Nanoscale Design of Biosensors for Toxicity Screening and Biomedical Applications

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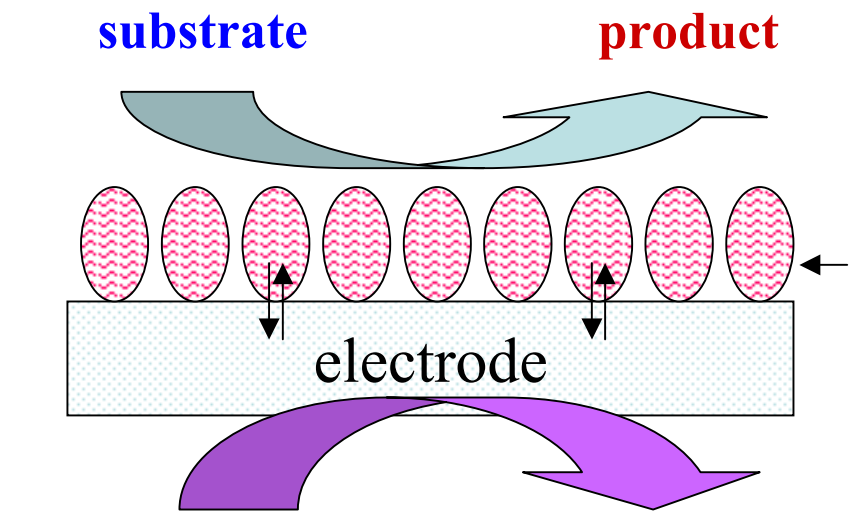
Traditional Electrochemical Biosensors



Enzyme, or
Label on Ab/Ag
Or DNA

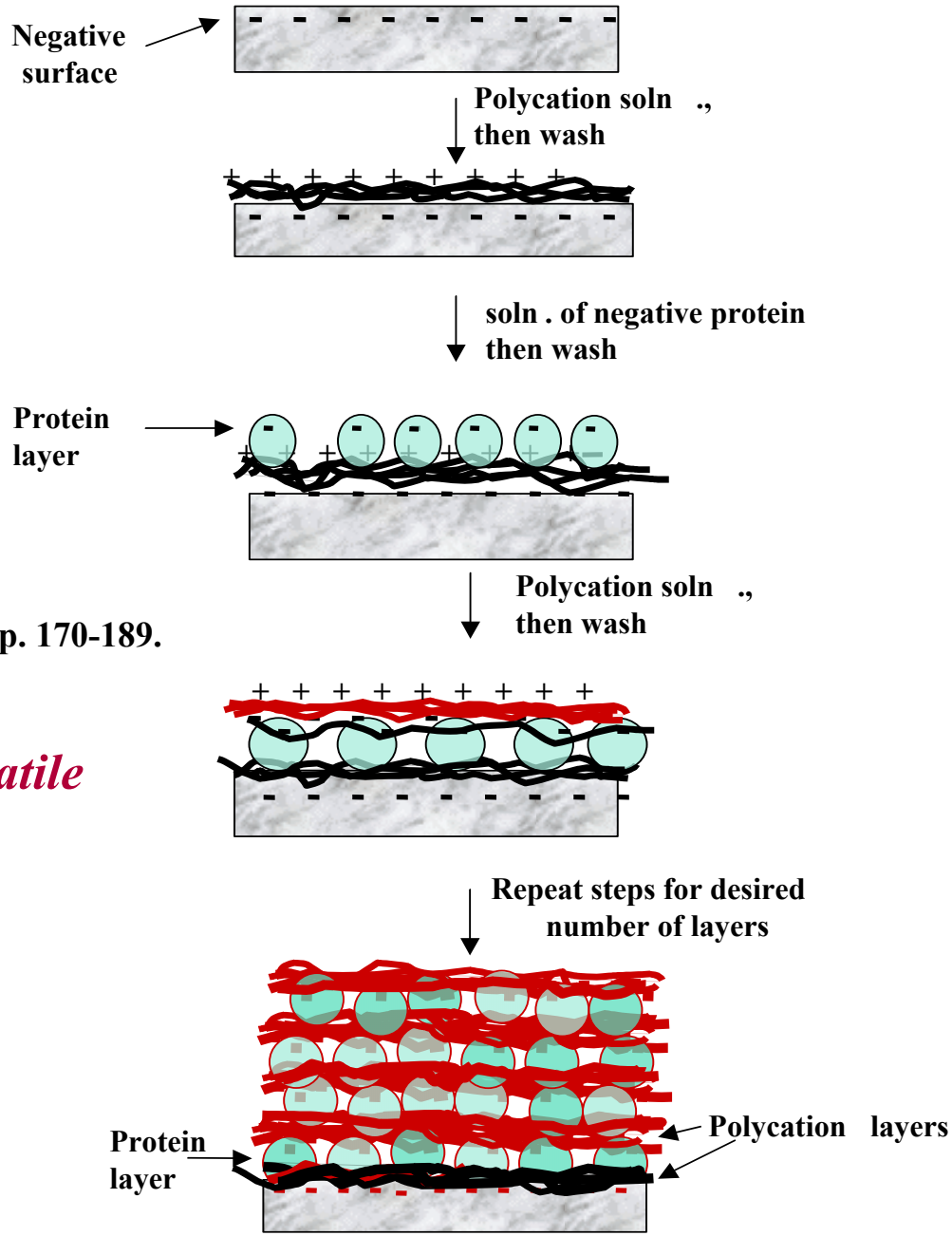
Apply voltage

Measure current prop.
to concentration of substrate

- nanoscale biosensing architecture
- patternable nanomaterials for arrays

Layer-by-layer Film assembly

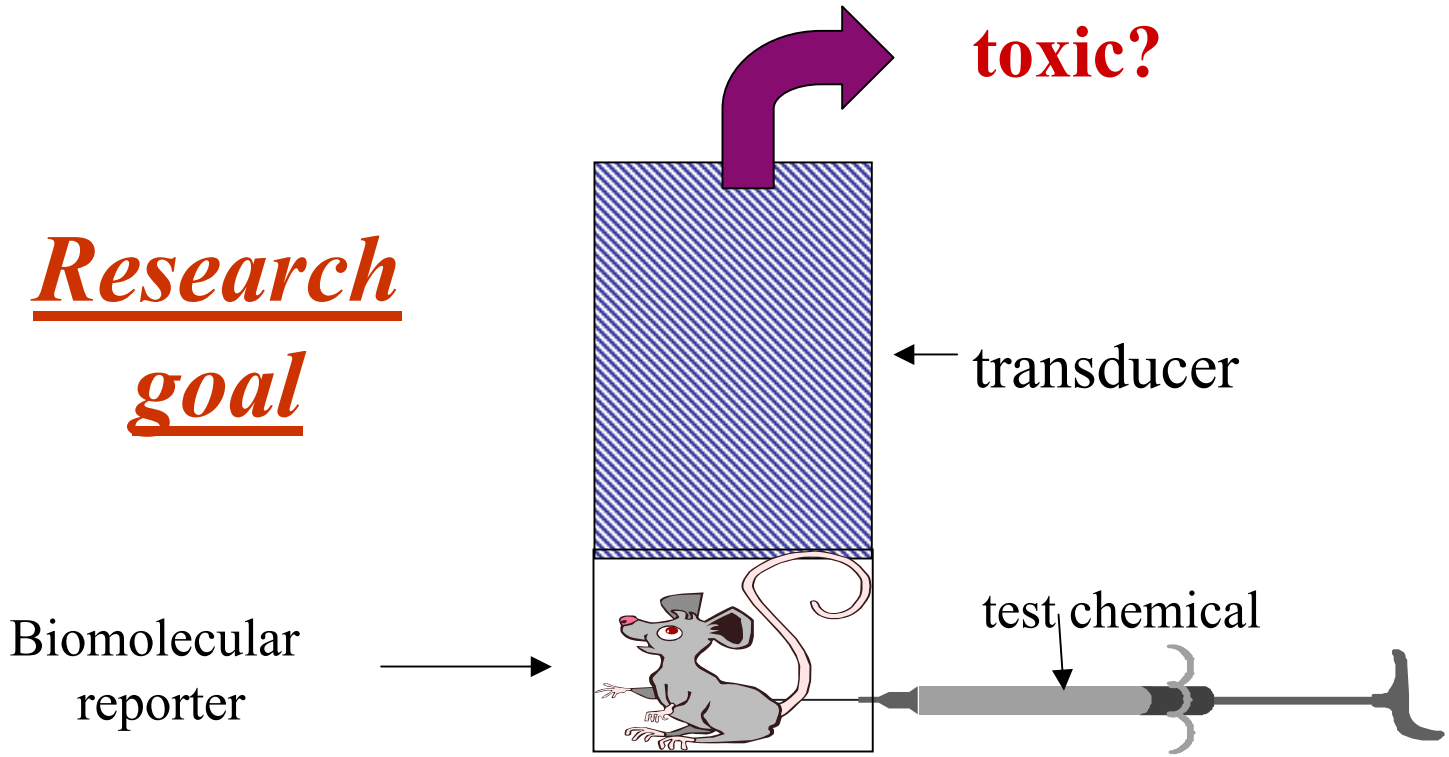
Lvov, Decher



Lvov, Y. in Nalwa, R.W.; Ed.;
Handbook Of Surfaces And Interfaces Of Materials, Vol. 3. Academic, 2001, pp. 170-189.

