

Bioelectrochemistry and new biomedical array technologies

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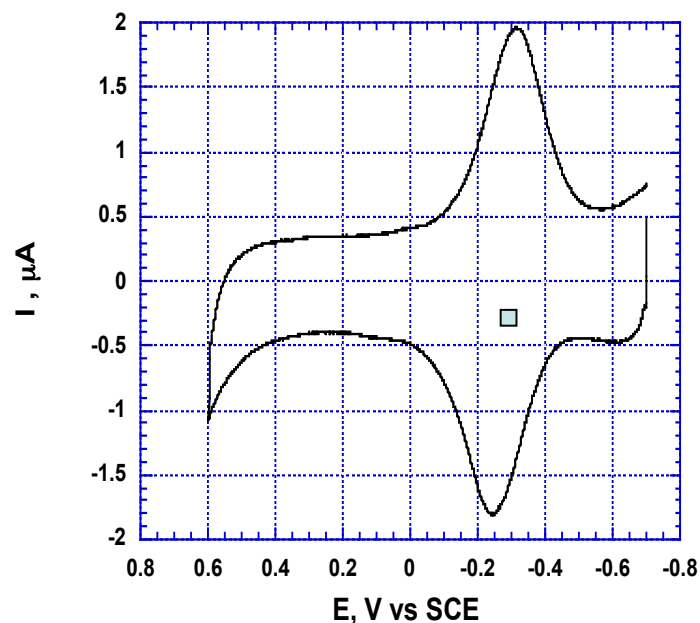
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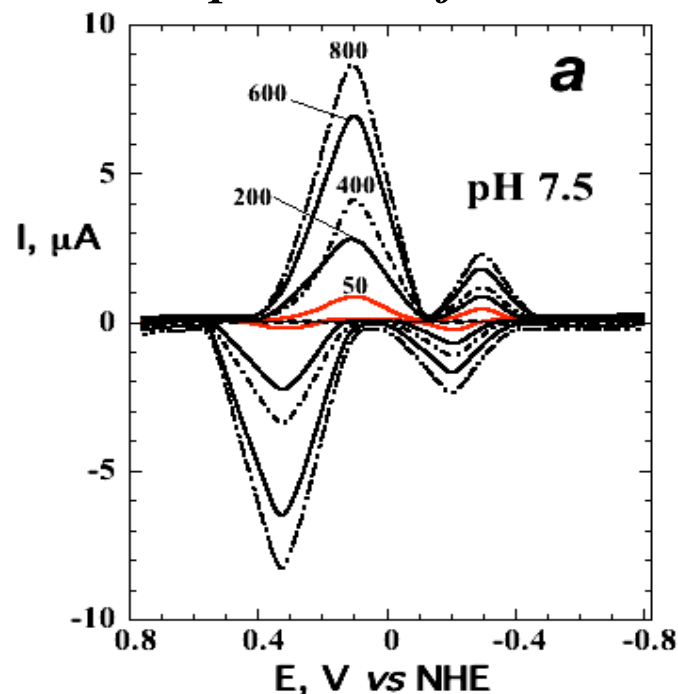


BIOELECTROCHEMISTRY: Protein Film Voltammetry

Tuberculosis KatG catalase-peroxidase in thin film.



Mn and quinone cofactors in PSII



Khrisna Alcantara, Bernard Munge, Zeus Pendon, Harry A. Frank, and James F. Rusling, "Thin Film Voltammetry of **Spinach Photosystem II**. Proton-gated electron transfer involving the Mn₄ cluster" *J. Am. Chem. Soc.*, **2006**, 128, 14930-14937.

