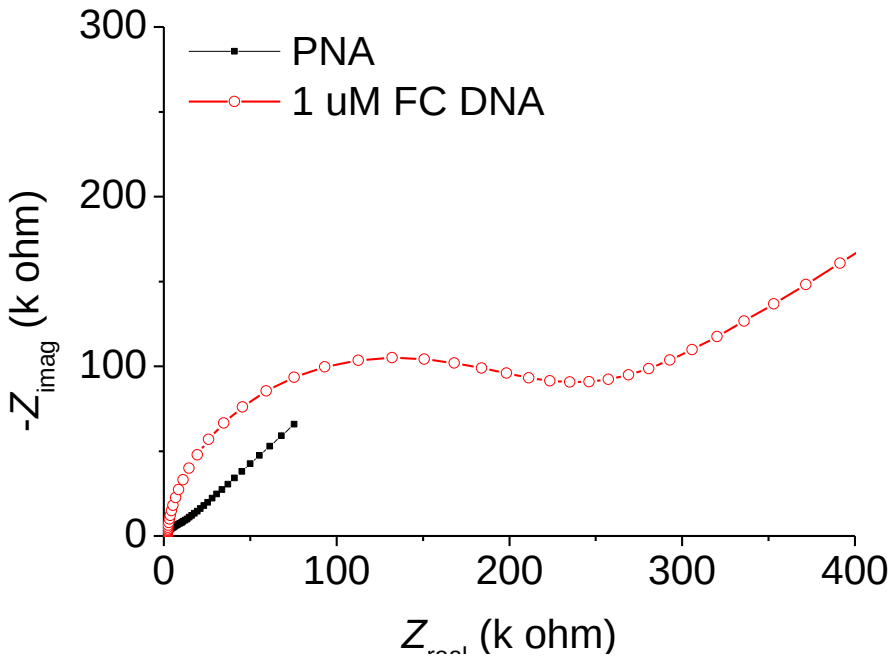
Peptide Nucleic Acids (PNA)

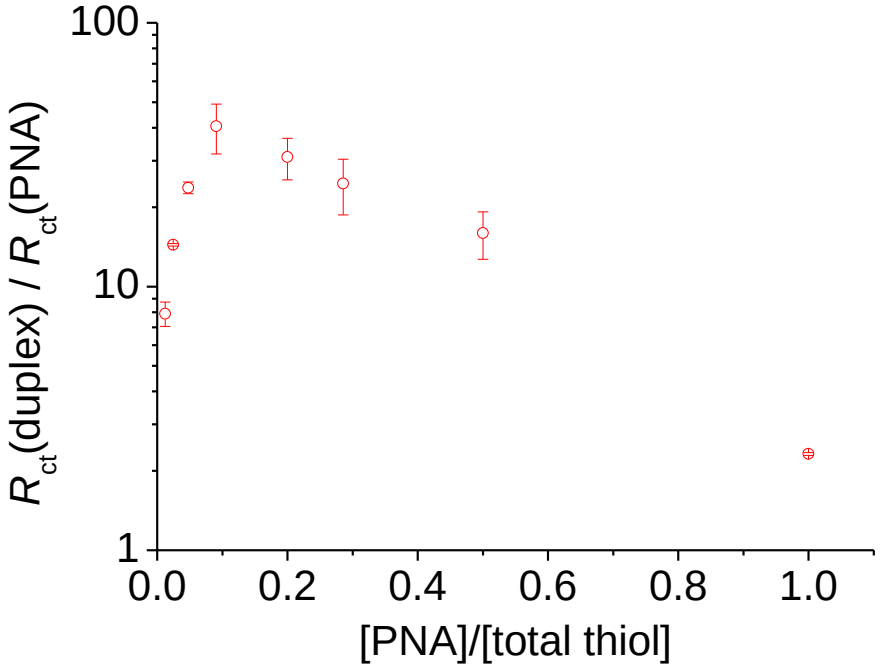




- Selectively hybridizes with ssDNA target
- Electrically neutral
- Greater PNA/DNA duplex stability than DNA/DNA duplex
- Significantly higher specificity
  - detection of single base-pair mismatches

PNA Probe – Optimization of Probe Density



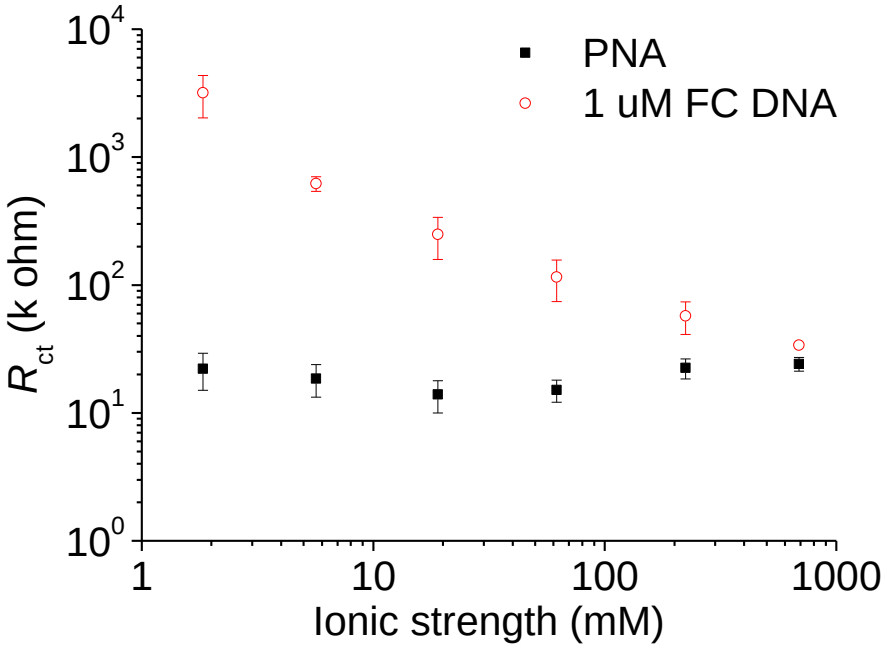


- Significant enhancement of R_{ct} change with hybridization compared to DNA probe (1.5 fold)
  - 40-fold change with optimized probe immobilization at 10mM PB

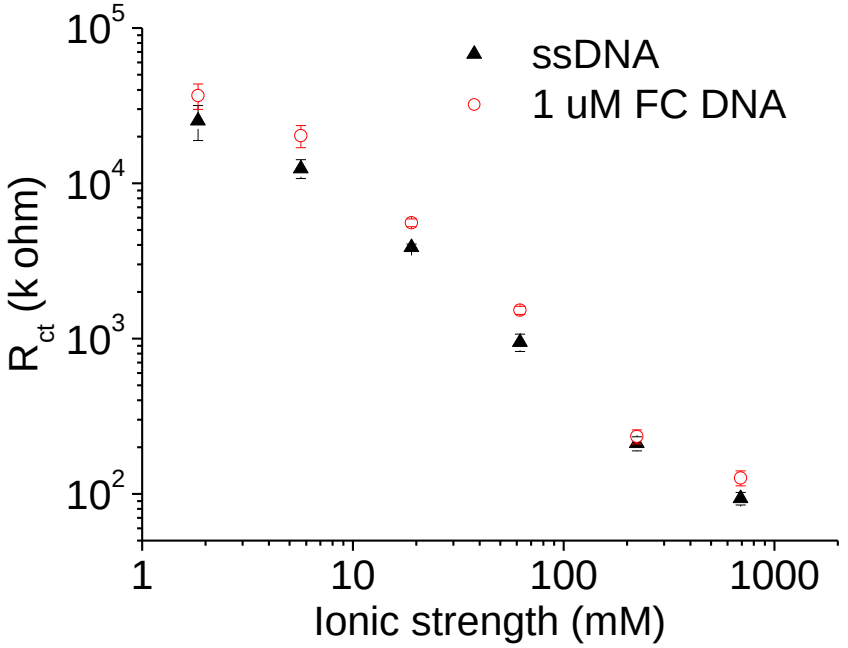
S.D. Keighley, P. Li, P. Estrela, P. Migliorato, Biosens. Bioelectron. 24 (2008) 906