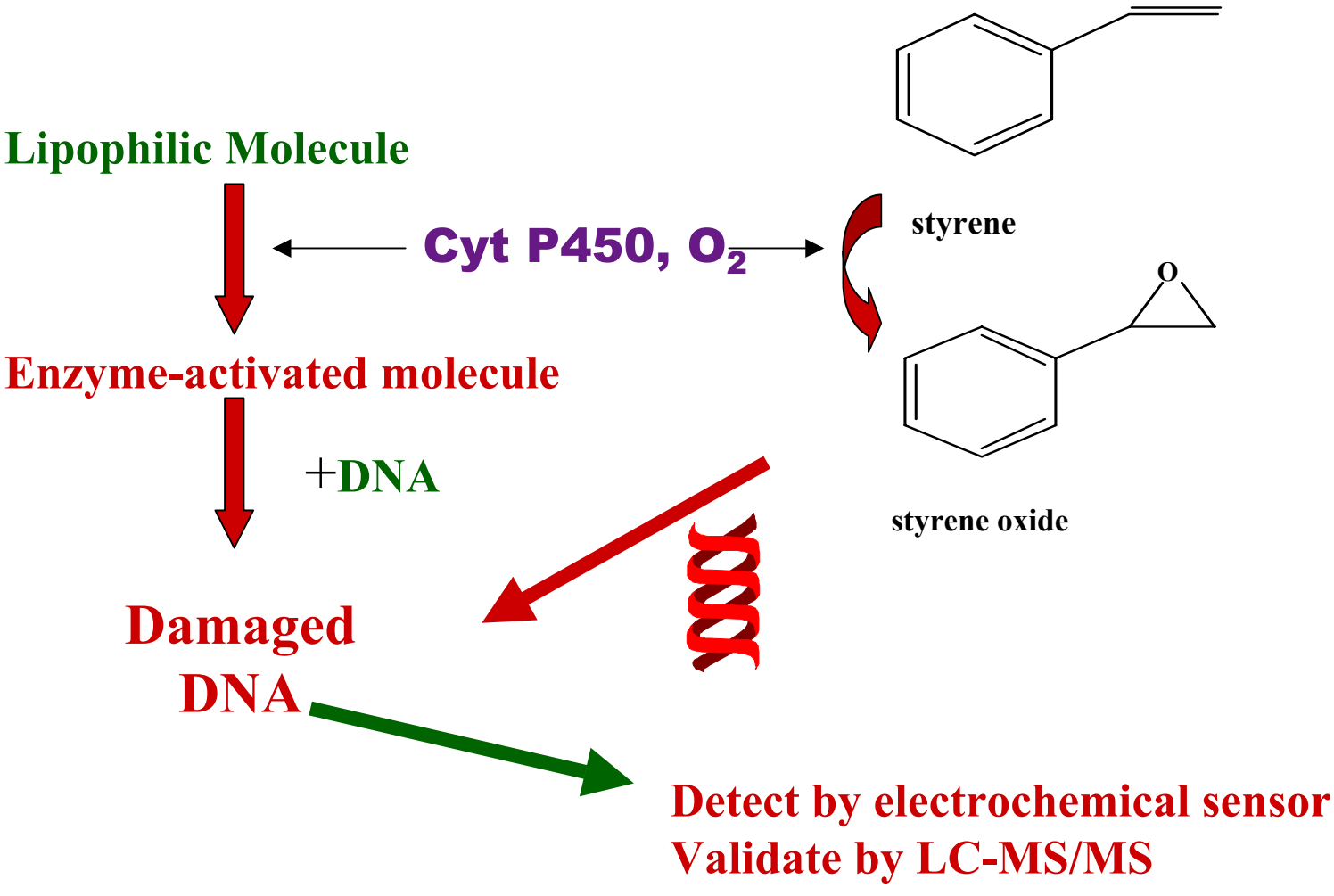
Stable, easily prepared, versatile

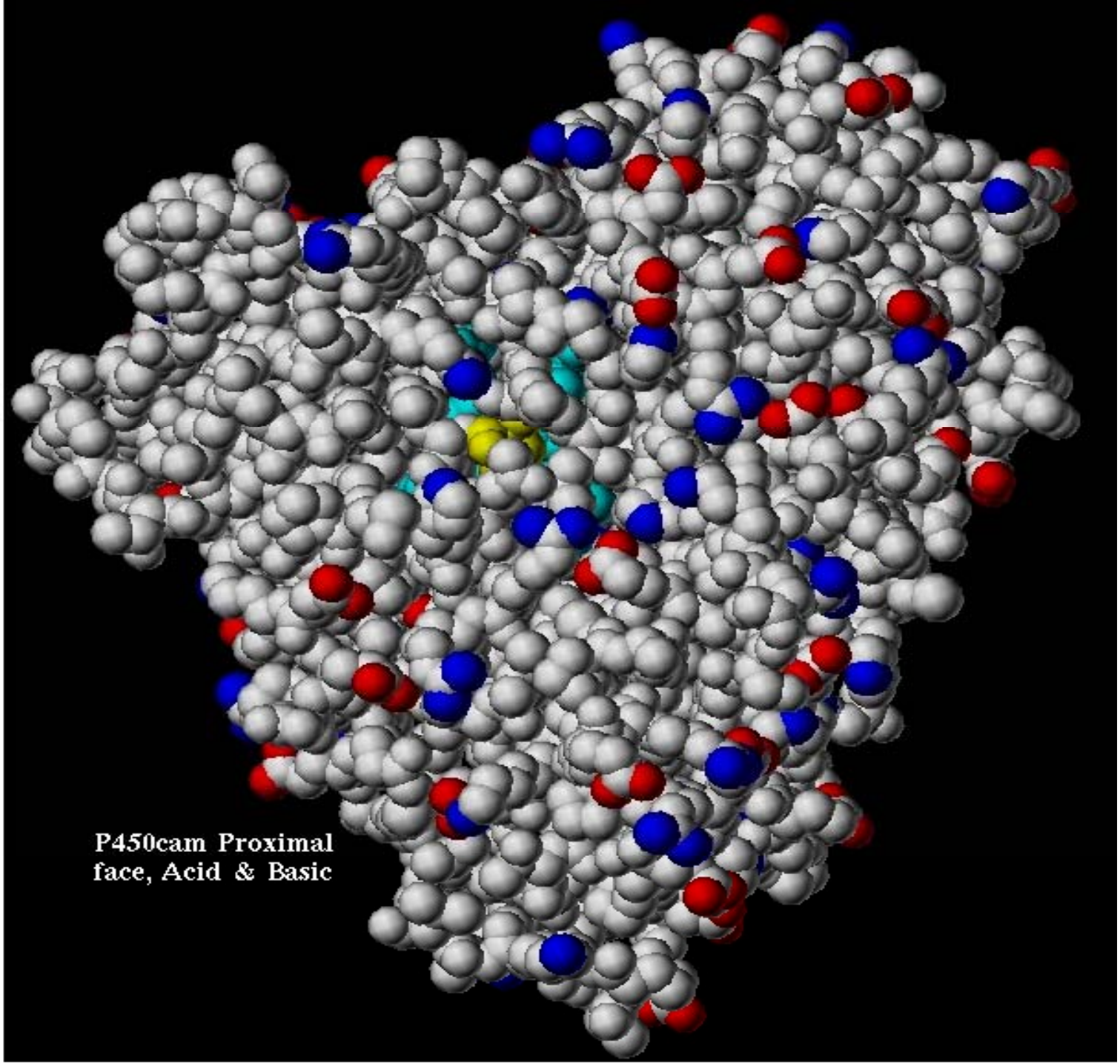
Research goal



- ***~ 30 % of drug candidates defeated by toxicity***
- ***Early screening could save drug development costs***

In vitro Toxicity Screening

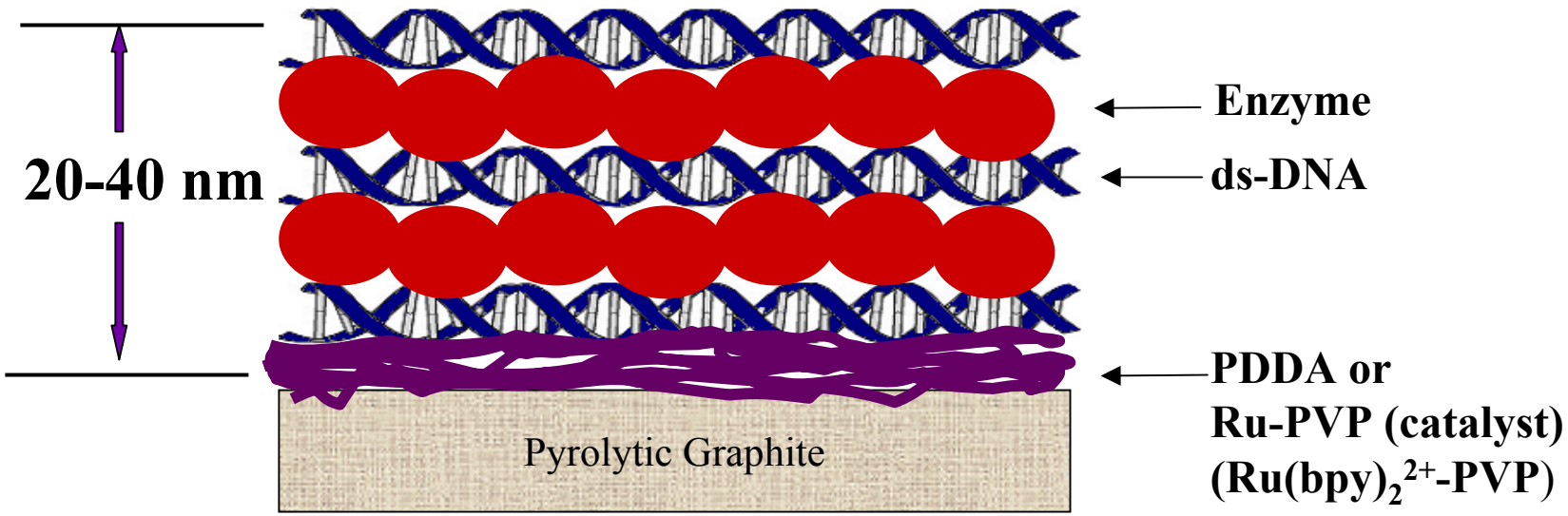



P450cam Proximal
face, Acid & Basic

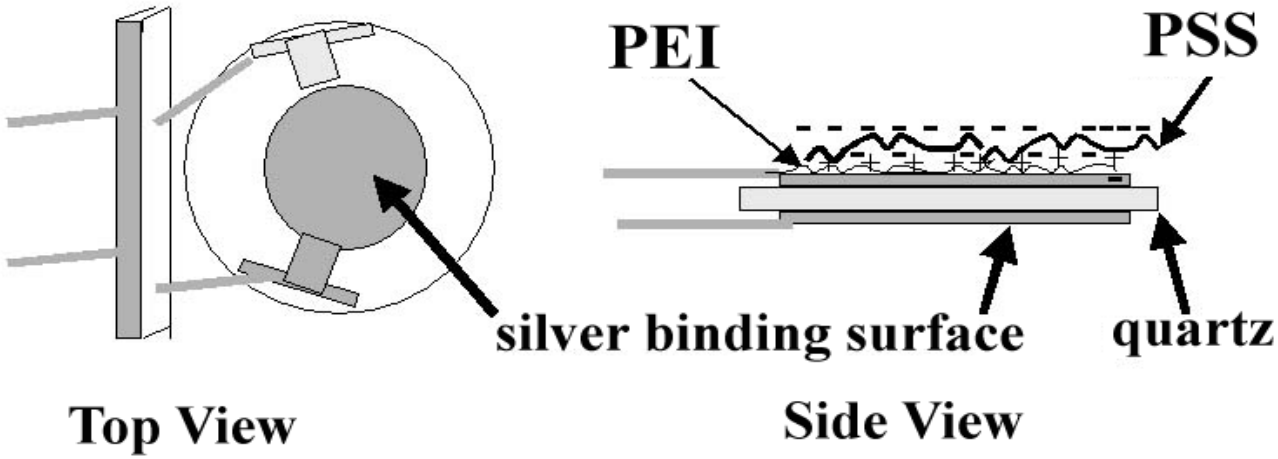
Collaboration with Prof. John Schenkman, Pharmacology, Uconn Health Center

Funding from NIH, NIEHS

Films for Toxicity Screening

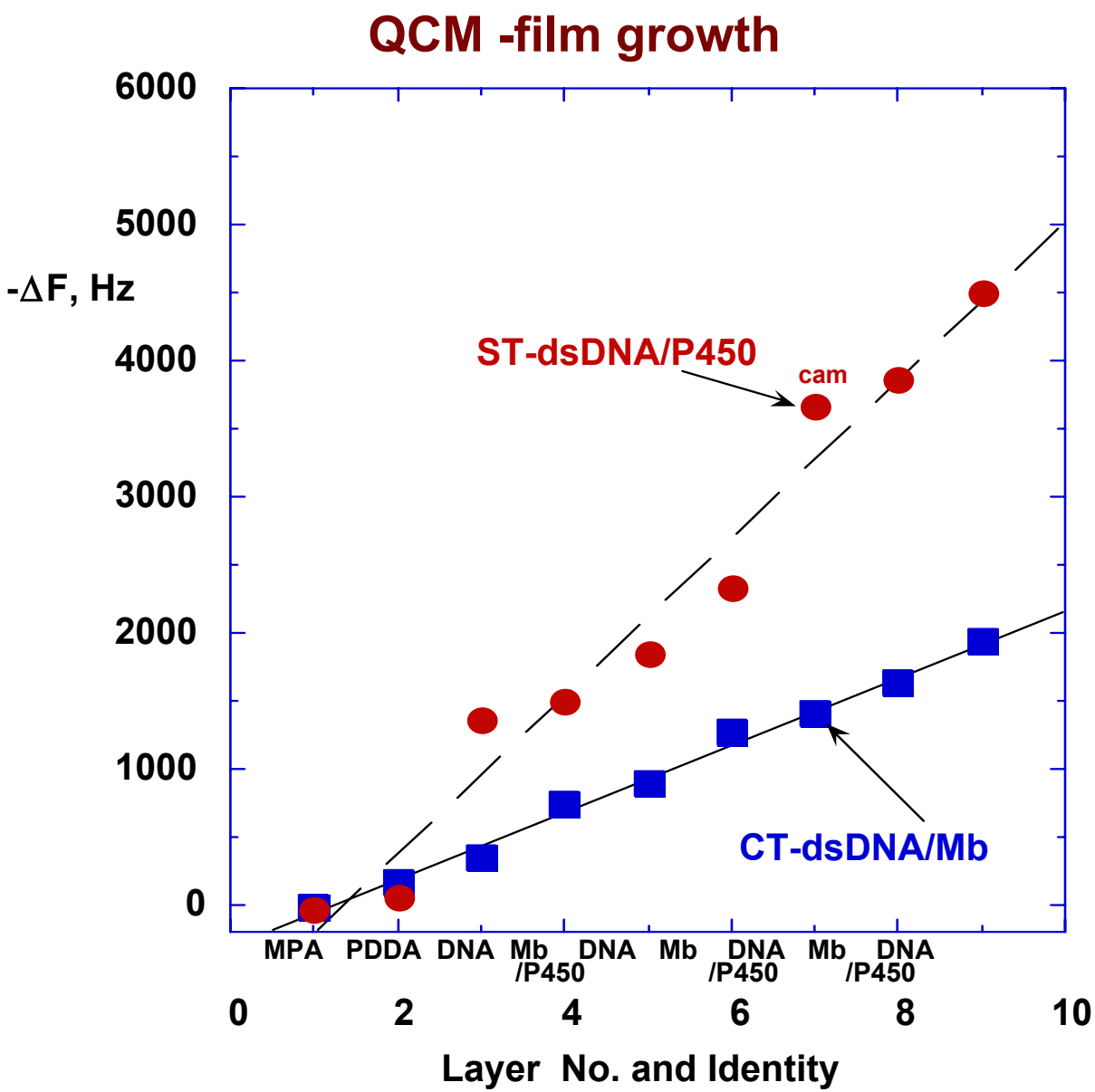


QCM Resonator

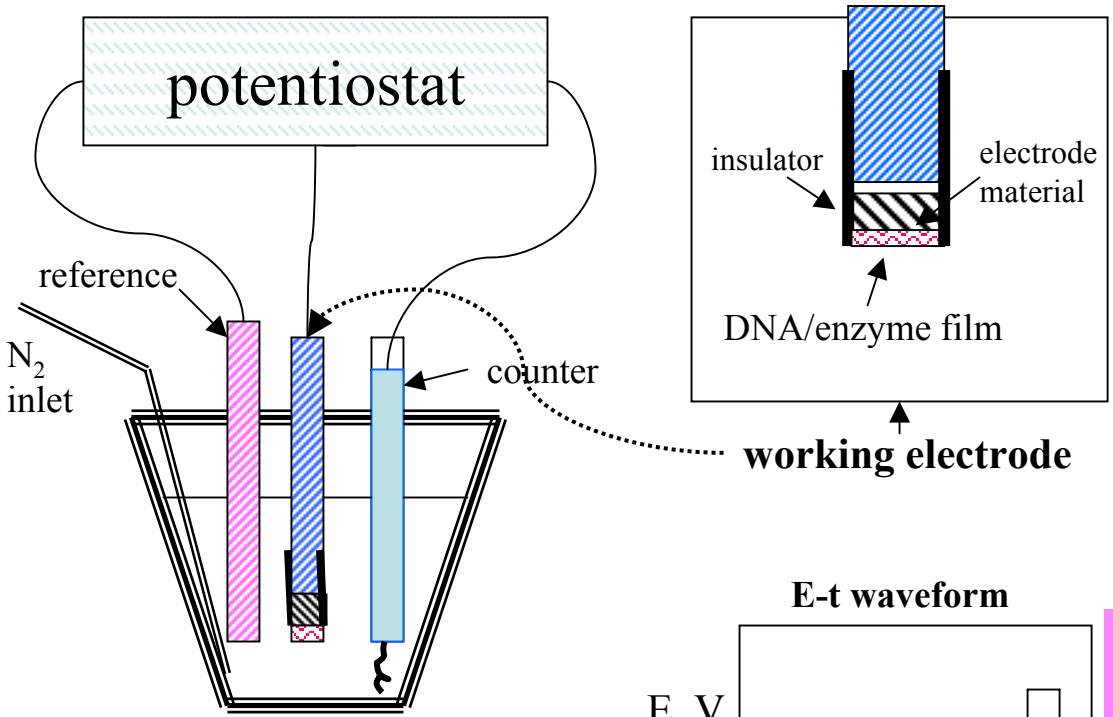


Mass: $M/A = -\Delta F/1.86 \times 10^8$

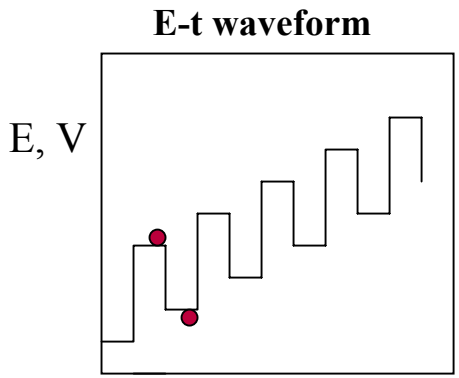
Thickness: $d = -(0.016)\,\Delta F$


QCM -film growth
6000
5000
-ΔF, Hz
4000
3000
2000
1000
0
ST-dsDNA/P450
cam
CT-dsDNA/Mb
MPA
PDDA
DNA
Mb /P450
DNA
Mb
DNA /P450
Mb
DNA /P450
0
2
4
6
8
10
Layer No. and Identity