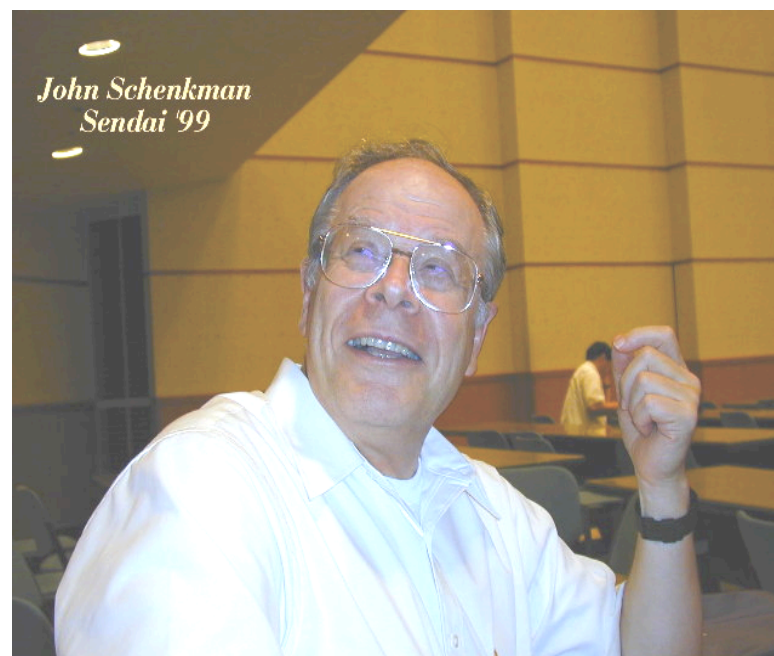
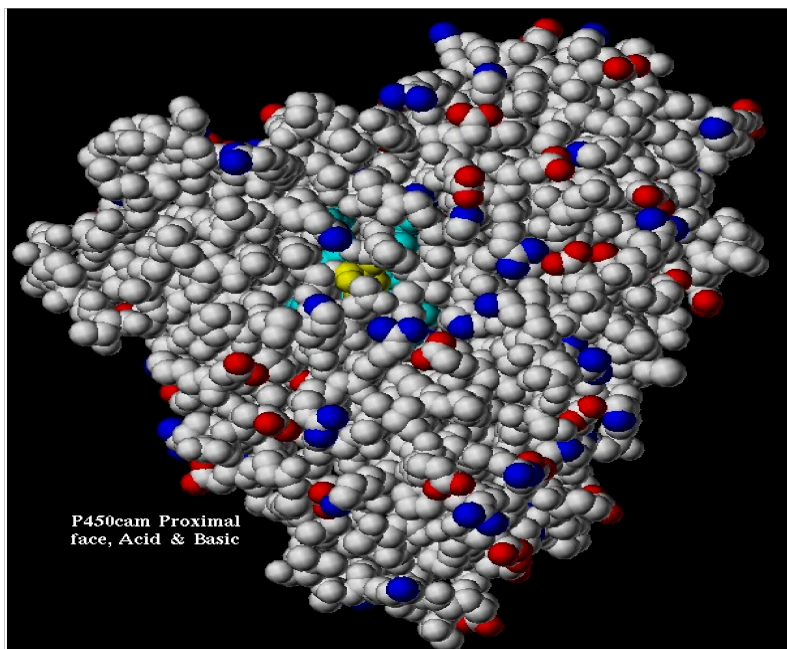
Funded by NSF, with H.A. Frank

Yun Zhang, Amy M. LaFountain, Nikki Magdaong, Marcel Fuciman, James P. Allen, Harry A. Frank, and James F. Rusling, Thin Film Voltammetry of Wild Type and Mutant Reaction Center Proteins from Photosynthetic Bacteria, *J. Phys. Chem. B*, **2011**, 115, 3226–3232.

Human Metabolic Enzymes: funded by NIH

- *cytochrome P450s - oxidative catalysts*
- *bioconjugation enzymes*
- *toxicity screening arrays*

***Prof. John Schenkman, Pharmacology,
Cell Biology, Uconn Health Center***



Sadagopan Krishnan, John B. Schenkman and James F Rusling, *Feature Article: Bioelectronic Delivery of Electrons to Cytochrome P450 Enzymes. J. Phys. Chem. B* **2011**, *115* 8371–8380. [dx.doi.org/10.1021/jp201235m](https://doi.org/10.1021/jp201235m)

316. Sadagopan Krishnan, Dhanuka Wasalathanthri, Linlin Zhao, John B. Schenkman, and James F. Rusling, Efficient Bioelectronic Actuation of the Natural Catalytic Pathway of Human Metabolic Cytochrome P450s, *J. Am. Chem. Soc.*, **2011**, *133*, 1459–1465.