PNA Probe – Optimization of Measurement Buffer Ionic Strength

PNA probes



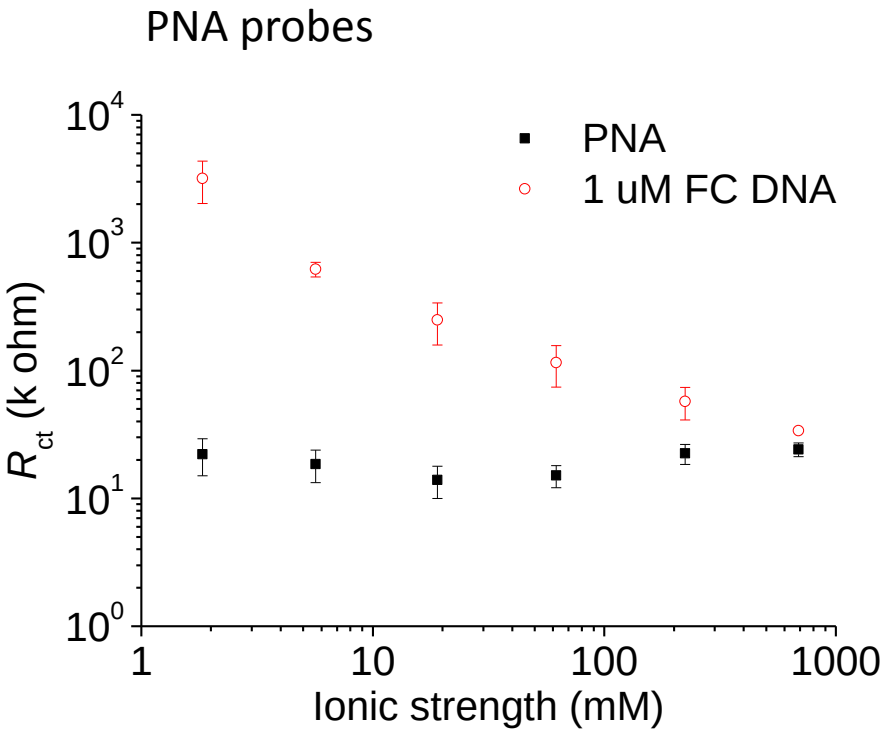
DNA probes

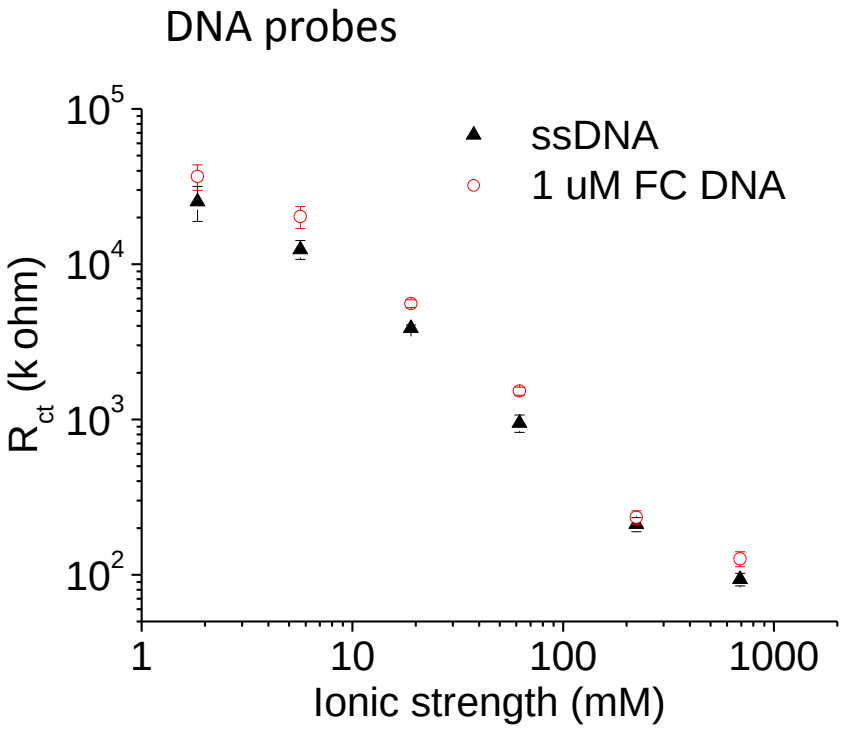


- Reducing ionic strength
  - PNA probes: R_{ct} constant
  - barrier after hybridization greatly increased
  - massive enhancement of R_{ct} change with hybridization
  - DNA probes: similar effect on charge screening of both ssDNA and dsDNA
  - both barriers increase
  - little effect on R_{ct} change

S.D. Keighley, P. Li, P. Estrela, P. Migliorato, Biosens. Bioelectron. 24 (2008) 906

PNA Probe vs. DNA Probe





- **PNA probes: 385-fold R_{ct} change with hybridization**
- **DNA probes: 0.65-fold R_{ct} change with hybridization**

S.D. Keighley, P. Li, P. Estrela, P. Migliorato, Biosens. Bioelectron. 23 (2008) 1291

S.D. Keighley, P. Li, P. Estrela, P. Migliorato, Biosens. Bioelectron. 24 (2008) 906

Protein Detection I

Motivation of protein detection

Detection platform:

Ferrocene redox self-assembled monolayer (SAM) detection platform

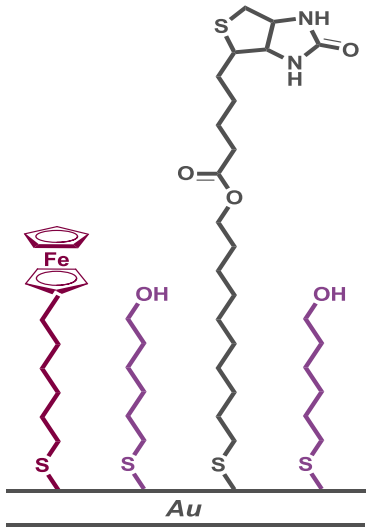
Method:

AC voltammetry

Theory on the detection principle

Specific interactions:

detection of biotin-streptavidin interaction



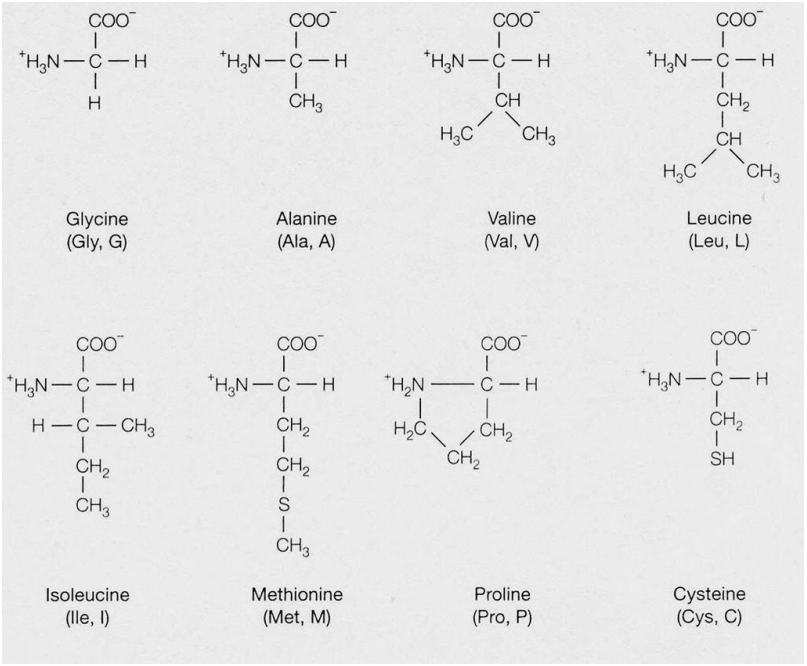
Proteins

Proteins have numerous functions: building components of muscles, carry oxygen in the blood, antibodies, enzymes, etc.

Proteins are chains of amino acids and can have different sizes (e.g. the synthetic sweetener in Diet Coke has only 2 amino acids, while hemoglobin is composed of a sequence of 600)

There are 20 possible amino acids

a few examples →



Amino Acids

DNA runs a series of “programs” (the *genes*) every time a cell needs to make given proteins – it tells the cell exactly what sequences of amino acids to produce

Every 3 nucleotides specifies 1 amino acid and some combinations give the instruction to STOP production

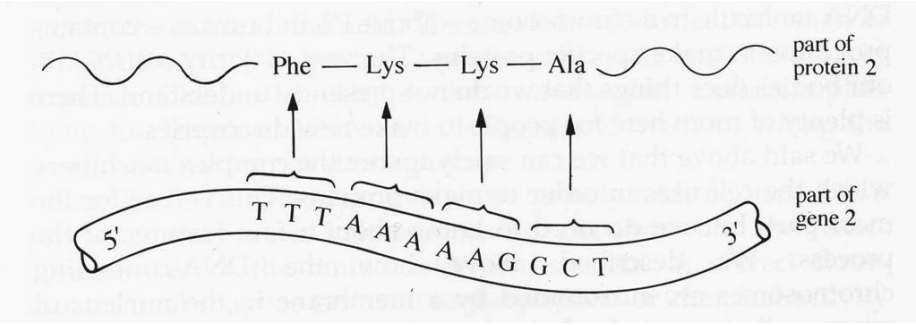
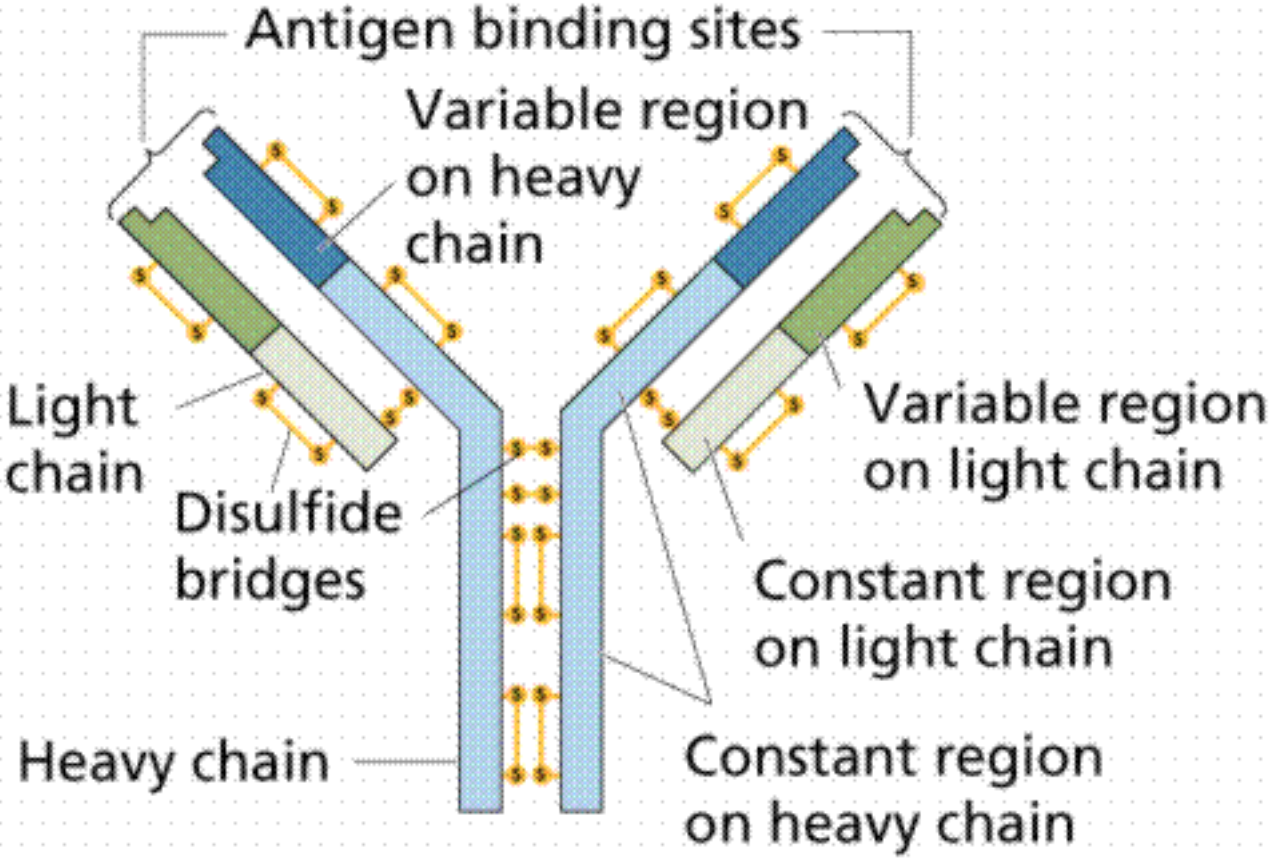