Equipment for toxicity biosensors



Electrochemical cell



Square-wave voltammetry

- **I measured, then subtracted**

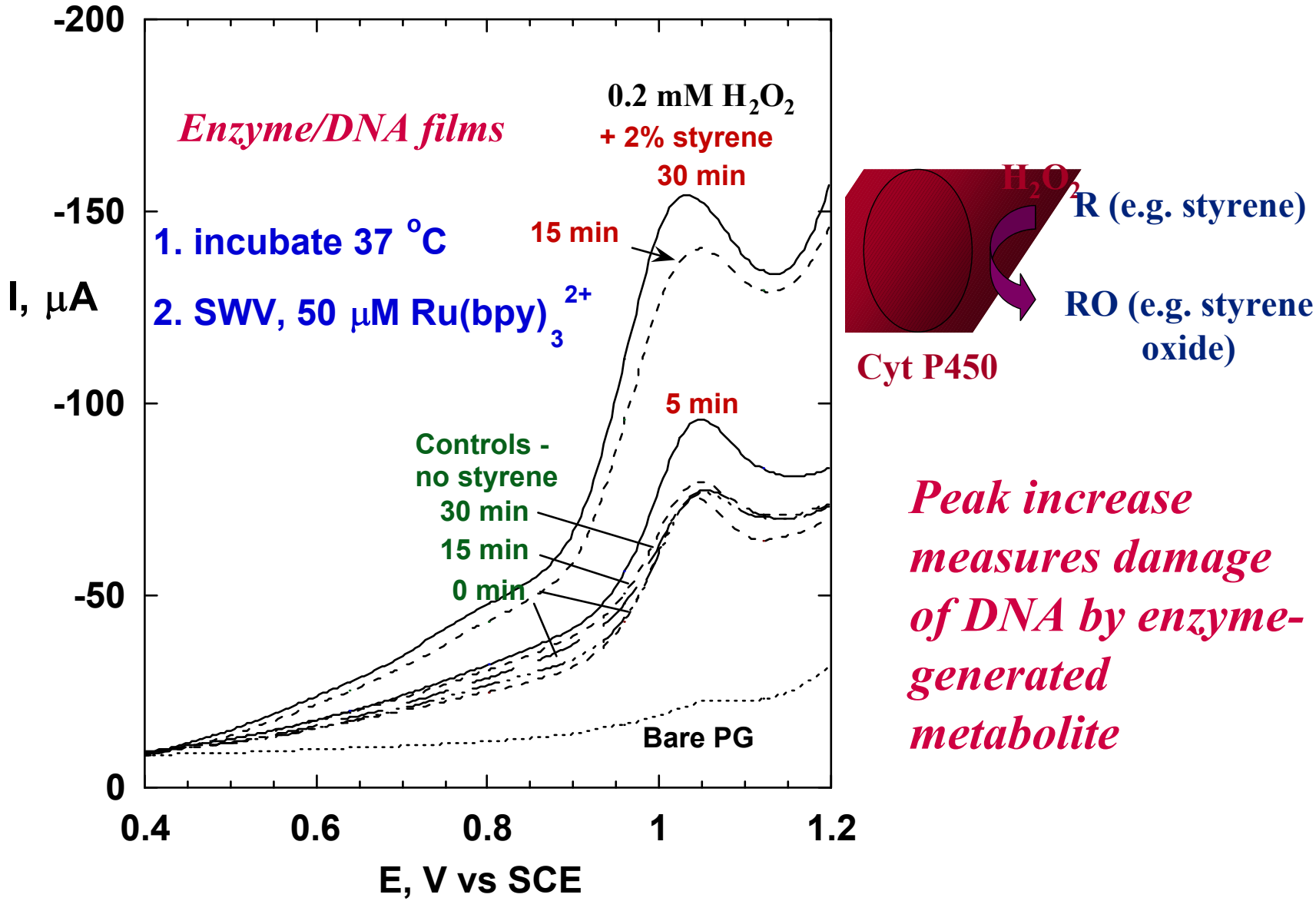
Screening Chemical Toxicity

Enzyme reaction - Incubate:
Reactant + H_2O_2-->metabolite

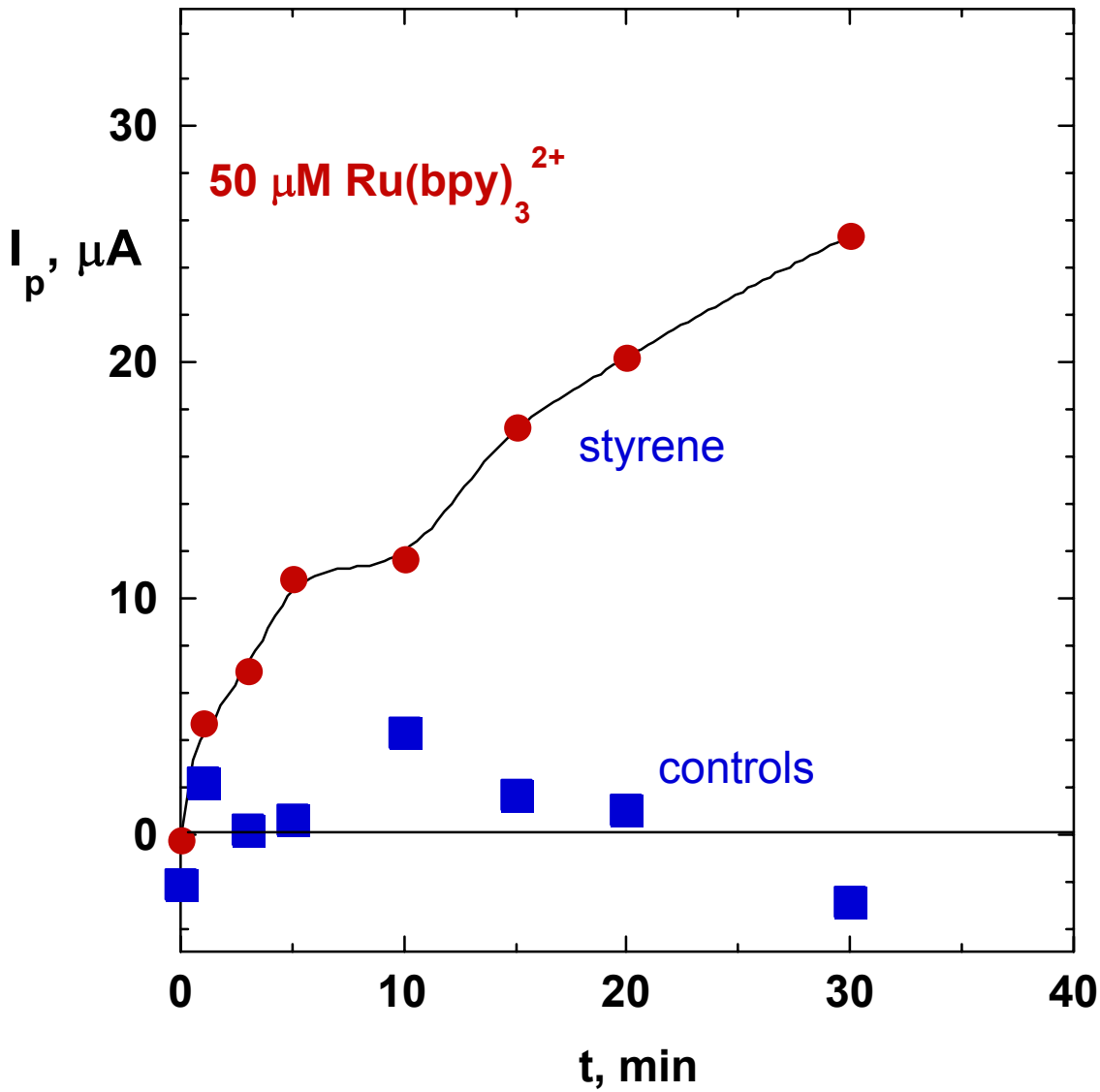
Analysis by catalytic SWV or electrochemiluminescence

$$RuL^{2+} = RuL^{3+} + e^-$$

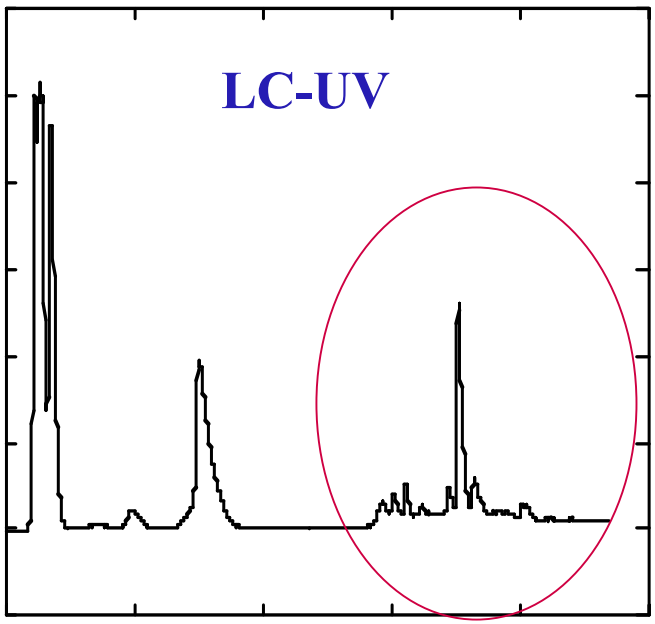
$$RuL^{3+} + \text{DNA-G} \rightarrow RuL^{2+} + \text{DNA-G}\bullet$$

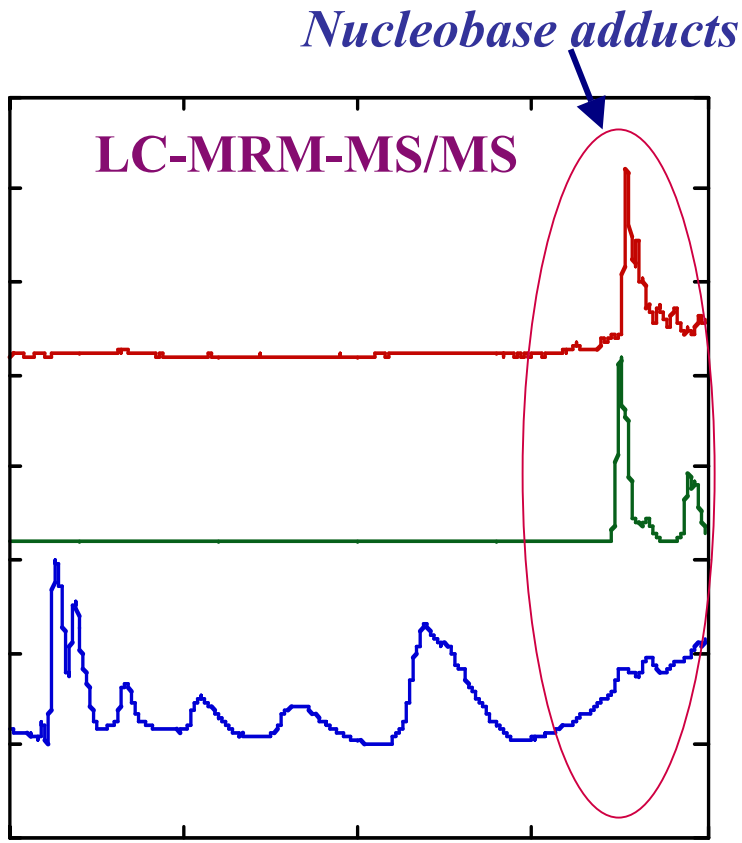

-200
-150
-100
-50
0
I, μA
0.4
0.6
0.8
1
1.2
E, V vs SCE
Enzyme/DNA films
1. incubate 37 °C
2. SWV, 50 μM Ru(bpy)3 2+
0.2 mM H2O2
+ 2% styrene
30 min
15 min
5 min
Controls -
no styrene
30 min
15 min
0 min
Bare PG
H2O2
R (e.g. styrene)
RO (e.g. styrene oxide)
Cyt P450
Peak increase measures damage of DNA by enzyme-generated metabolite