Chemical toxicity: a critical public health problem

- **New drugs & environmental chemicals must be proven safe.**
- **\$2 billion to develop a single drug!**
- **clinical failures approach 30% due to toxicity**
- **existing bioassays give no structural details**
- **animal models often fail; interspecies differences**

- **Our approaches - electro-optical arrays, LC-MS/MS, CE-LIF**

In vitro Genotoxicity Screening

Lipophilic Molecule



Enzyme-activated molecule



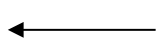
Damaged DNA

+DNA

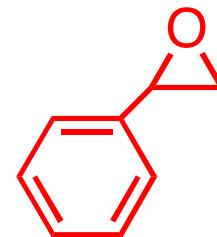


*Detect by electro-optical arrays,
LC-MS/MS, CE-LIF*

Cyt P450, O₂



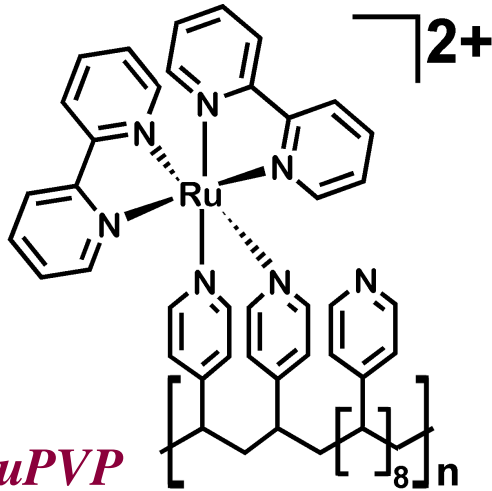
styrene



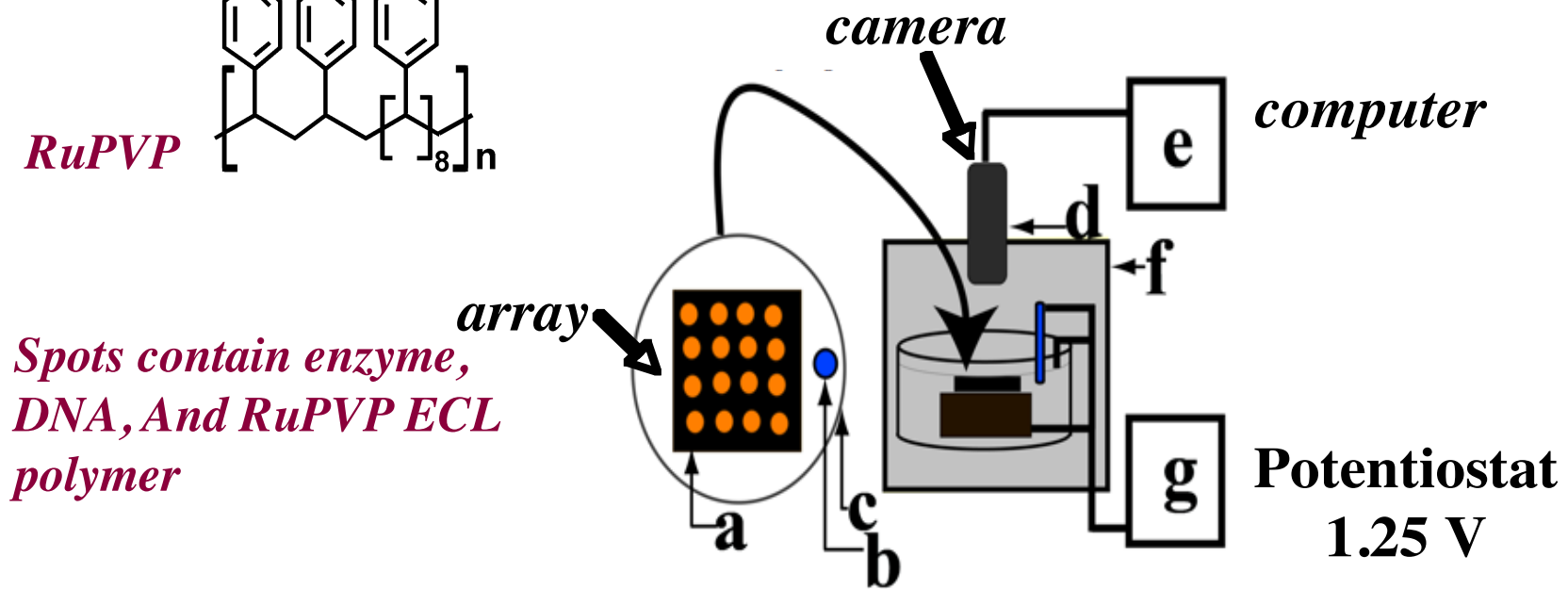
styrene oxide

*Reactive