Table 1.1 The Genetic Code

1st base	2nd base				3rd base
	T	C	A	G	
T	Phe	Ser	Tyr	Cys	T
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	T
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Protein-Protein: Antibody-Antigen Interaction



Motivation of protein detection

Biomarker identification

Protein interaction studies

Immunological profiling

Vaccine development

Protein Microarray

- Valuable platform for proteomic analysis
- Simultaneously high throughput analysis

Challenge: lack of a PCR-equivalent amplification process
-> high sensitive detection for low concentration proteins in complex mixtures

Label-based methods

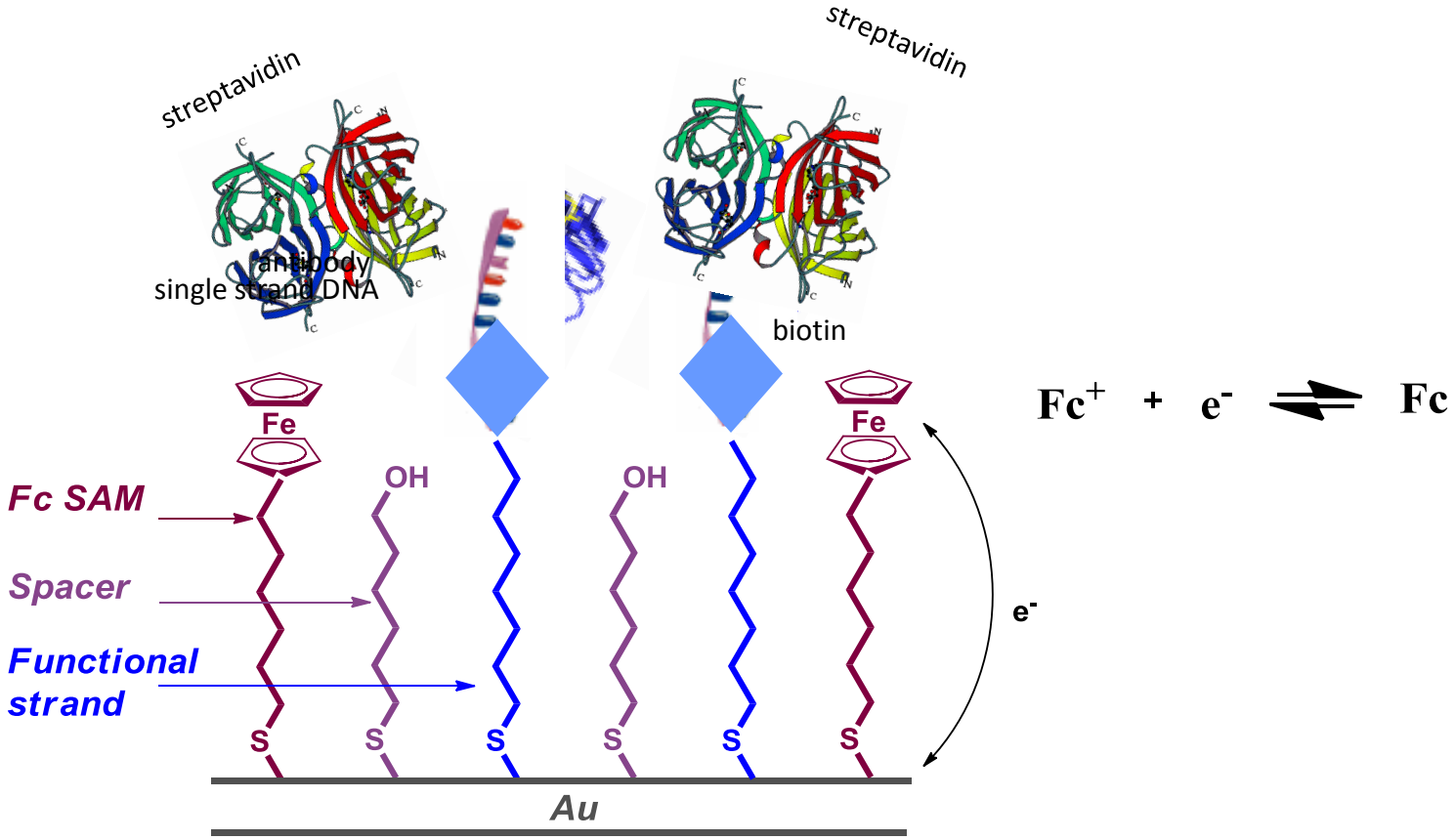
Fluorescence-
Radioactive isotopes-
Bead-based-
SERS nanoparticle-
Au nanoparticle-
Quantum dots-

Label-free methods

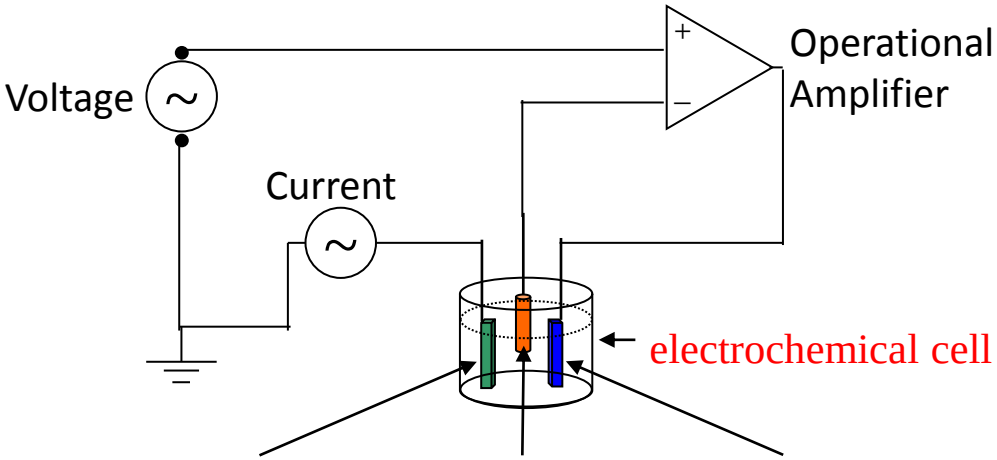
-Surface plasmon resonance
-Nanotubes / nanowires
-Microcantilevers
-Redox active SAM
-Peptide aptamer
(subpico molar detection)

Generic Redox SAM Platform

- Ferrocene redox self-assembly monolayer (SAM)
  - flexible
  - label-free
- Intrinsic charge of the target molecules modifies the electrochemical characteristic, formal potential $E^{o'}$, of the redox SAM



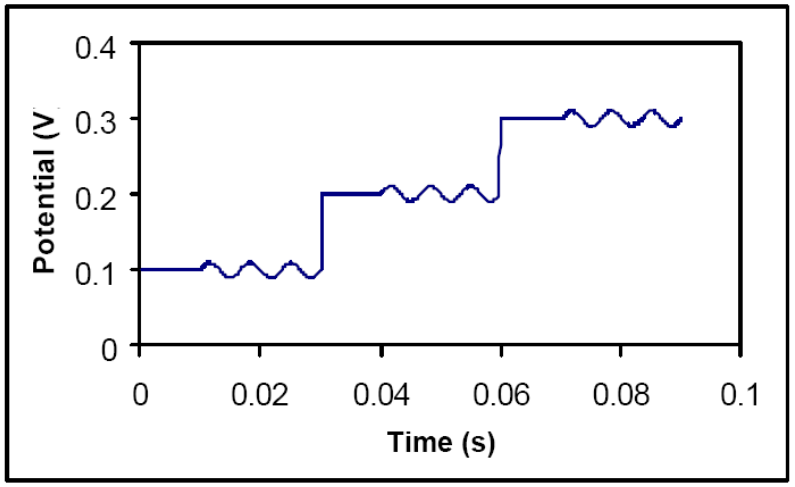
Instrumentation – AC voltammetry

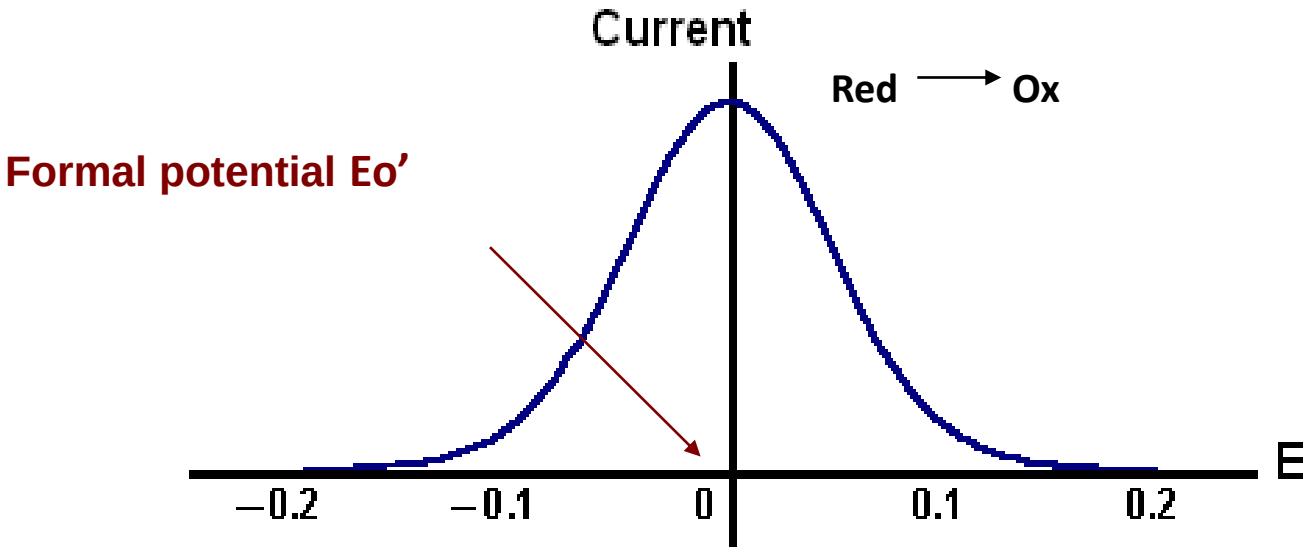


Mixed SAM functionalised Au working electrode

reference electrode

counter electrode





Protein Detection I

Motivation of protein detection

Detection platform:

Ferrocene redox self-assembled monolayer (SAM) detection platform

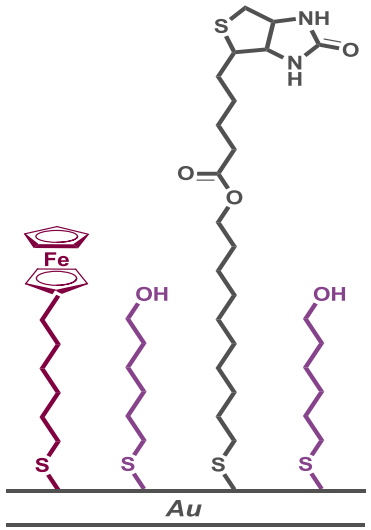
Method:

AC voltammetry

Theory on the detection principle

Specific interactions:

detection of biotin-streptavidin interaction

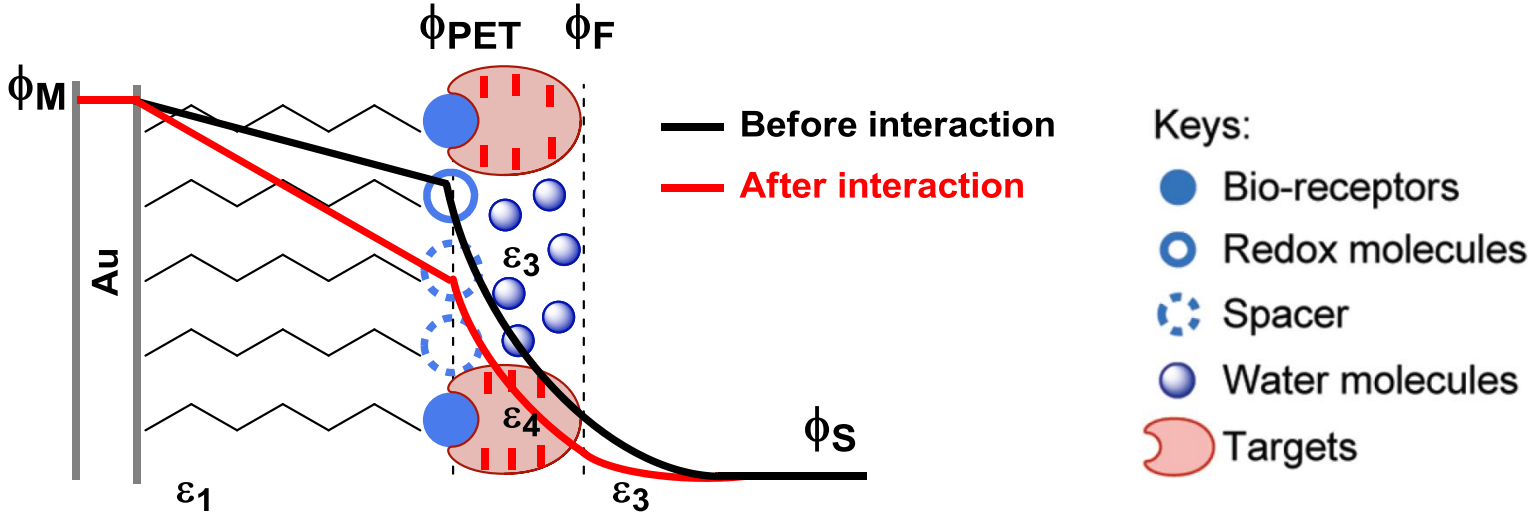


Theory - Detection Principle

Detecting the change in formal potential, $E^{o'}$

- intrinsic charge of the target molecules causes a change in ϕ_{PET}

-> directional shift in formal potential ($E^{o'} = E^{o} + \phi_{PET} - \phi_{S}$)



ϕ_M : potential at the metal

ϕ_{PET} : potential at plane of electron transfer, PET

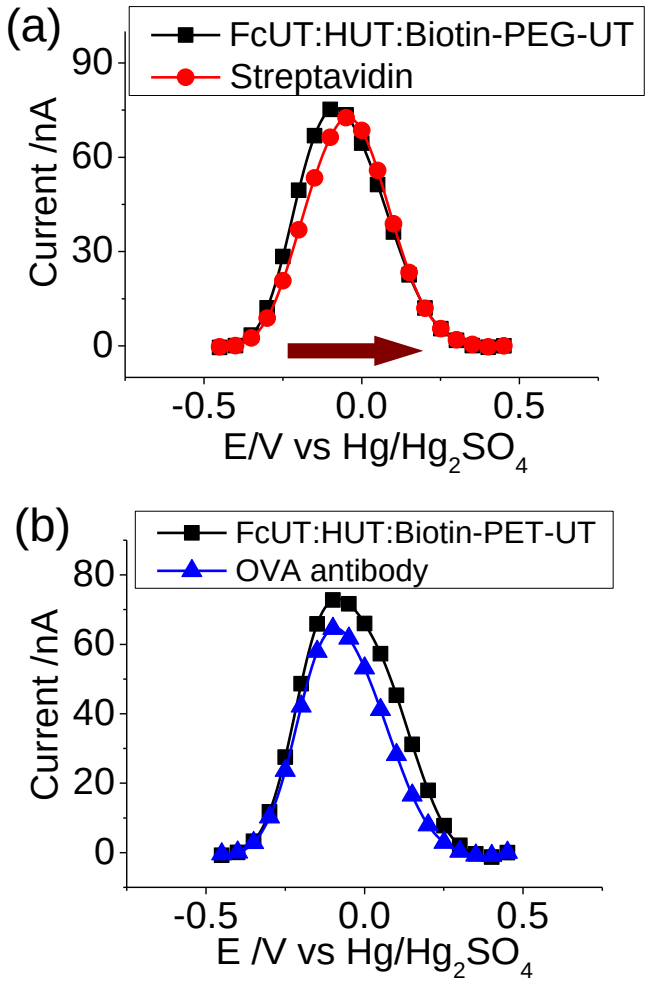
ϕ_S : potential at the solution

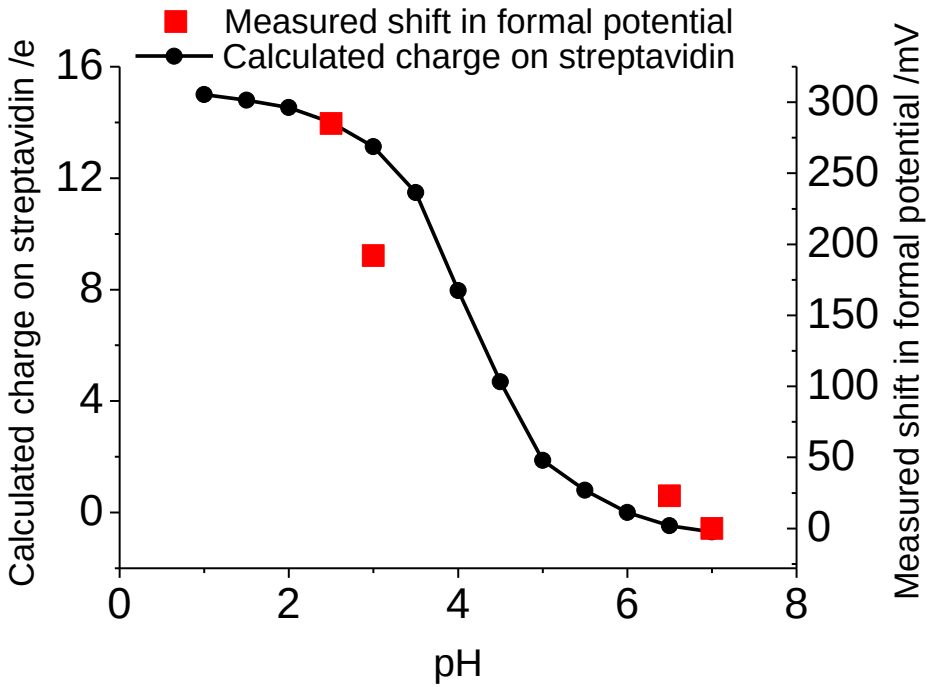
ε_1 : dielectric constant of the SAM

ε_3 : dielectric constant of the electrolyte

Detection of Biotin-Streptavidin Interaction

- A positive shift in $E^{o'}$ was observed upon interaction due to the net positive charge of streptavidin at pH2.5 13mM phosphate buffer





Good agreement was found between the shift in $E^{o'}$ and the theoretical calculation of the net charge of streptavidin[1]

2. Proteine M.M., pI, composition, titrage programme (ABIM, France: http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim/

Protein Detection II

Peptide aptamer detection platform

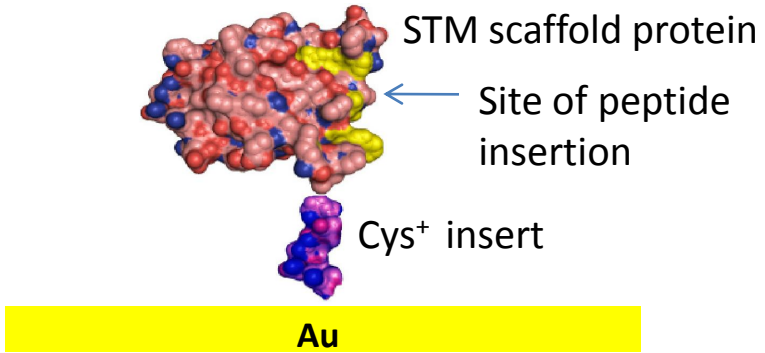
Field effect transduction methods:

- open circuit potential (OCP)
- metal oxide semiconductor field effect transistor (MOSFET)