Cyt P450cam/DNA film + 0.2 M H_2O_2

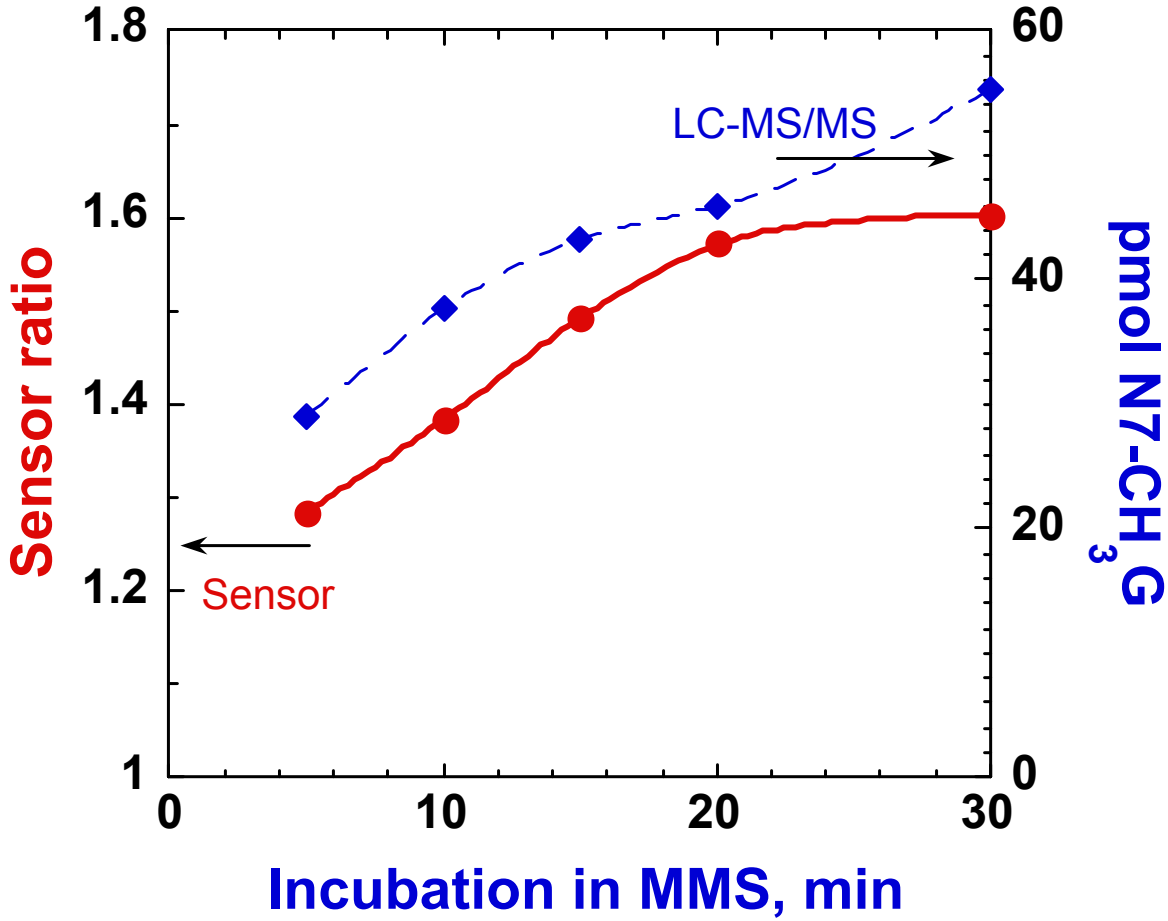


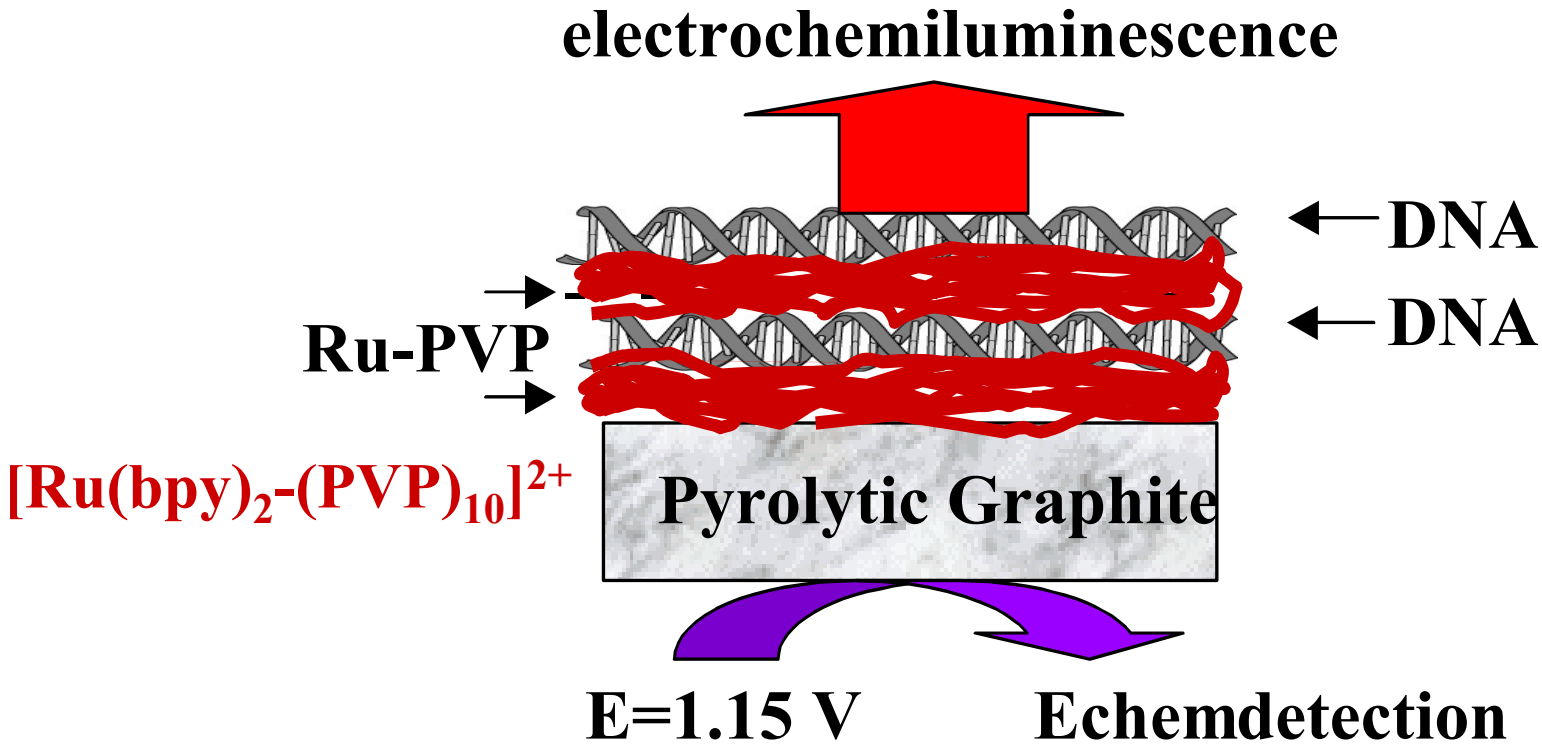
Detection of DNA-styrene oxide adducts after incubations of films + hydrolysis





Comparison of toxicity sensors with LC-MS For DNA damage by methylmethane sulfonate

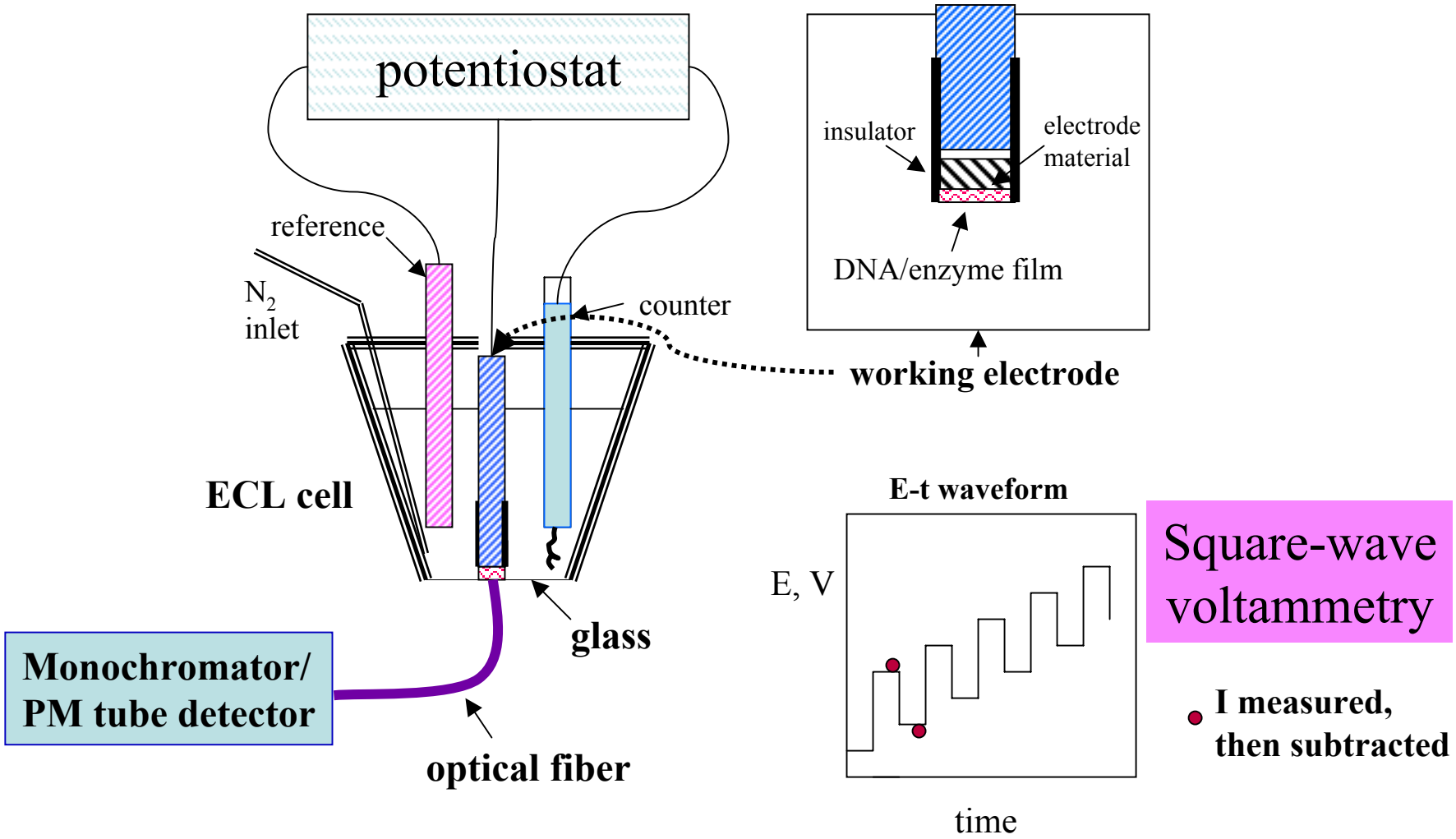




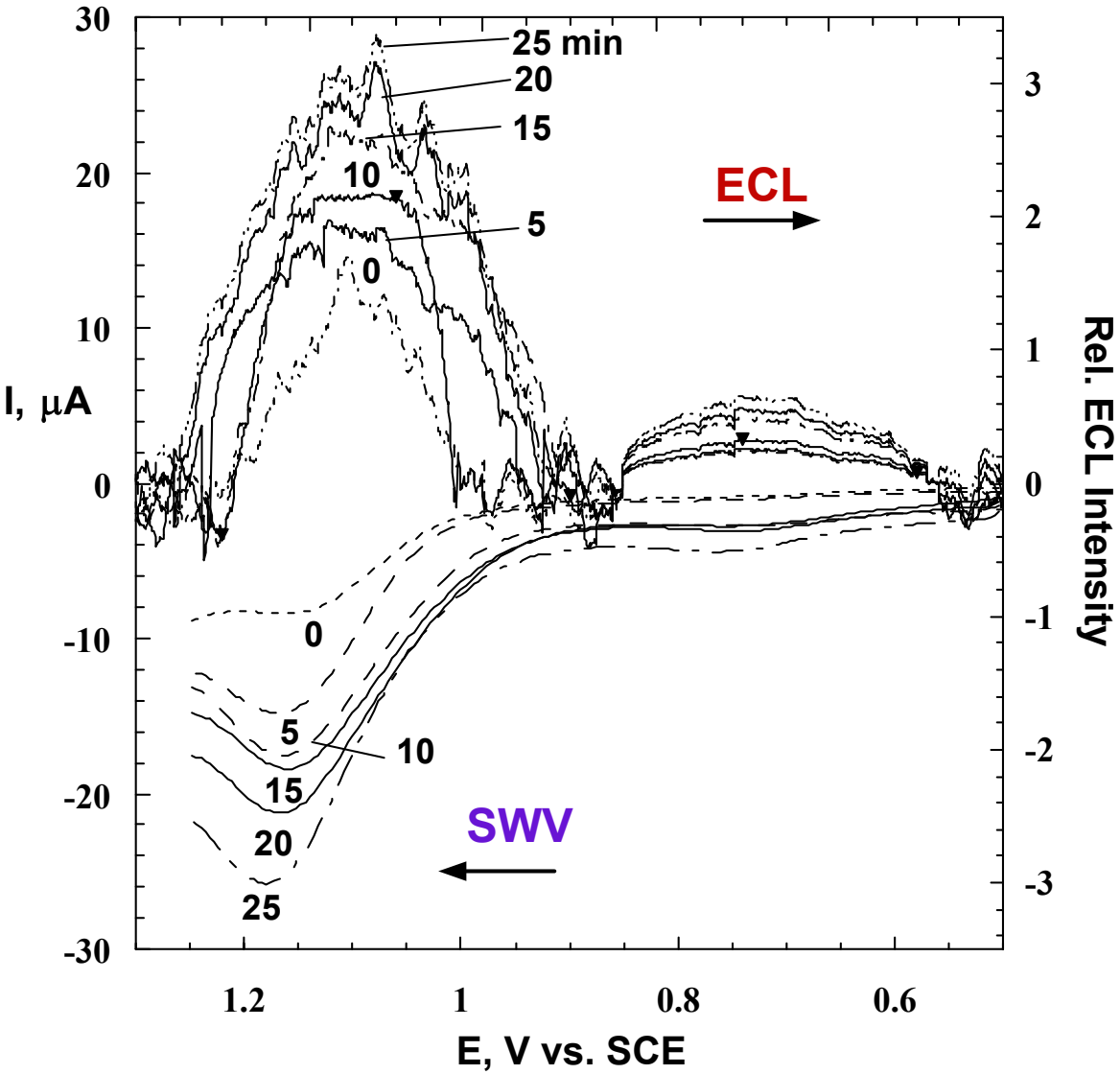
Lynn Dennany, Robert J. Forster and James F. Rusling, "Simultaneous Direct Electrochemiluminescence and Catalytic Voltammetry Detection of DNA in Ultrathin Films" *J. Am. Chem. Soc.* **2003**, *125*, 5213-5218.

Collaboration with NCSR, Dublin City Univ.