metabolite*





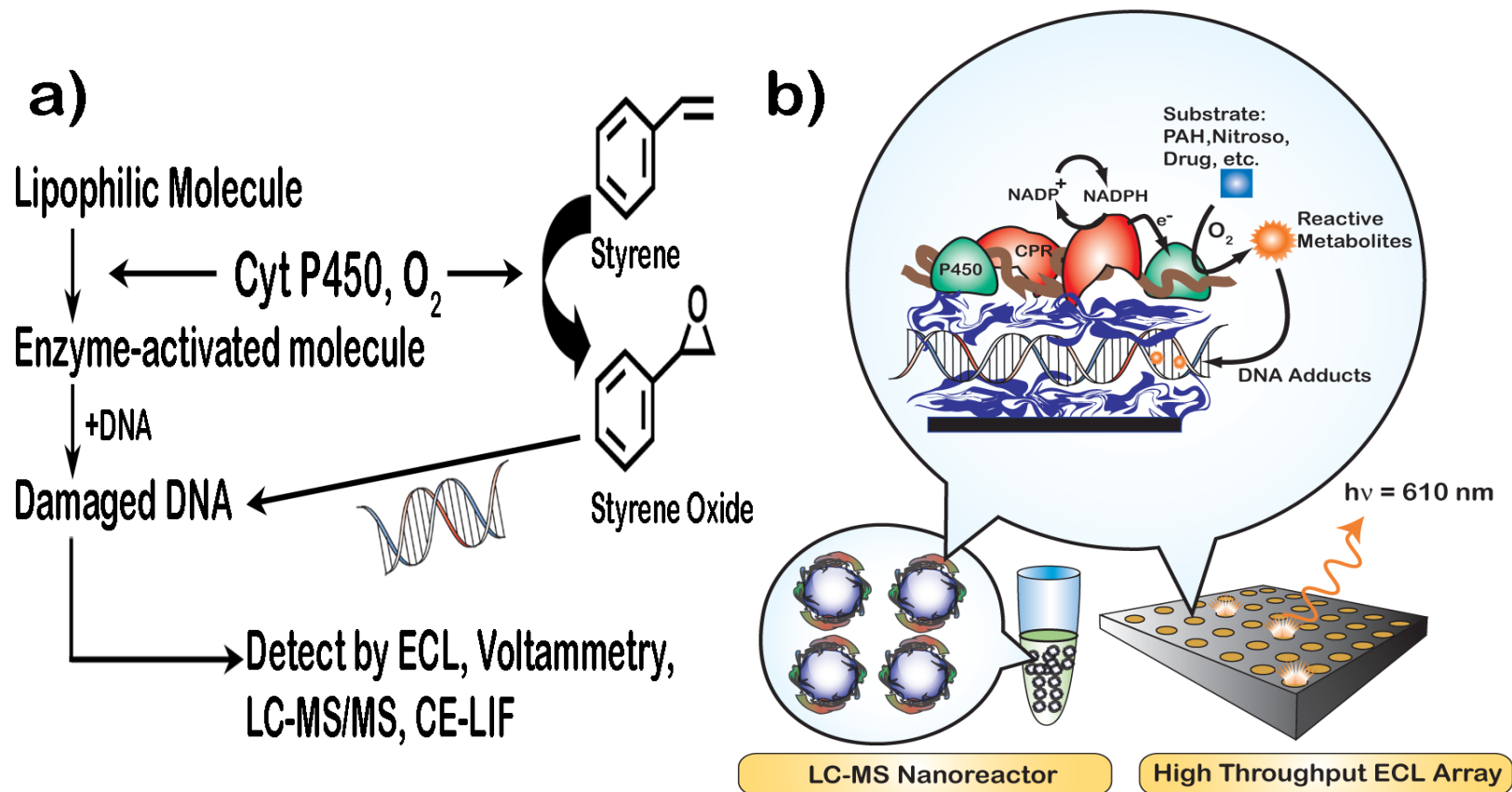
Chemical toxicity screening arrays



ECL ARRAY with CCD camera for detection

Funded by NIH

Using rat or human liver microsomes as multi-enzyme sources



Screening for Reactive Metabolites

1. Enzyme reaction - Incubate:
2. ---> metabolites

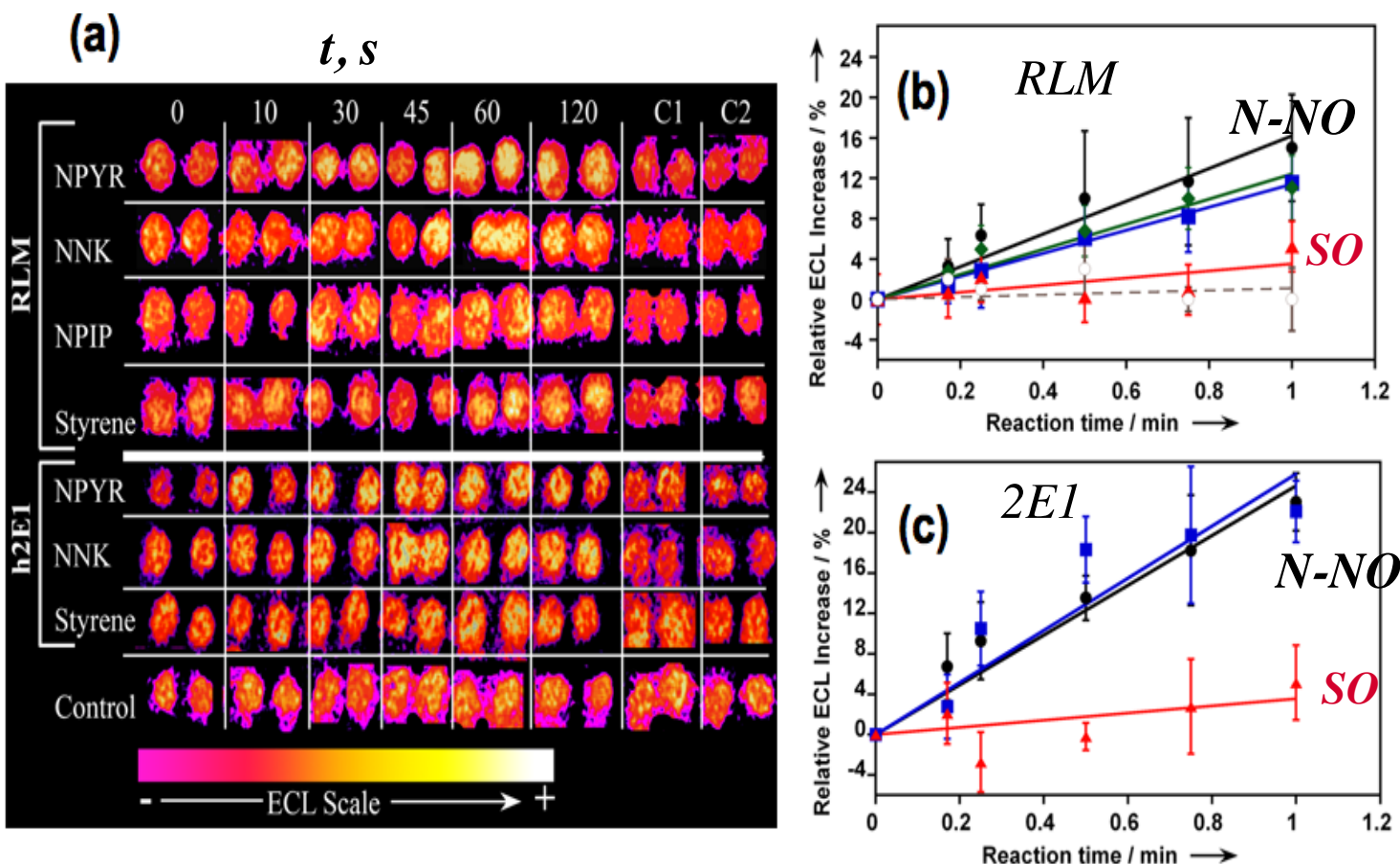


2. Analysis by
electrochemiluminescence (ECL)