Real time detection with OCP

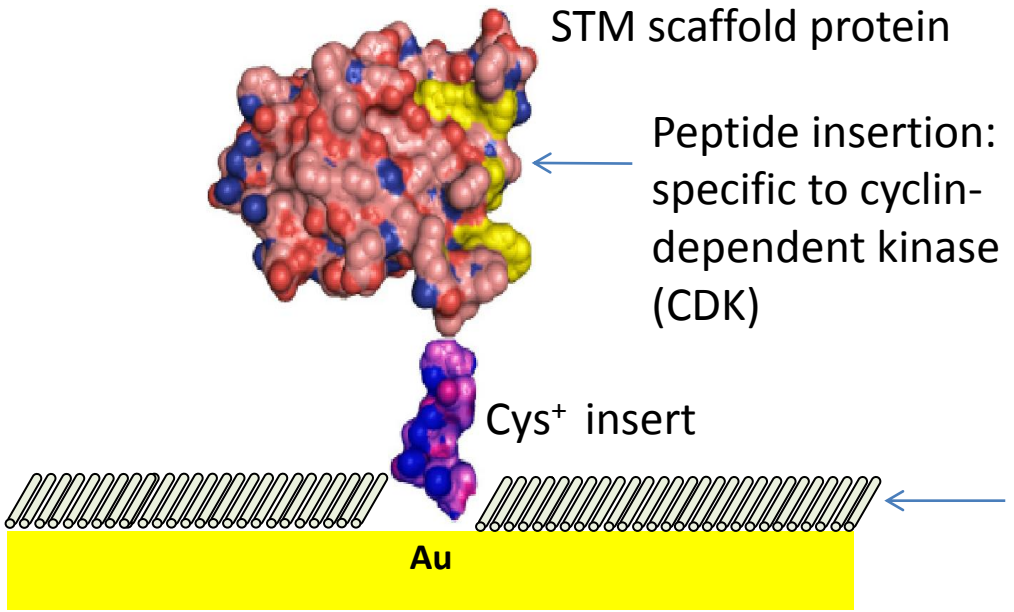
Linear array MOSFET

- detection principle
- fabrication and layout
- detection of CDK



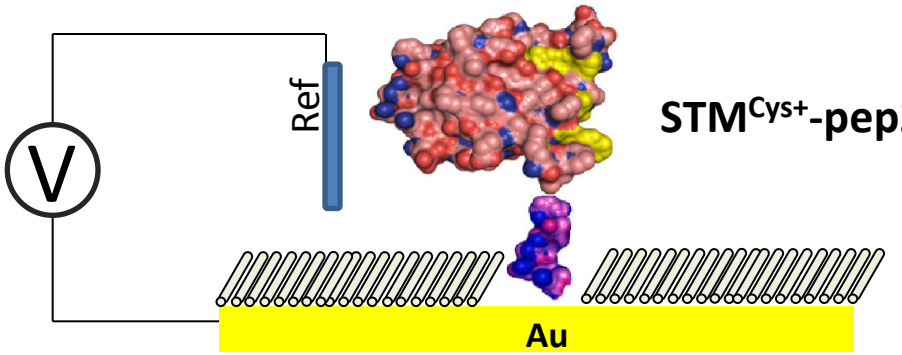
Peptide Aptamer Platform

- Scaffold protein: STM with Cys insert -> -SH group for Au immobilization
- Advantages over antibodies:
  - Does not lose specificity when immobilized on surfaces
  - STM scaffold lacks affinity for human proteins
  - Flexibility: immobilization of STM scaffold protein is optimized, just need to change the peptide insertion for different targets



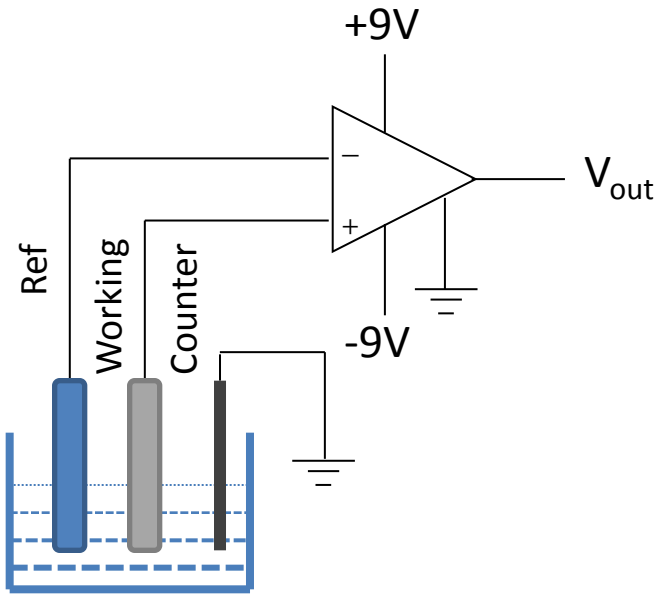
Field Effect Detection with Peptide Aptamer

Peptide aptamer platform:

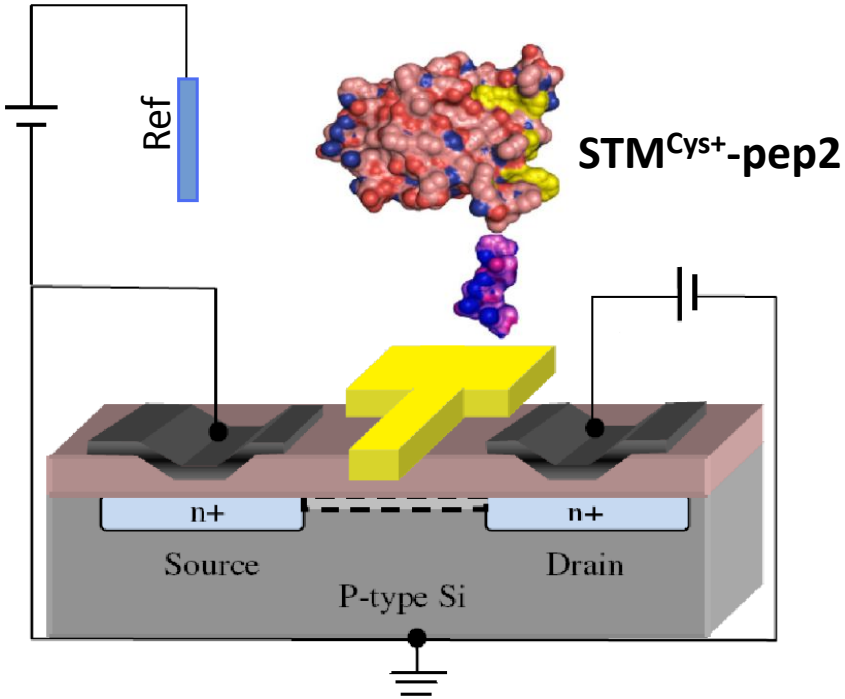


Field effect transduction methods:

i) Open circuit potential (OCP)



ii) Metal oxide semiconductor field effect transistors (MOSFET)



Protein Detection II

Peptide aptamer detection platform

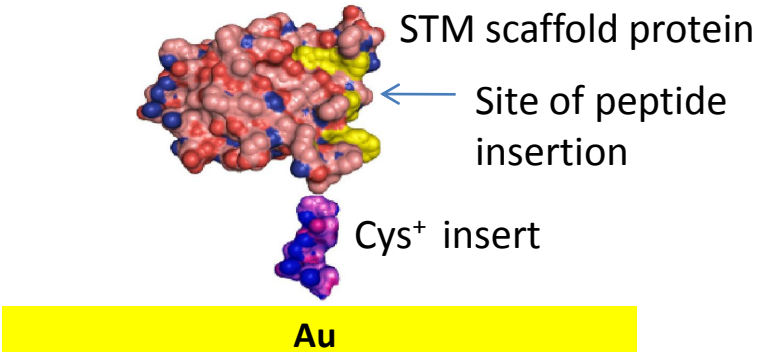
Field effect transduction methods:

- open circuit potential (OCP)
- metal oxide semiconductor field effect transistor (MOSFET)