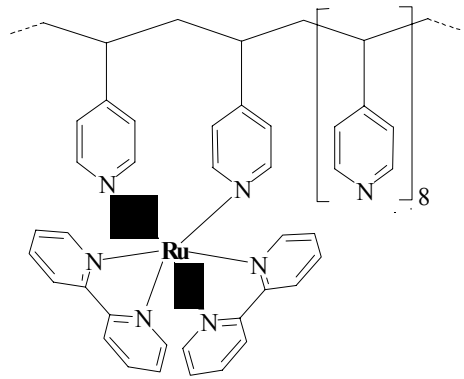
Equipment for ECL toxicity sensors



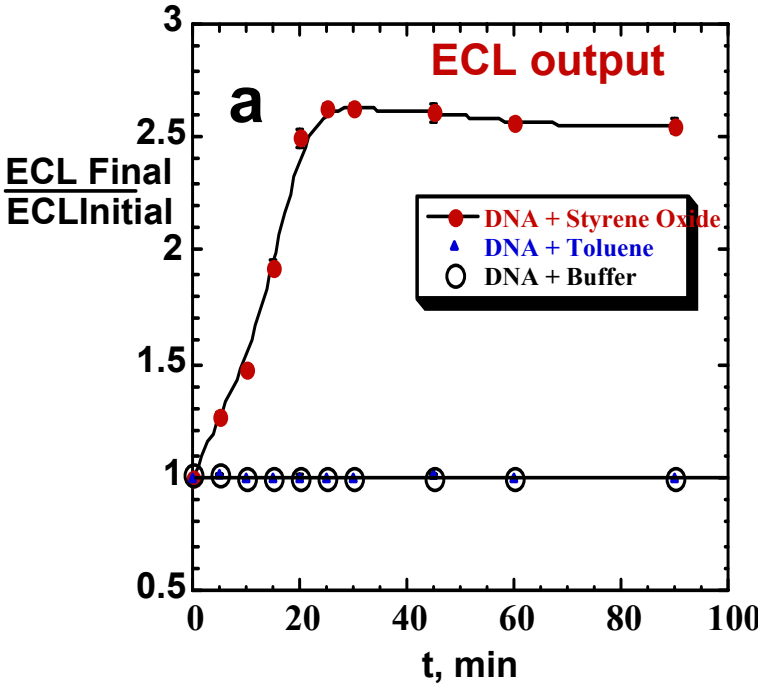
Incubations with styrene oxide

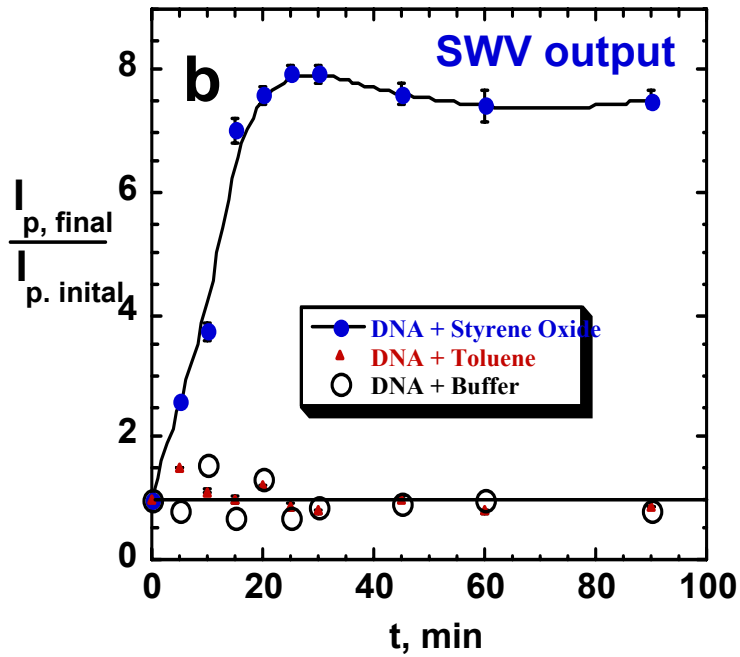


DNA +



Incubation of Ru-PVP/DNA films with styrene oxide





Direct ECL generation from DNA

$RVP\text{-}RuL^{2+} = PVP\text{-}RuL^{3+} + e^-$

$PVP\text{-}RuL^{3+} + DNA\text{-}G \rightarrow PVP\text{-}RuL^{2+} + DNA\text{-}G\bullet$

Then?

$PVP\text{-}RuL^{3+}$ oxidizes DNA-G• to give

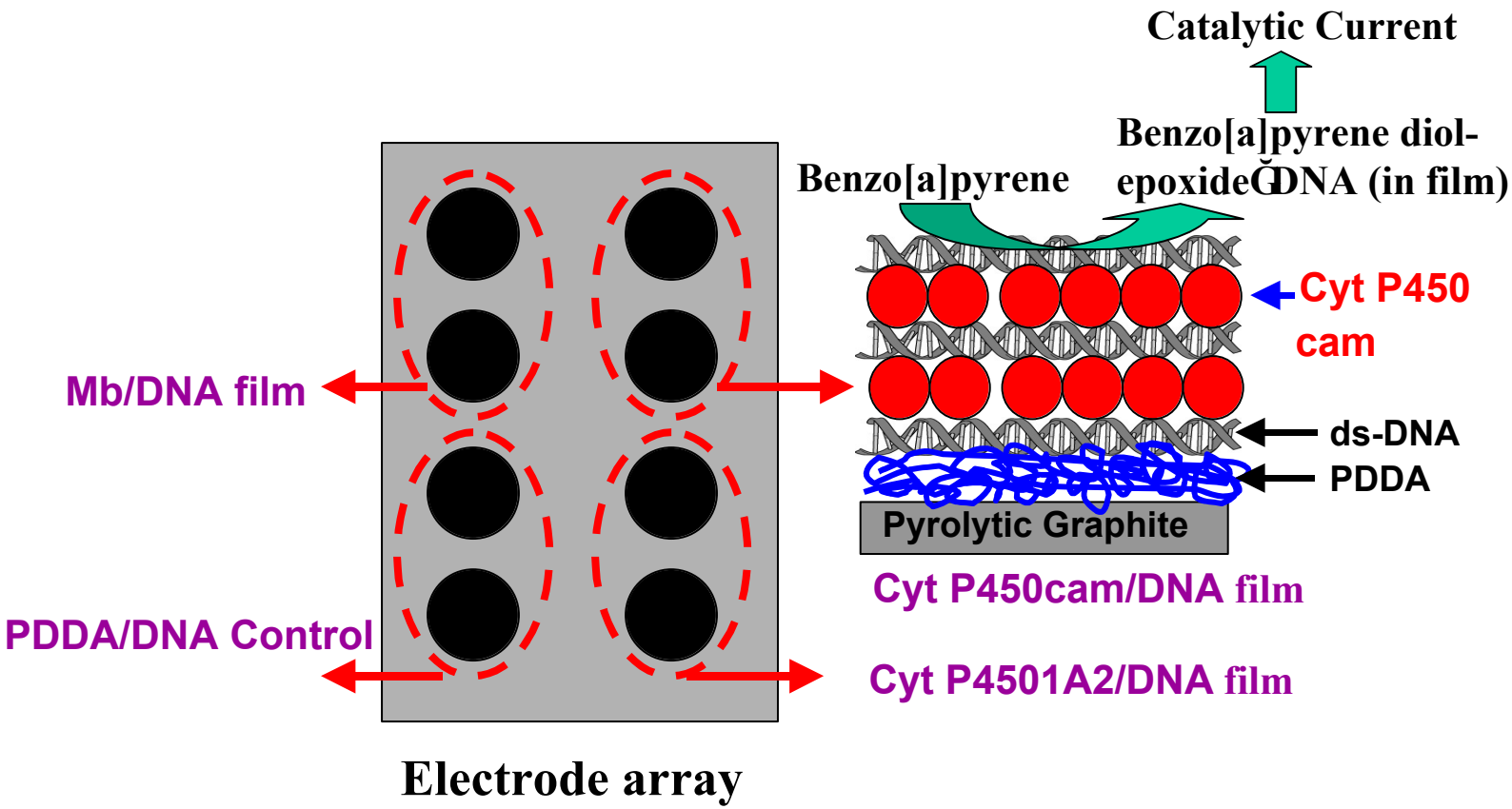
Photoexcited $PVP\text{-}[RuL^{2+}]^*$

Or

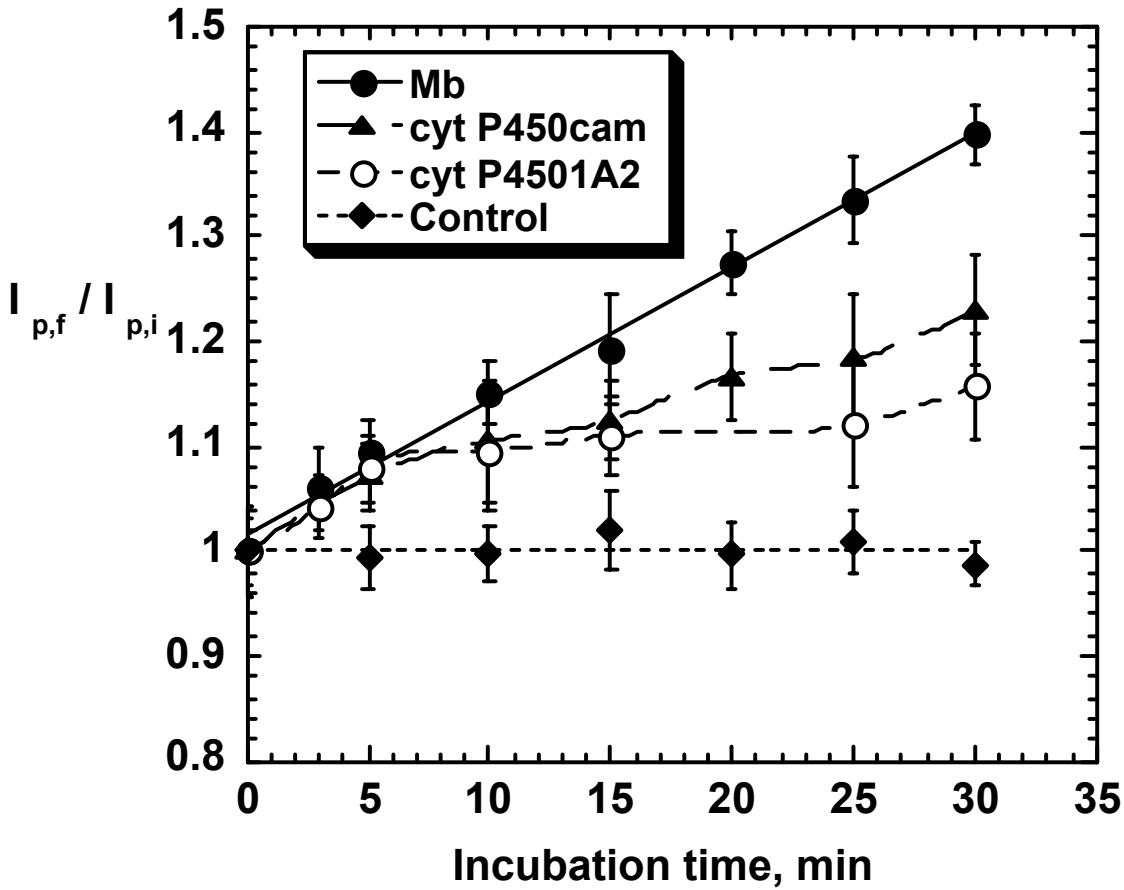
DNA-G• reduces $PVP\text{-}RuL^{2+}$ to $PVP\text{-}RuL^{+}$,

$PVP\text{-}RuL^{3+} + PVP\text{-}RuL^{+} \rightarrow PVP\text{-}[RuL^{2+}]^*$

Arrays: Which Liver Cytochrome P450s - generate toxic Benzo[a]pyrene Metabolites?



Arrays detect in-vitro DNA damage from metabolites of different enzymes in DNA/enzyme films



Rel. turnover rate, 1/min (nmol E)

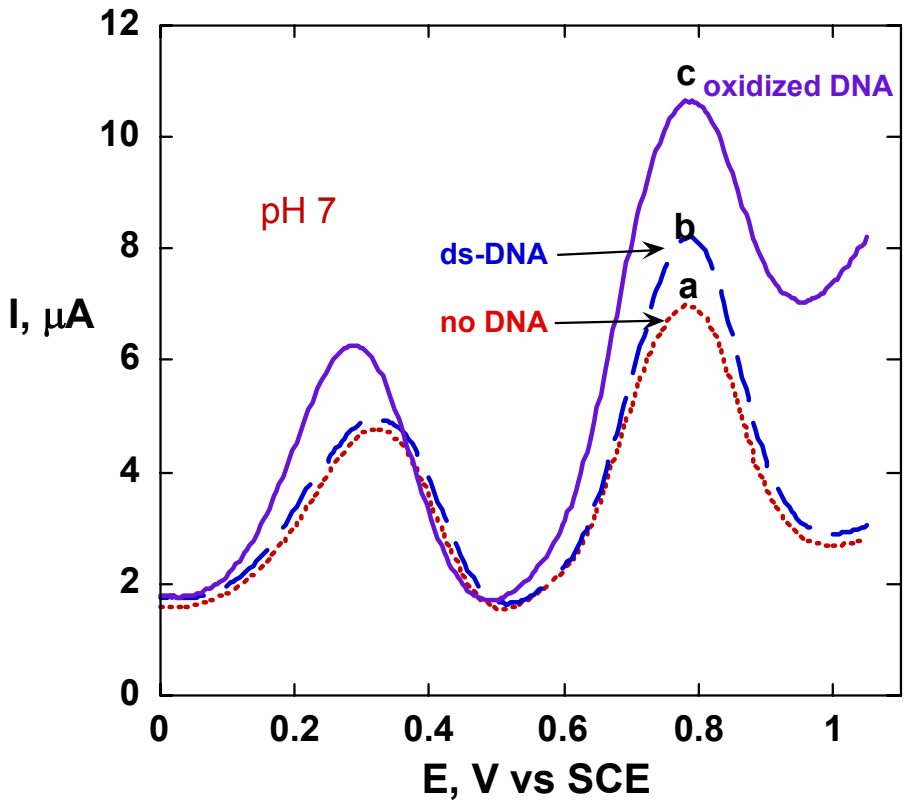
Mb	0.9
P450cam	3.0
P450 1A2	3.5

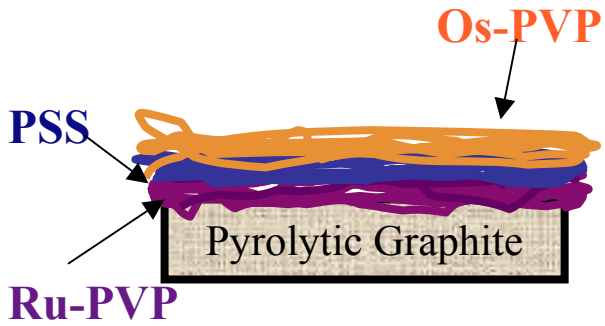
Drug discovery applications

Figure 7. Influence of incubation time with 50 μM benzo[a]pyrene and 1 mM H_2O_2 on the peak current ratios from SWV of PDDA/DNA/(enzyme/DNA)$_2$ films Control is PDDA/DNA/(Mb/DNA)$_2$ film in 50 μM benzo[a]pyrene alone.