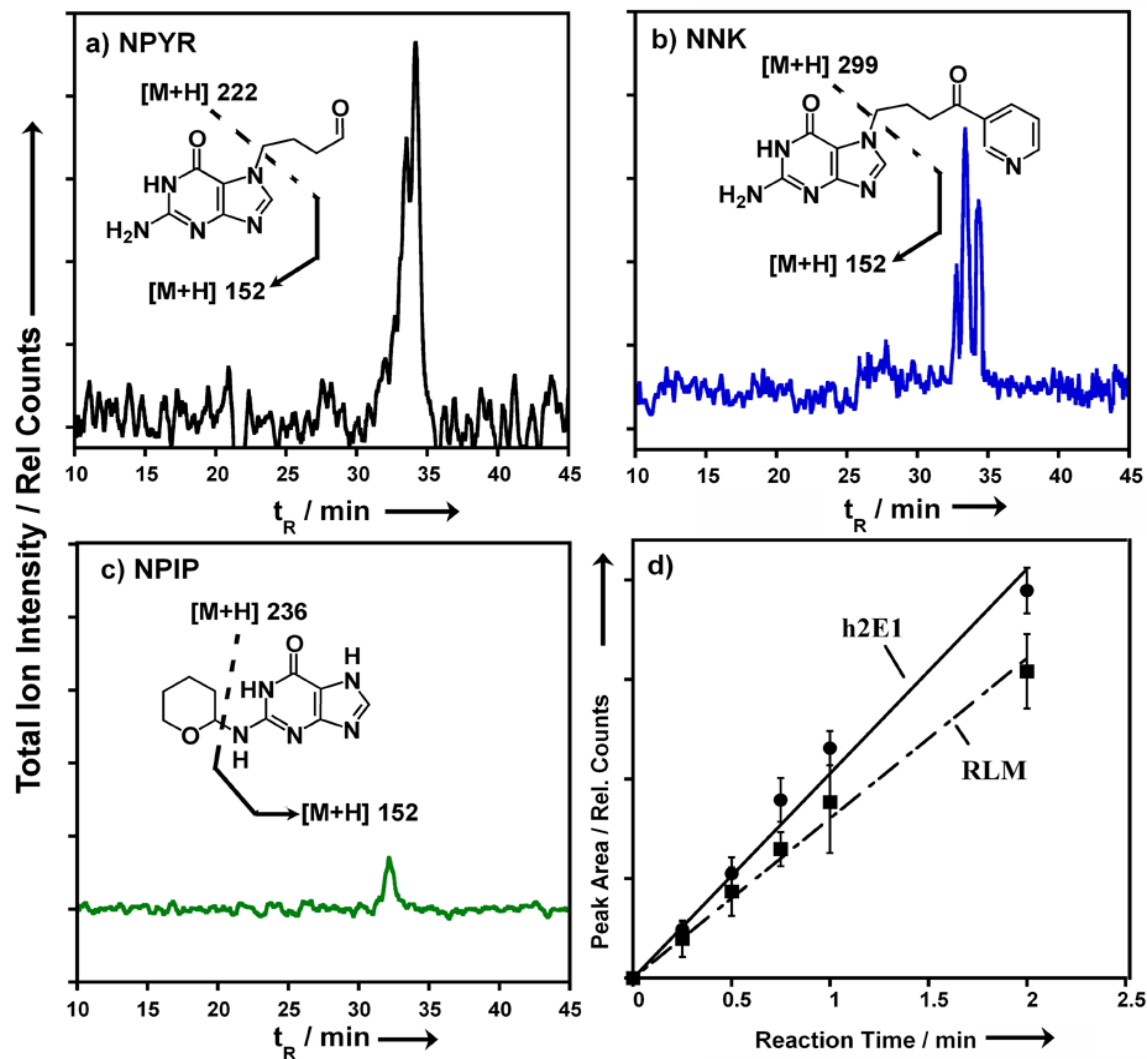


Films contain enzymes, RuPVP and DNA

Microsomes are multi-enzyme source in ECL arrays



S. Krishnan, B. Bajrami, E. G. Hvastkovs, D. Choudhary, J. B. Schenkman, and J. F. Rusling, Synergistic Metabolic Toxicity Screening Using Microsome/DNA Electrochemiluminescent Arrays and Nanoreactors, *Anal Chem.*, **2008**, *80*, 5279–5285.