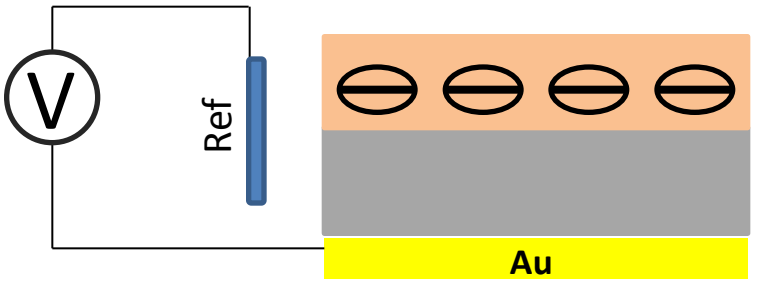
Real time detection with OCP

Linear array MOSFET

- detection principle
- fabrication and layout
- detection of CDK

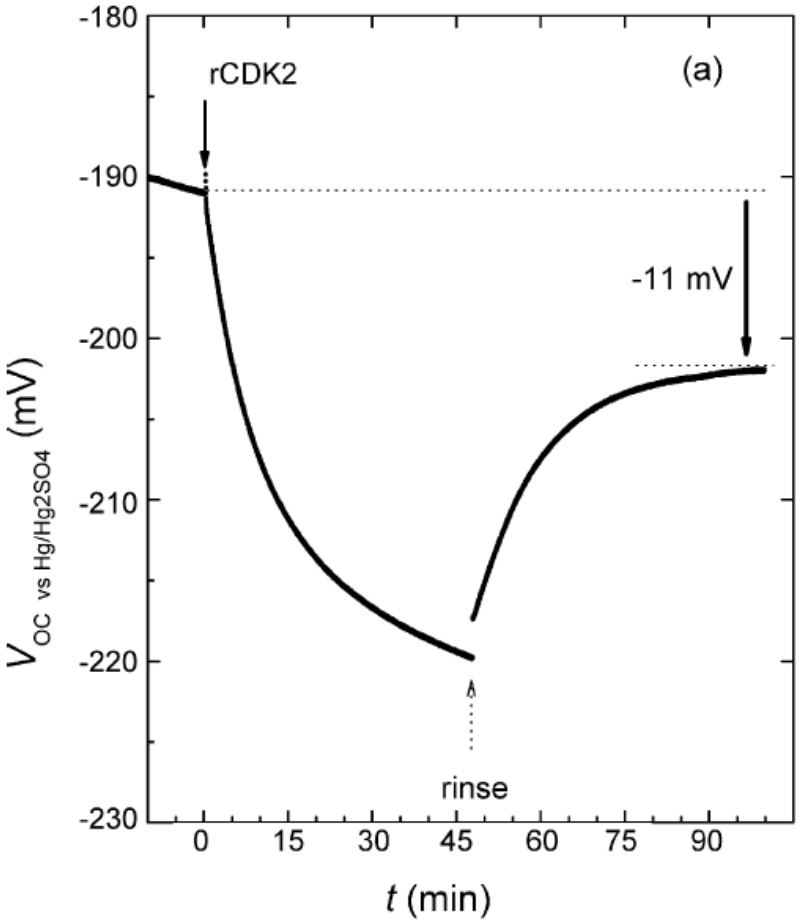


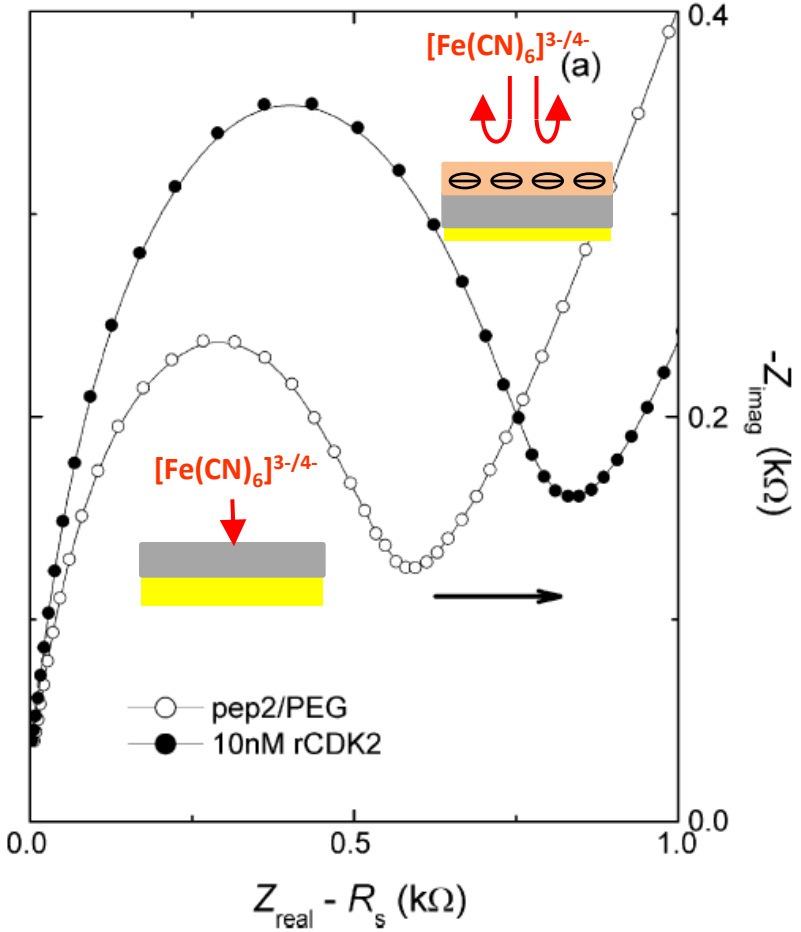
Open circuit voltage (OCP) – Specific Interaction with CDK2



Negatively charged
Cyclin-dependent kinase (CDK2)

STM^{Cys+}-pep2





P Estrela, D Paul, P Li, S D Keighley, P Migliorato, Electochimica Acta **2008**, 53, 6489

Protein Detection II

Peptide aptamer detection platform

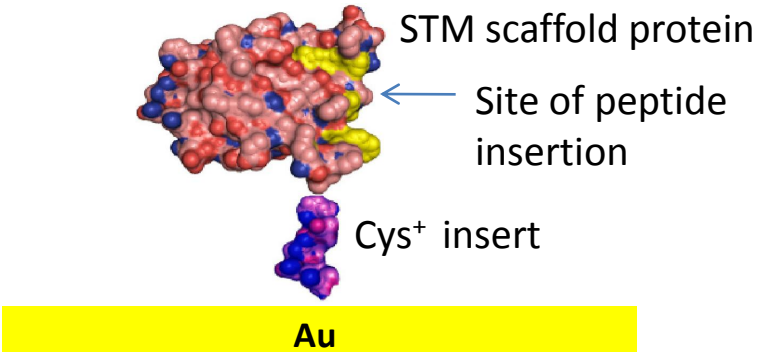
Field effect transduction methods:

- open circuit potential (OCP)
- metal oxide semiconductor field effect transistor (MOSFET)

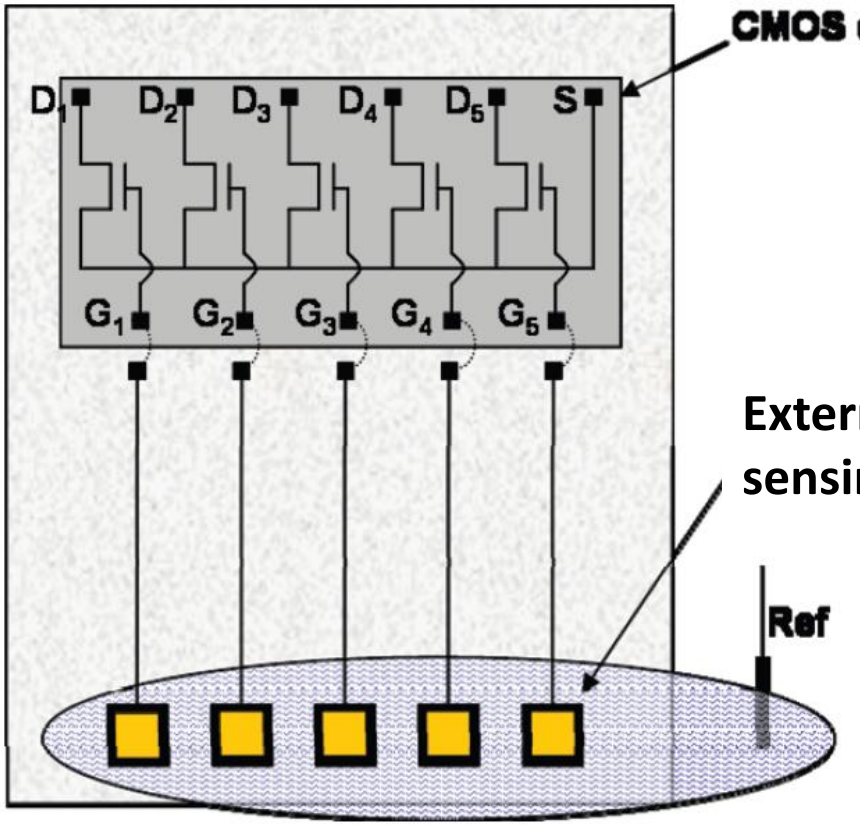
Real time detection of CDK with OCP

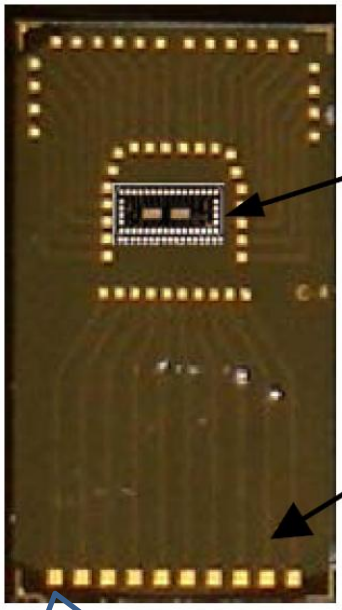
Linear array MOSFET

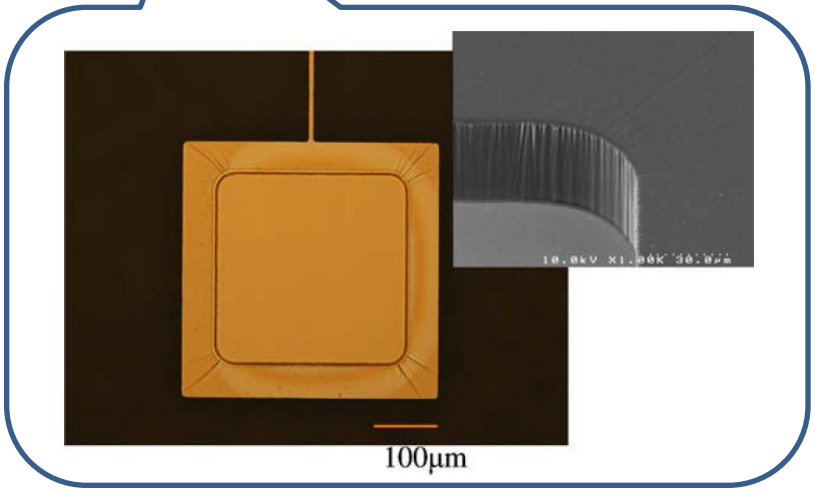
- detection principle
- fabrication and layout
- detection of CDK