Sensors for oxidative stress via oxidized DNA

SWV (10 Hz) of PVP-Ru/PSS/PVP-Os film (a) in buffer;
(b) + 0.2 mg/mL CT ds-DNA
(c) + 0.2 mg/mL CT ds-DNA after 80 min. in Fenton reagent





ECL detection in films:

Lynn Dennany, Robert J. Forster, Blanaid White, Malcolm Smyth and James F. Rusling, **Am. Chem. Soc.,** *2004,* **126,** ***8835-8841.***

Amos Mugweru, Bingquan Wang, and James F. Rusling "Voltammetric Detection of Oxidized DNA Using Ultrathin Films of Os and Ru Metallopolymers", *Anal Chem* **2004**, 4555-7563.

Summary: DNA damage detection/toxicity sensors

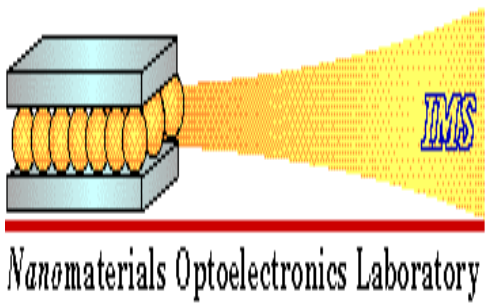
- **Catalytic voltammetry and ECL toxicity sensors**
- **sensors produce metabolites, damage DNA**
- **Can detect 5-10 damaged bases/10,000**
- **can detect DNA oxidation - 8-oxoguanine (1/6000)**
- **Future: extensions to many compounds, cyt P450 arrays, ECL arrays, drug toxicity**

Single-walled carbon nanotube forests as a basis for immunosensors

James F. Rusling and Xin Yu,
Depts. of Chemistry and Pharmacology, Univ. Connecticut

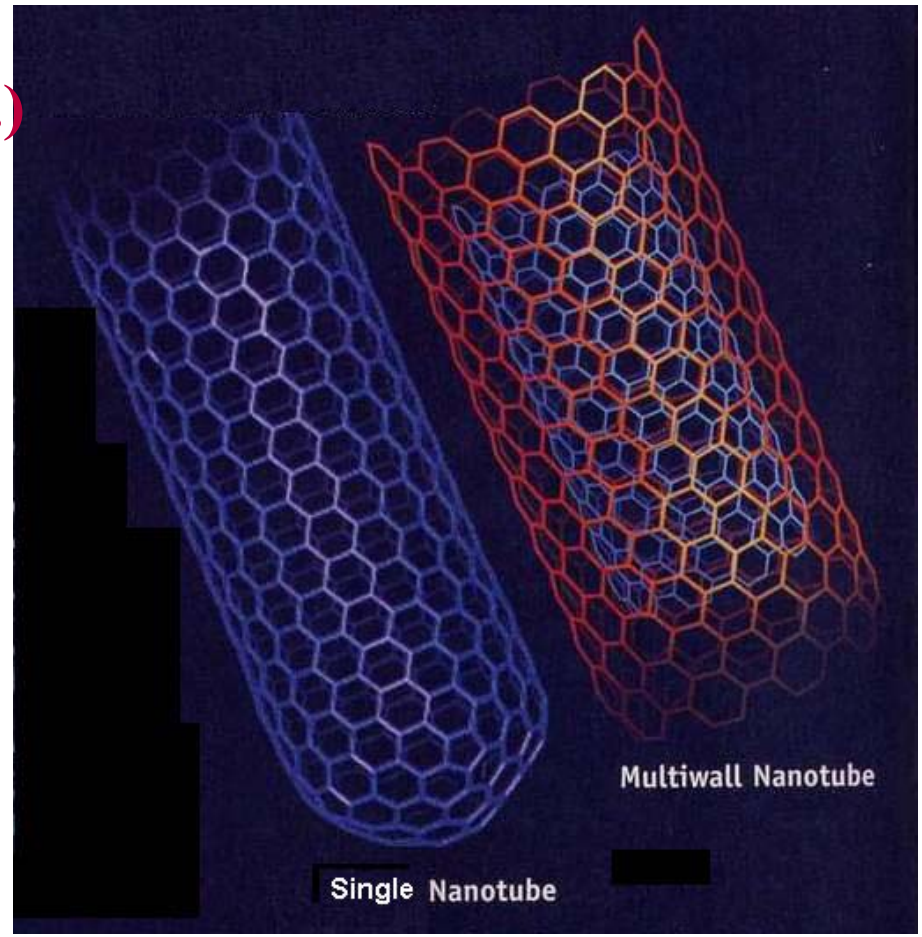
Maire O'Connor, Anthony Killard, Malcolm Smyth
NCSR, Dublin City University

Sang Nyon Kim, Fotis Papadimitrakopoulos
Institute of Materials Science, University of Connecticut



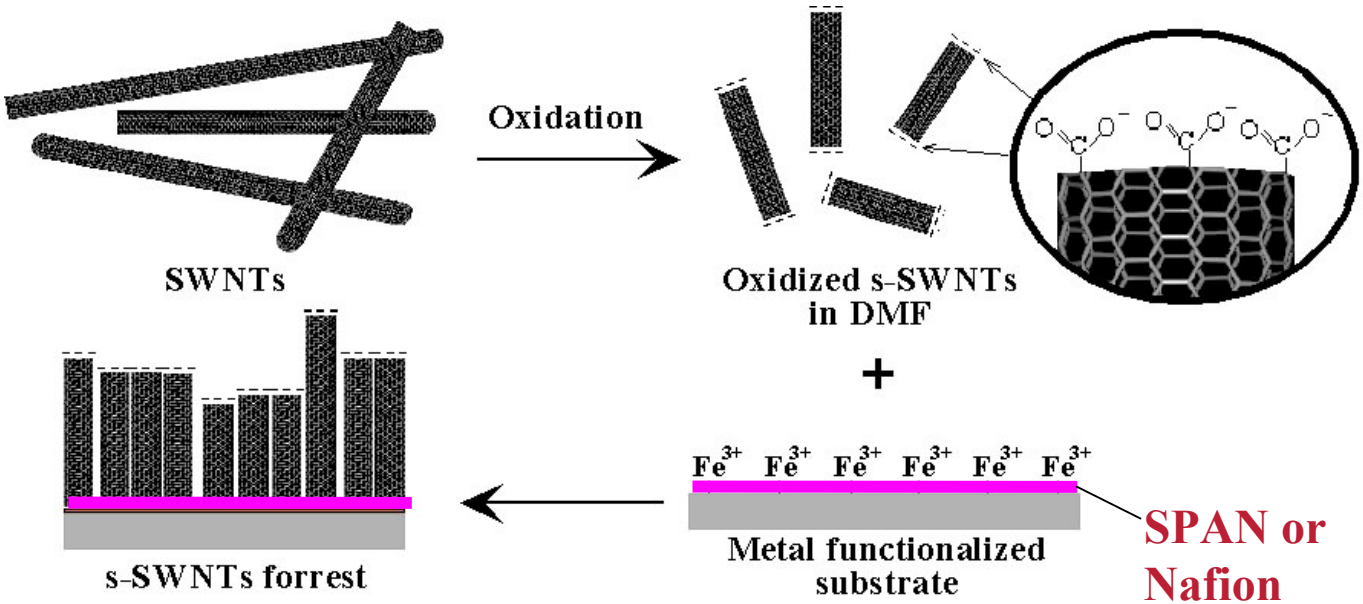
Carbon Nanotubes

- **Single walled (1.4 nm o.d.) and multi-walled**
- **Highly conductive, flexible, strong, patternable**
- **Commercially Available**



Single-Walled Carbon Nanotube Forests: Antigen-Antibody Sensing

~1.4 nm diameter, high conductivity

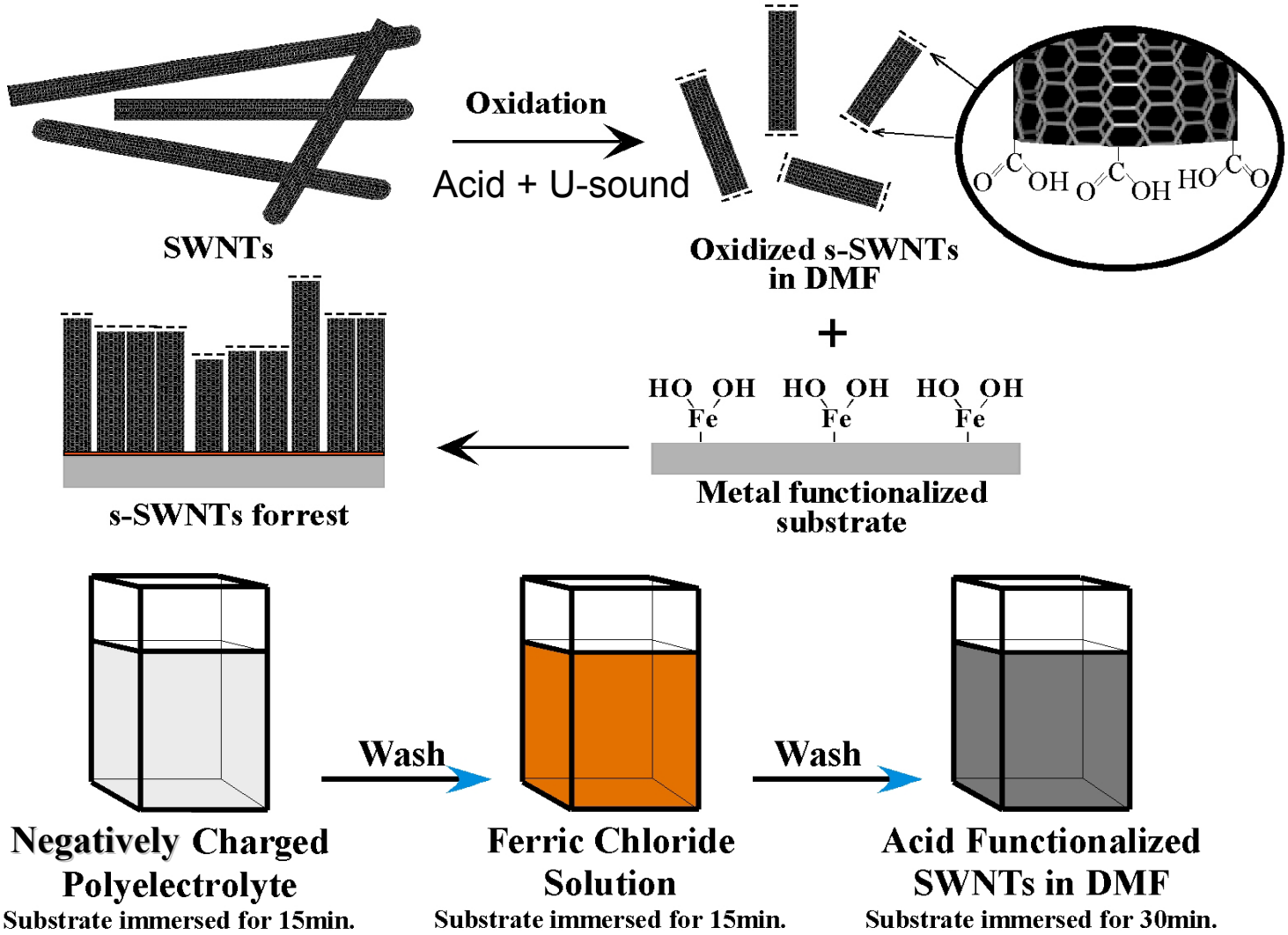


Chattopadhyay, Galeska, Papadimitrakopoulos, *J. Am. Chem. Soc.* 2001, *123*, 9451.

End COOH groups allow chemical attachment to proteins (antibodies)

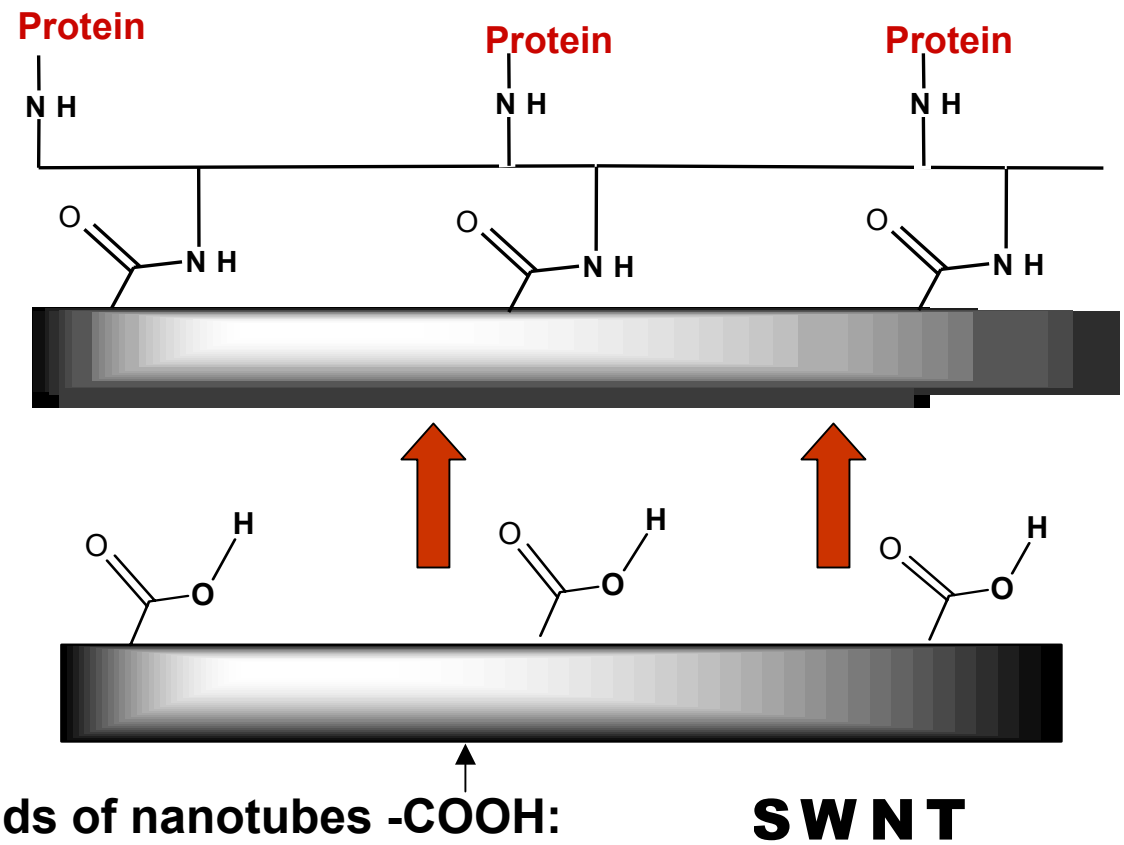
High conductivity to conduct signal (e's) from enzyme label to meas. circuit