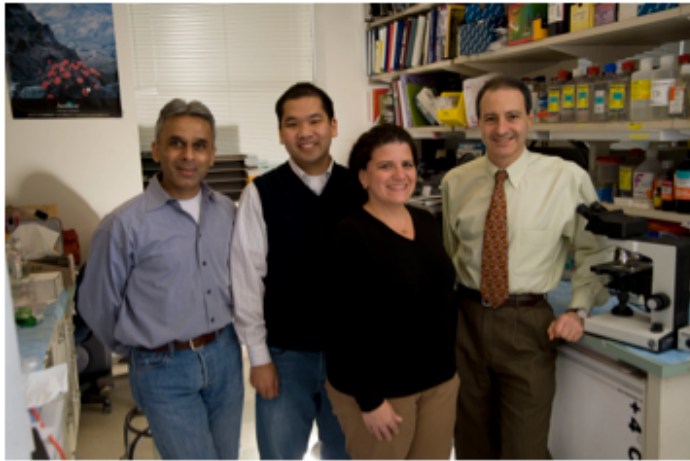
Using microsomes as enzyme source:

- RLM-metabolic enzymes
- *cyt P450s + CPR*
- *driven by NADPH*
- *h2E1 = cyt P450 2E1 + CPR*
- *same DNA adducts as reported previously*

SRM-MS/MS chromatograms measuring total ion current for predominant guanine adducts (m/z shown in inset) produced via exposure of a) NPYR, b) NNK, and c) NPIP to RLM/DNA nanoreactors followed by neutral thermal DNA hydrolysis. Inset shows guanine adduct and fragmentation pattern consistent with each chromatogram. d) Integrated peak area measured by UV detection increased for NPYR-guanine adducts with reaction time from both RLM and h2E1 films. Substrates: 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPYR).

Nanoparticle-Based Arrays for Sensitive Detection of Cancer Biomarker Proteins

- **Ultrasensitive detection of multiple cancer biomarkers**
- **Long term objectives**
 1. **early cancer detection, therapy monitoring**
 2. **tools for cancer research; surgical decisions**



Dr. Vyomesh Patel

Dr. Silvio Gutkind

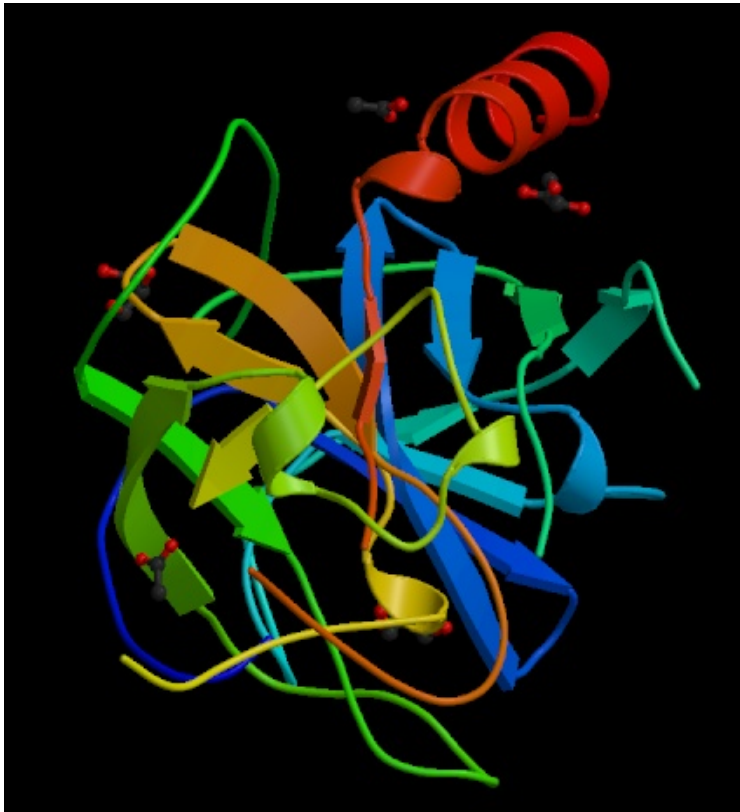
National
Institutes of Health,



Prof. Fotios Papadimitrakopoulos, Inst.
Materials Sci., Univ. of Connecticut

Funded by NIH

Biomarker Targets: 1. Prostate Specific Antigen



Adapted From Brookhaven Protein Databank

➤ PSA - Single chain glycoprotein , MW 33 kDa. Biomarker for prostate cancer

➤ Detection of PSA in serum: clinical detection of prostate cancer: **4-10 ng/mL, normal <3 ng/mL in serum**