MOSFET Chip Design – Extended Gate Structure





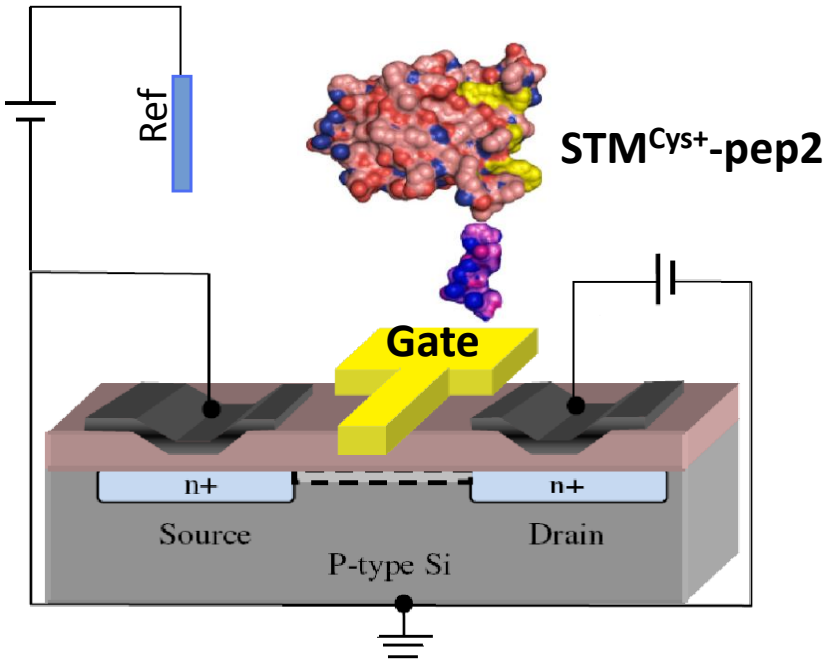


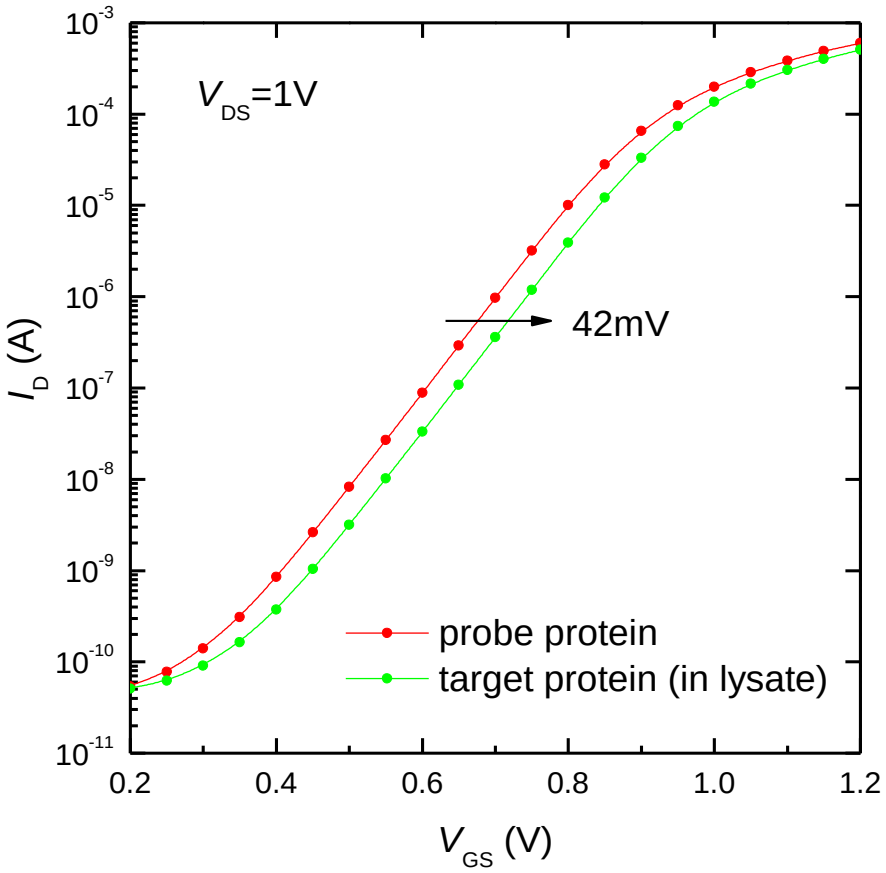
P Estrela, D Paul, J J Davis, P Migliorato,

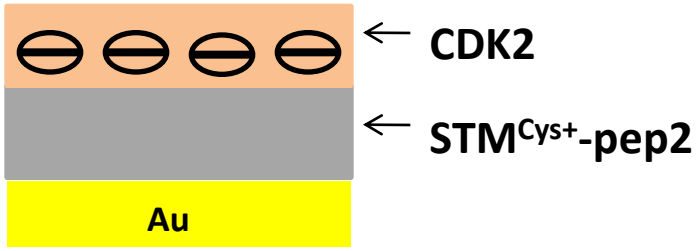
Anal. Chem. 2010, *82, 3531–3536*

L Wang, P Estrela, Ejaz Huq, P Li, P Migliorato, Microelectronic Engineering 2010, 87, 753

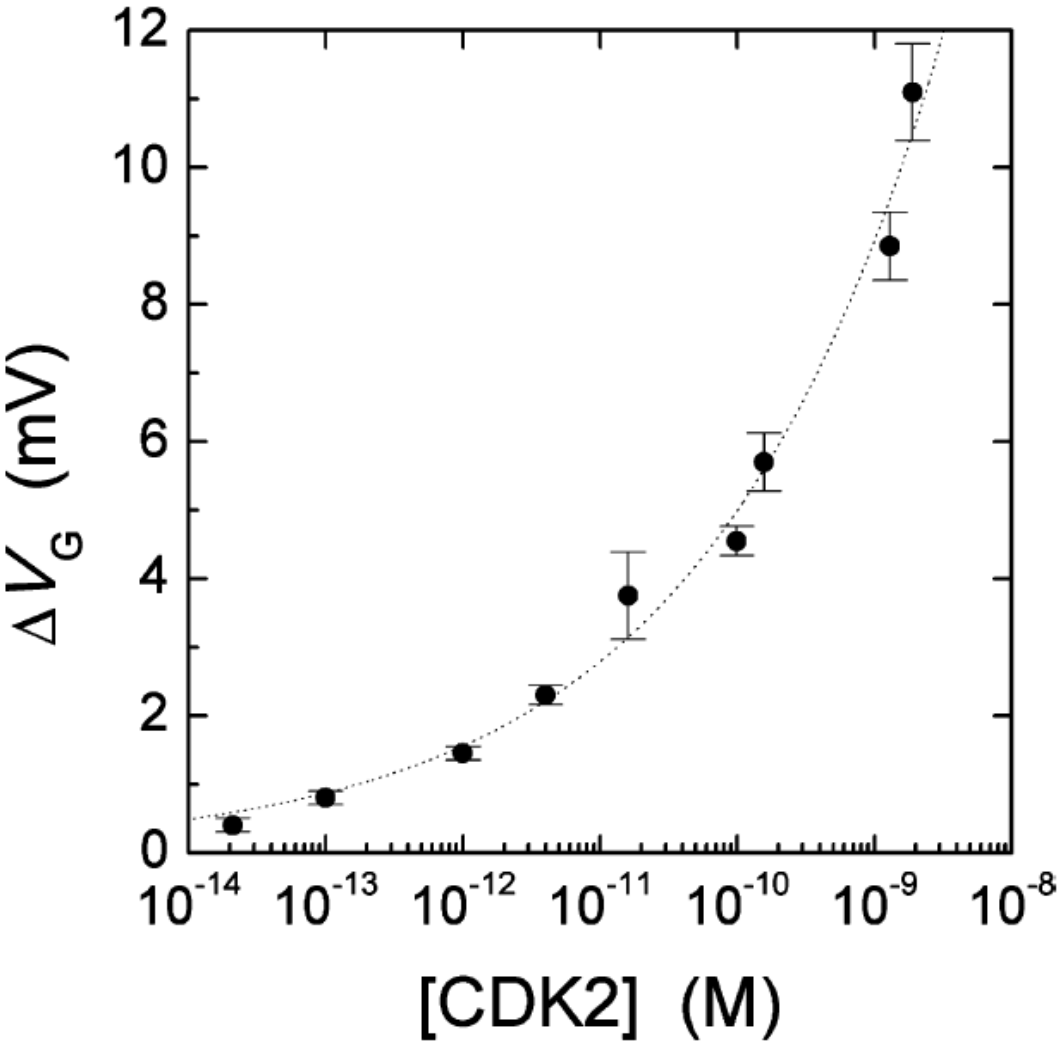
MOSFET Detection







MOSFET – Specific Interaction with CDK2



Sensitivity : 100 fM, within clinical range

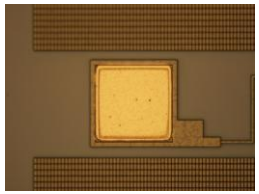
P Estrela, D Paul, J J Davis, P Migliorato, Anal. Chem. 2010, *82, 3531–3536*

Conclusion

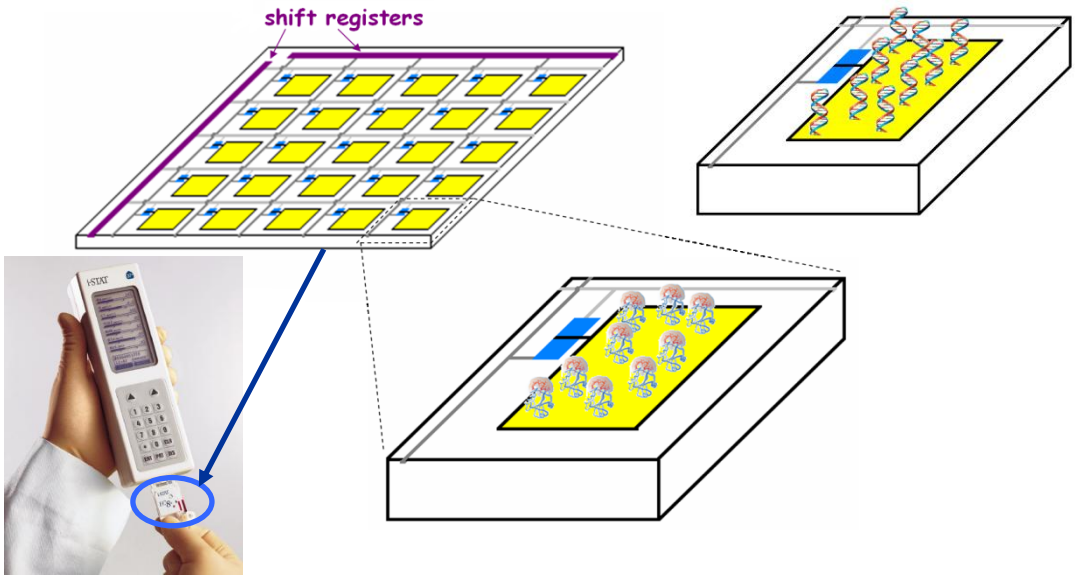
Our platforms and electrical detection methods allows:

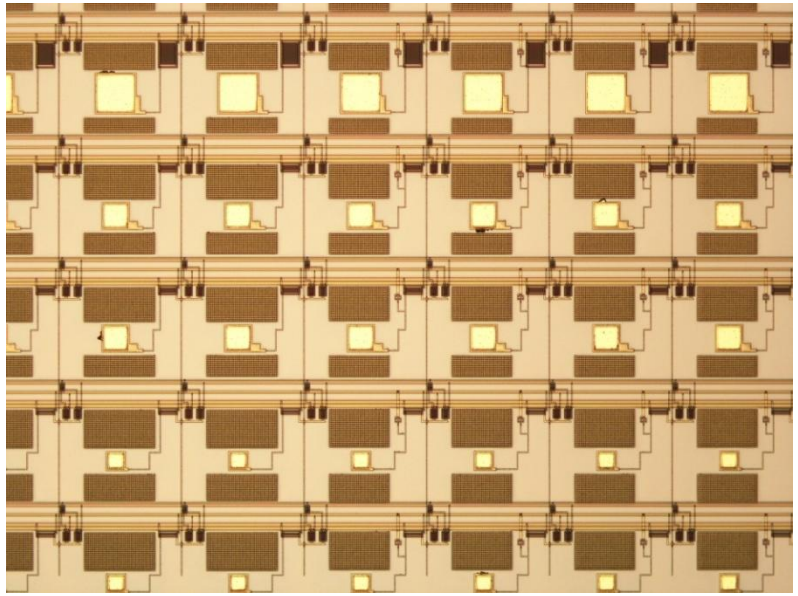
- Label free detection
- Low cost disposable micro-arrays
- Multiplexed
- Portable systems
- Simultaneous detection
- High throughput

Sensing pad with SU8 well 80x80µ

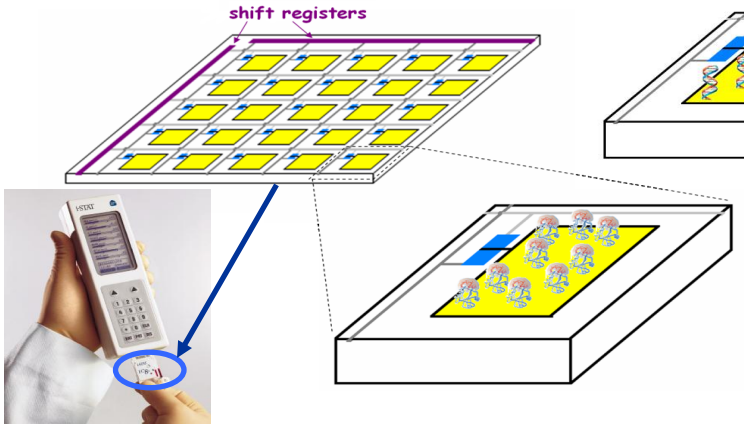


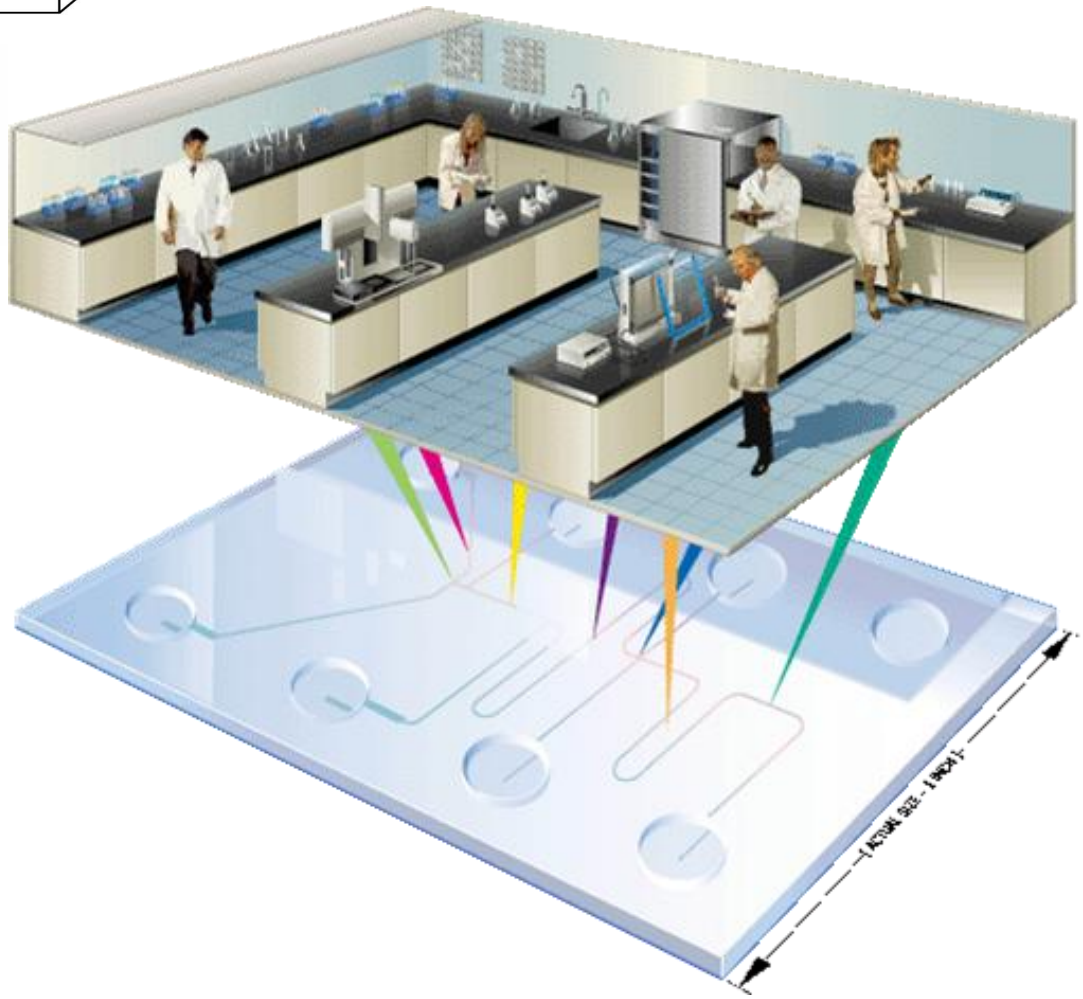
DNA / Protein Multiplexed Microarry





Future direction: Lab-on-chip



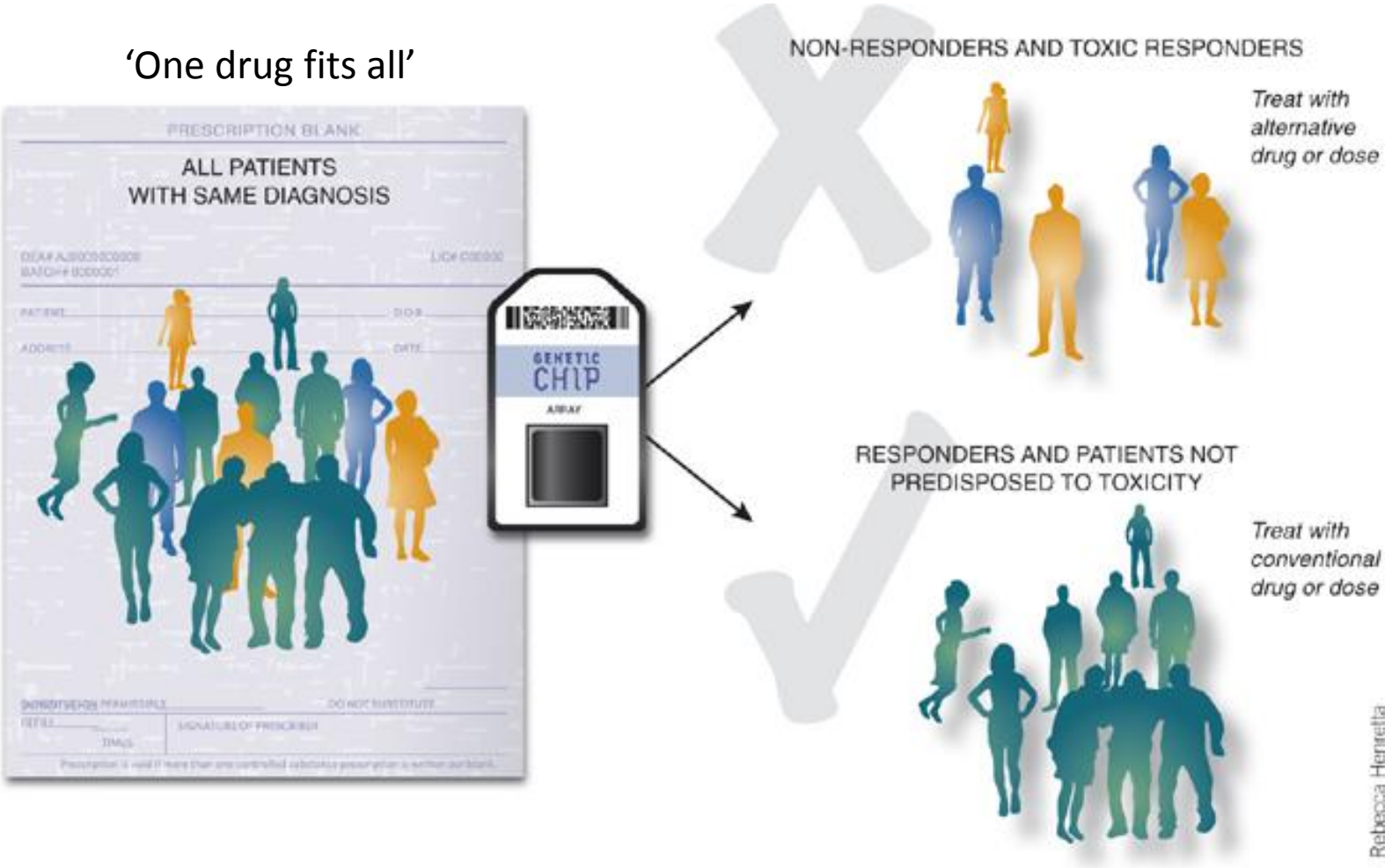


www.gene-quantification.de

Future direction: personalized medicine

- **Right drug, right dose, right time to the right patient**

‘One drug fits all’



Drug therapy is chosen for each patient based on their particular genetic profile

M. Piquette-Miller, D. M. Grant, *Clinical Pharmacology & Therapeutics* (2007) **81,** 311–315.