Experimental Procedure for SWNT Forest Assembly



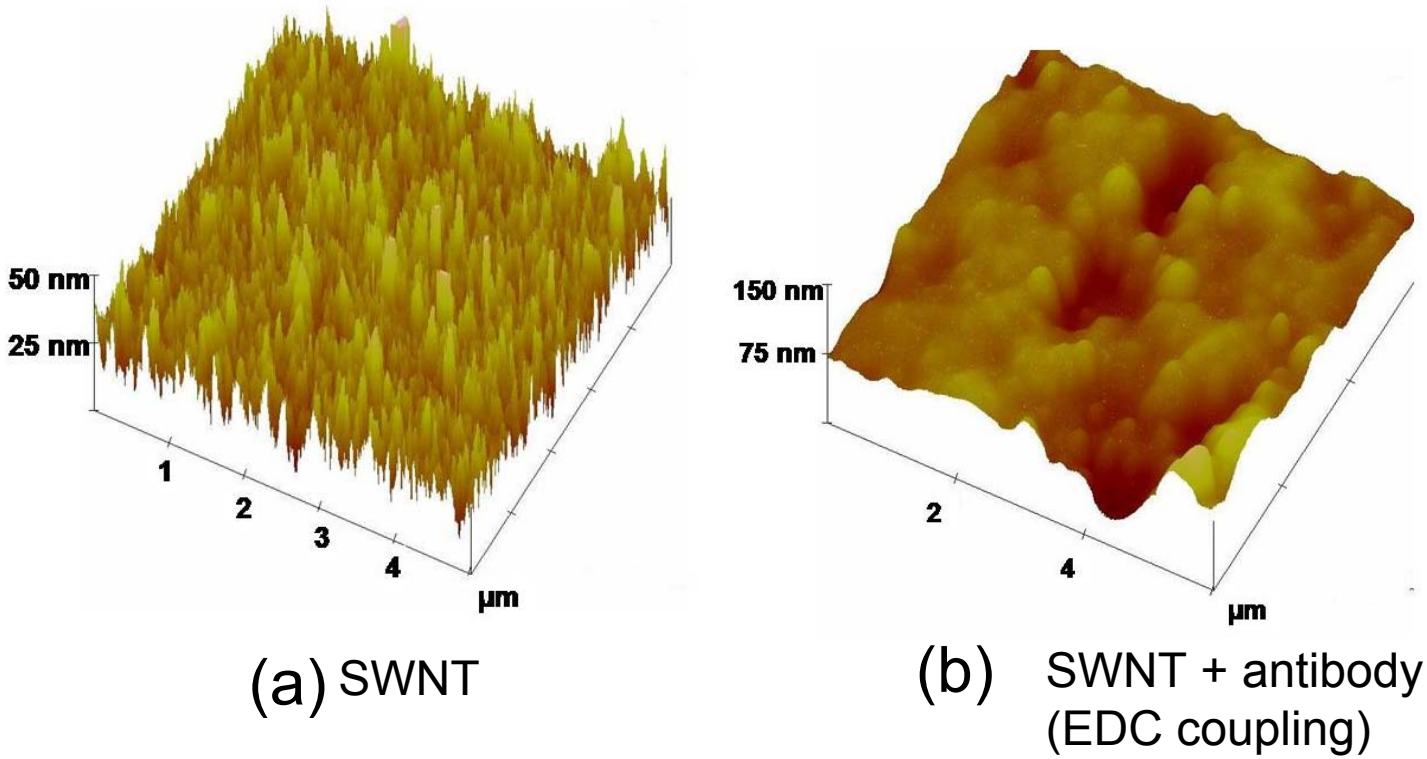
Chattopadhyay, Galeska, Papadimitrakopoulos, *J. Am. Chem. Soc.* 2001, *123*, 9451.

Covalently Binding Protein to SWNT



water-soluble carbodiimide (1-(3-(dimethylamino) propyl)-3-ethylcarbodiimide hydrochloride, EDC, or EDC + NHSS

AFM of SWNT forest with and without antibiotin attached

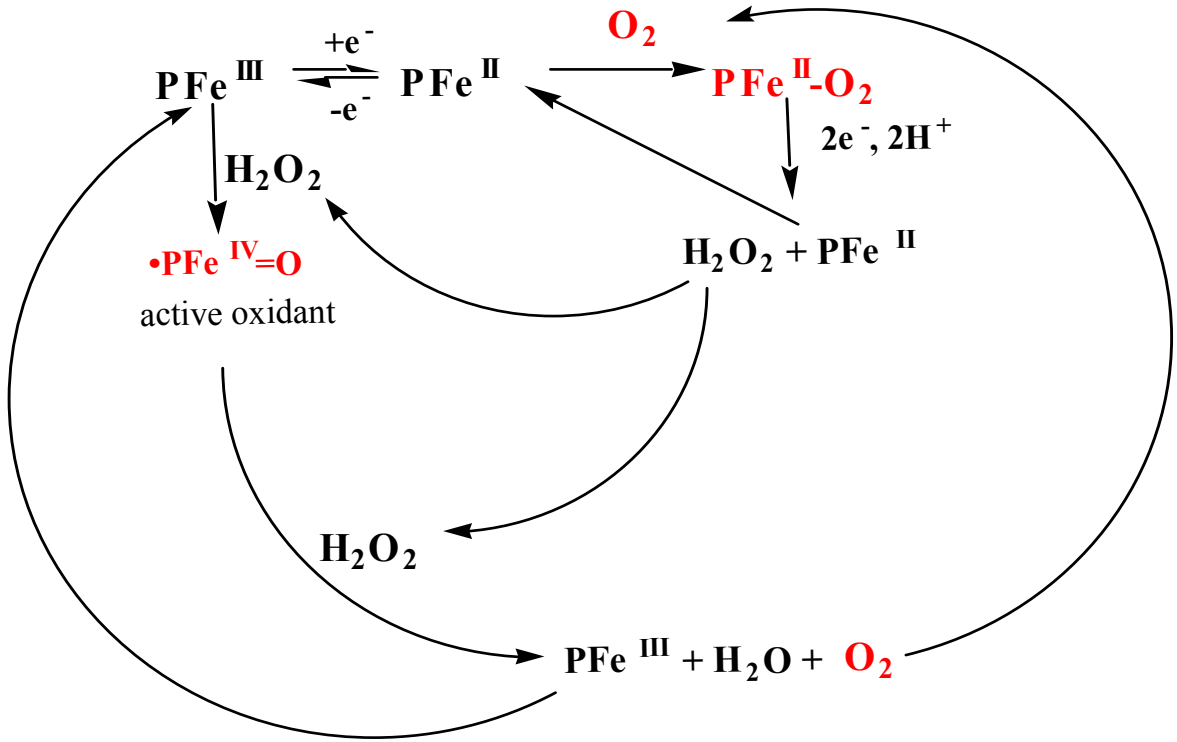


(a) SWNT

(b) SWNT + antibody (EDC coupling)

(a) SWNT forest on smooth silicon and (b) Anti-biotin antibody functionalized SWNT on smooth silicon

Electrochemical Response of Peroxidases



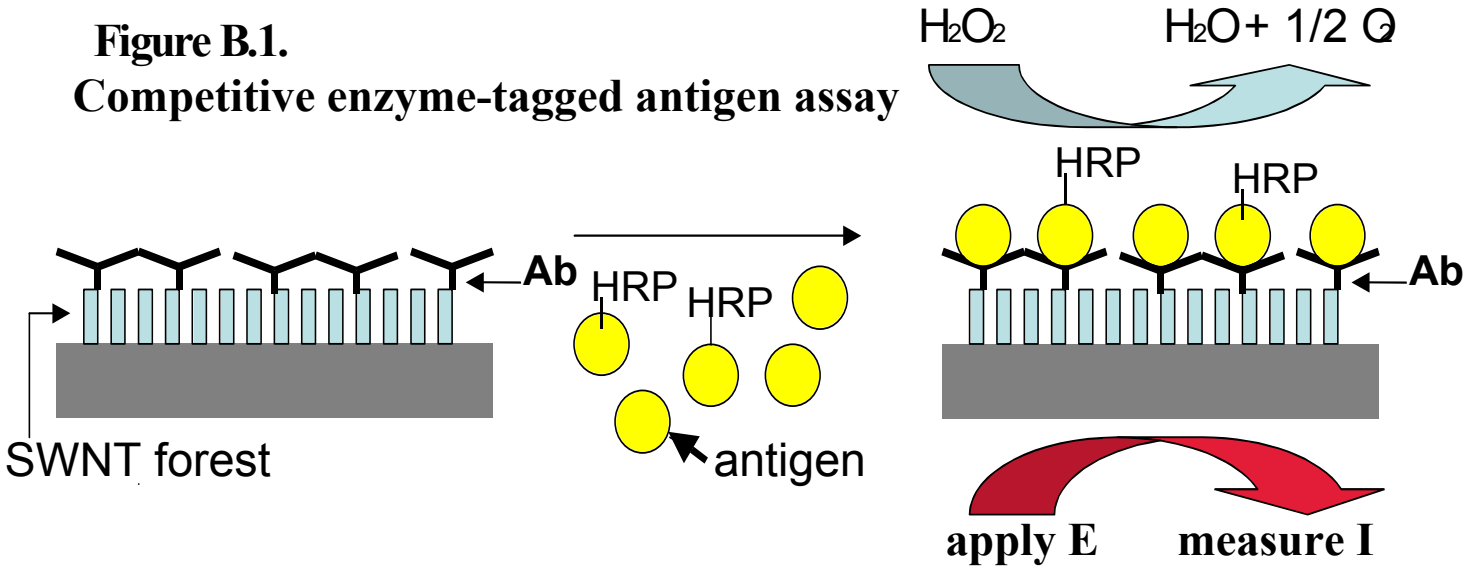
Possible reduced species in red

HRP on electrodes: + H_2O_2 = current signal

Zhe Zhang, Salem Chouchane, Richard S. Magliozzo,and James F. Rusling, "Direct Voltammetry and Enzyme Catalysis with *M. tuberculosis* Catalase-Peroxidase, Peroxidases and Catalase in Lipid Films", *Anal. Chem.*, **2002**, *74*, 163-170.

Competitive Immunoassay

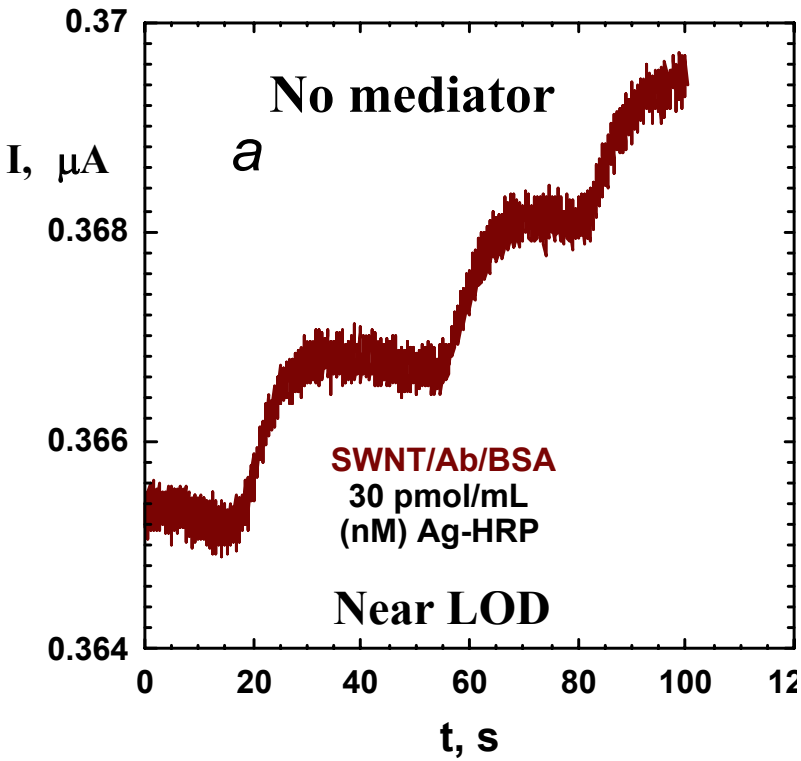
Figure B.1.
Competitive enzyme-tagged antigen assay

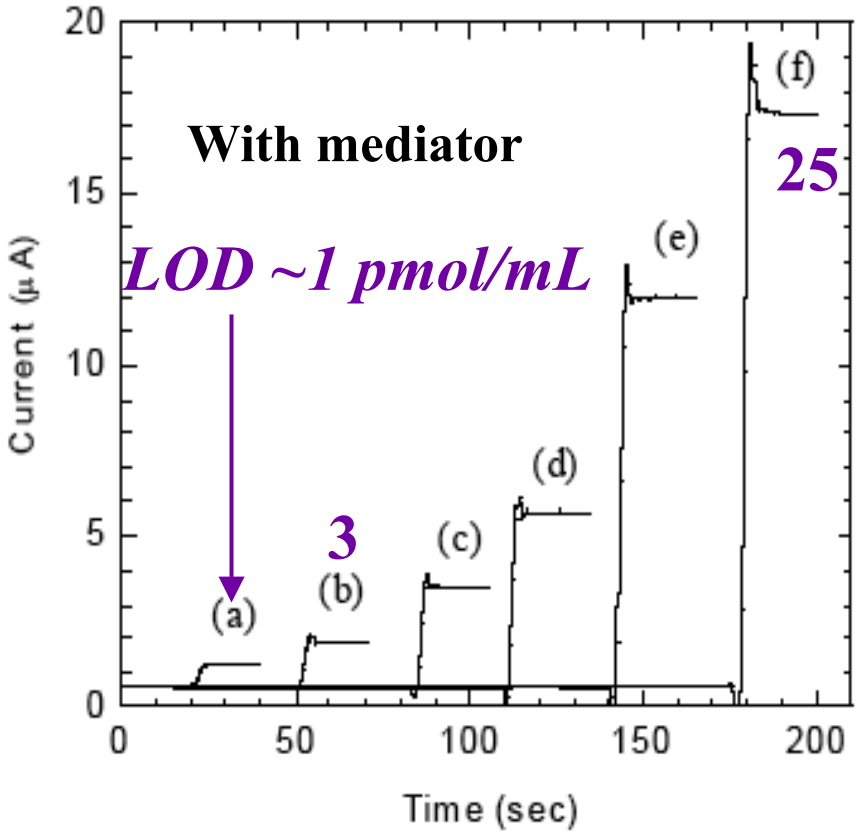


Catalytic current should be inversely proportional to the amount of non-labeled Ag, depending on binding constant, Ag was pre-bound on Ab

Anti-biotin/biotin-HRP test system

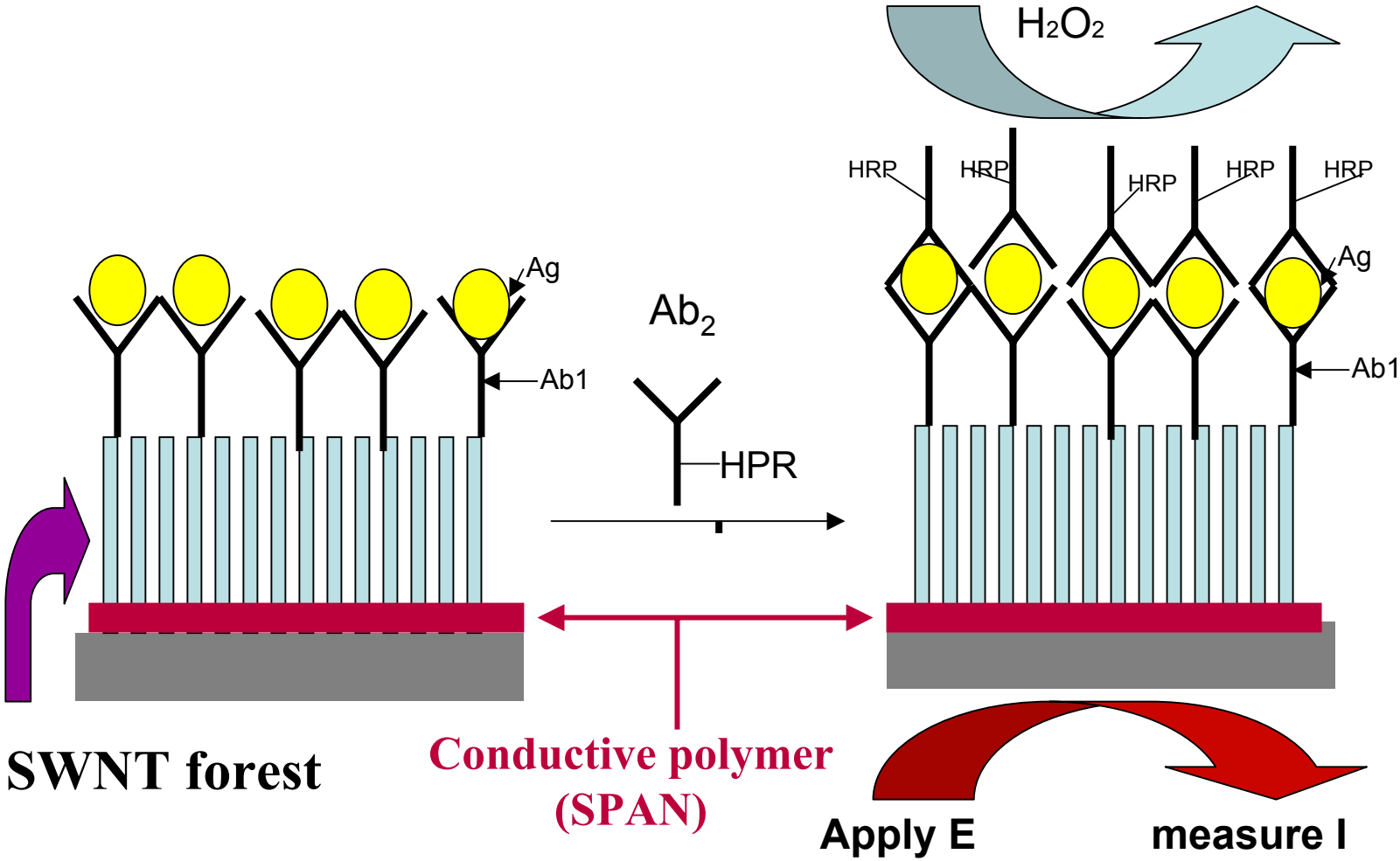
(H_2O_2 present)



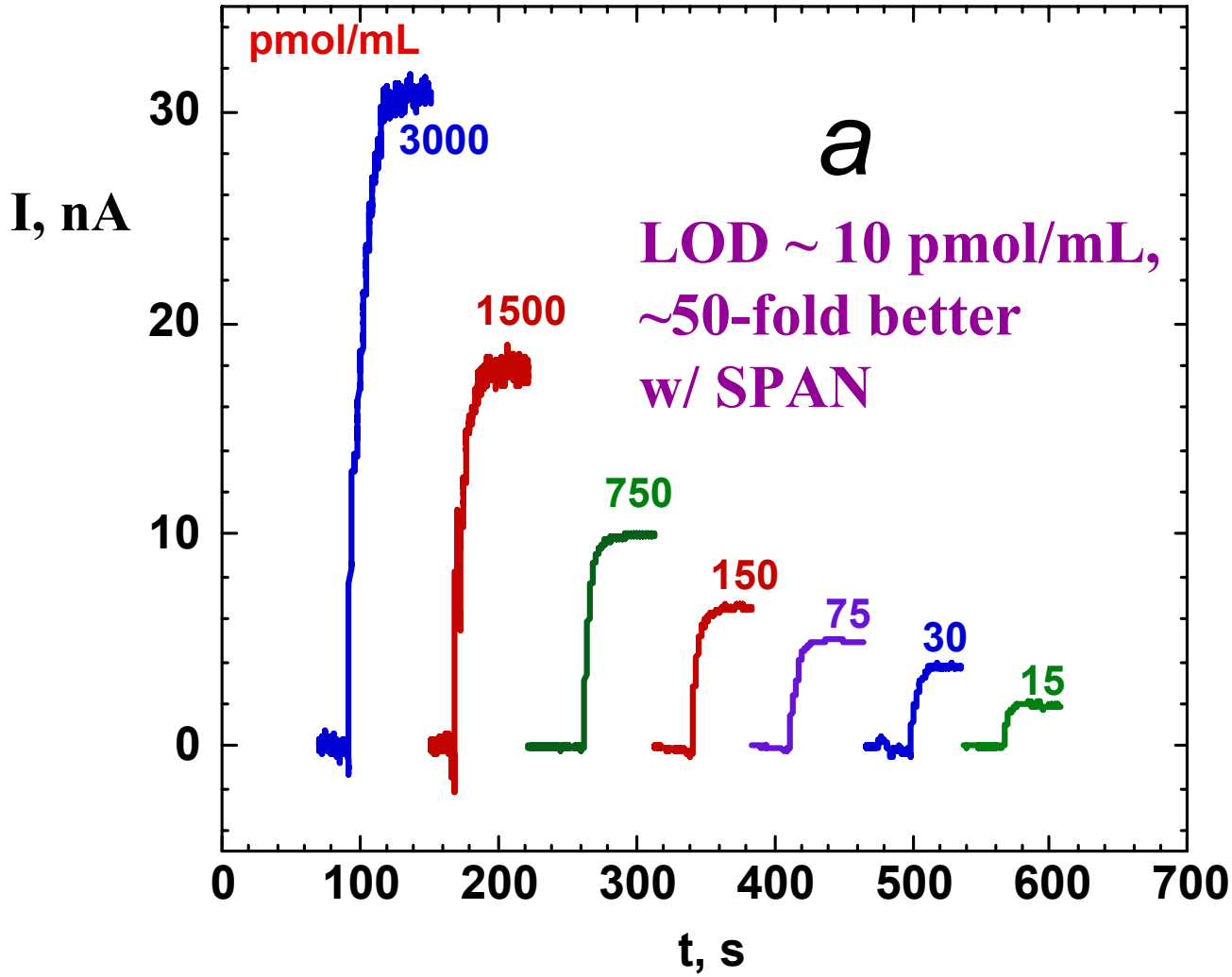


not all the HRP label was communicating with the measuring circuit - soluble mediator shuttles electrons from HRP label more efficiently

Sandwich Assay for Human Serum Albumin

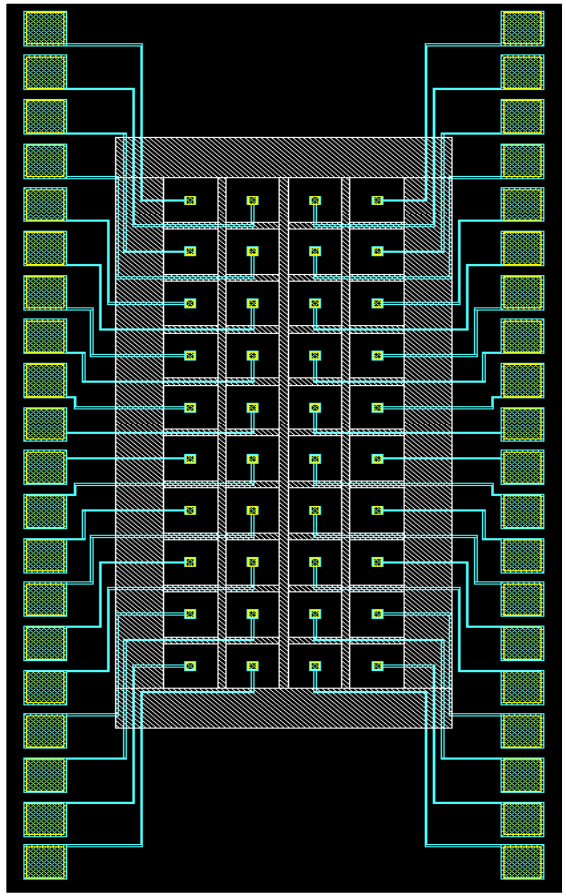


Detection of Human Serum albumin in 10 μL drops on SWNT forest immunosensor



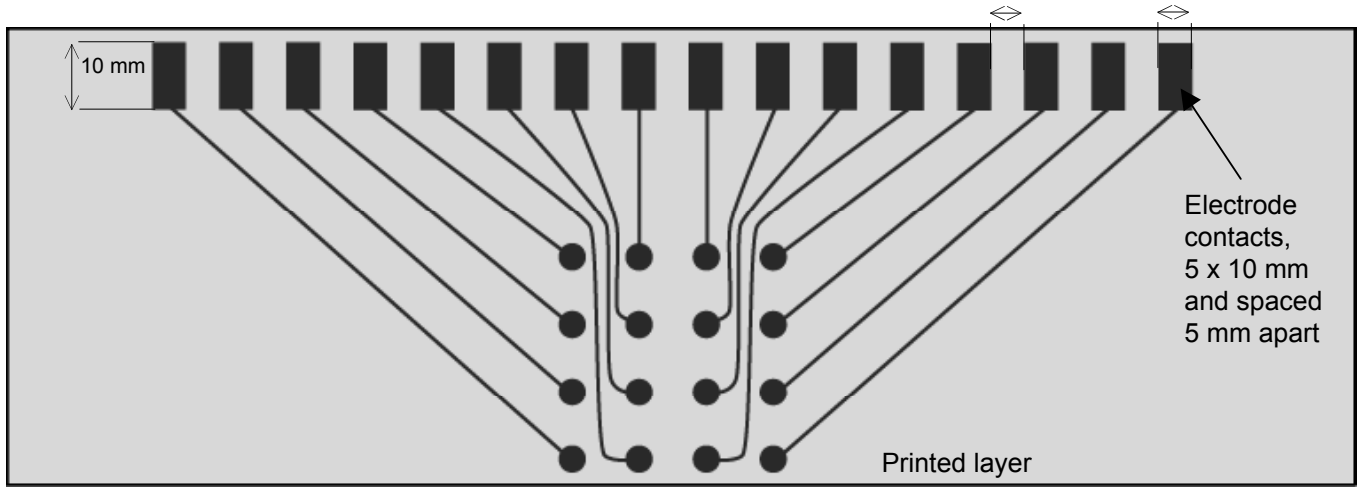
Design approaches to future arrays

1. **Layer-by-layer approach general, simple**
2. **Stable films, complex architecture, any surface**
3. **Sensors for toxicity, oxidative stress**
4. **Ambient T solution processable**
5. **SWNT forests patterned by solution process**
6. **Excellent LOD and sensitivity using conductive polymer bed (SPAN)**
7. **Possibility of automated array formation**
8. **Applications to proteins, pathogens, etc.**



Future work: pattern SWNT forest arrays onto microchi

collaboration with Univ. of Edinburgh Genomics Inst. (GT



Also, screen printed carbon arrays, Lab 901, Edinbugh

Detection of Protein biomarkers for Cancer:

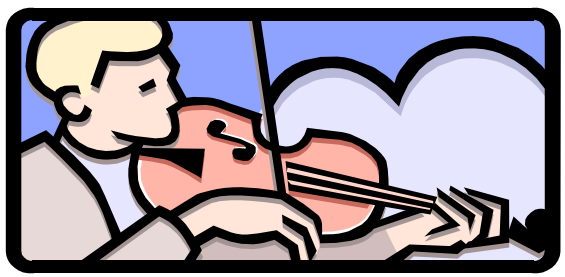
- **NIH, NIDCR**
- **prostate, squamous cell, and breast cancers**

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Thanks to NIH, NSF and ARO for funding!

Thanks to all our coworkers and collaborators
http://web.uconn.edu/rusling/

Thanks to YOU for listening!

Thanks to intangible creative factors

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