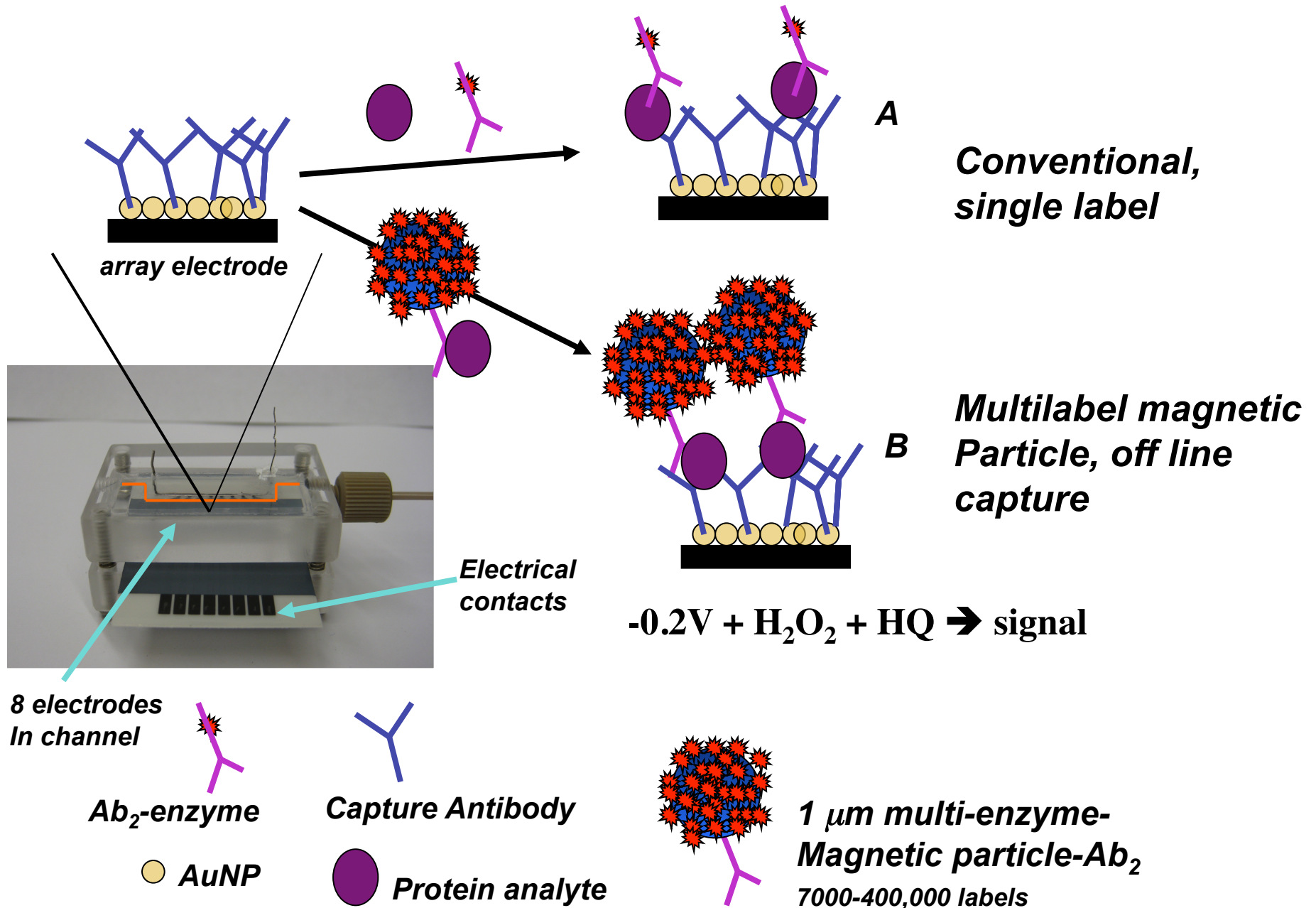
➤ Less invasive treatment protocols, avoid surgery

2. Interleukin 6 (IL-6)

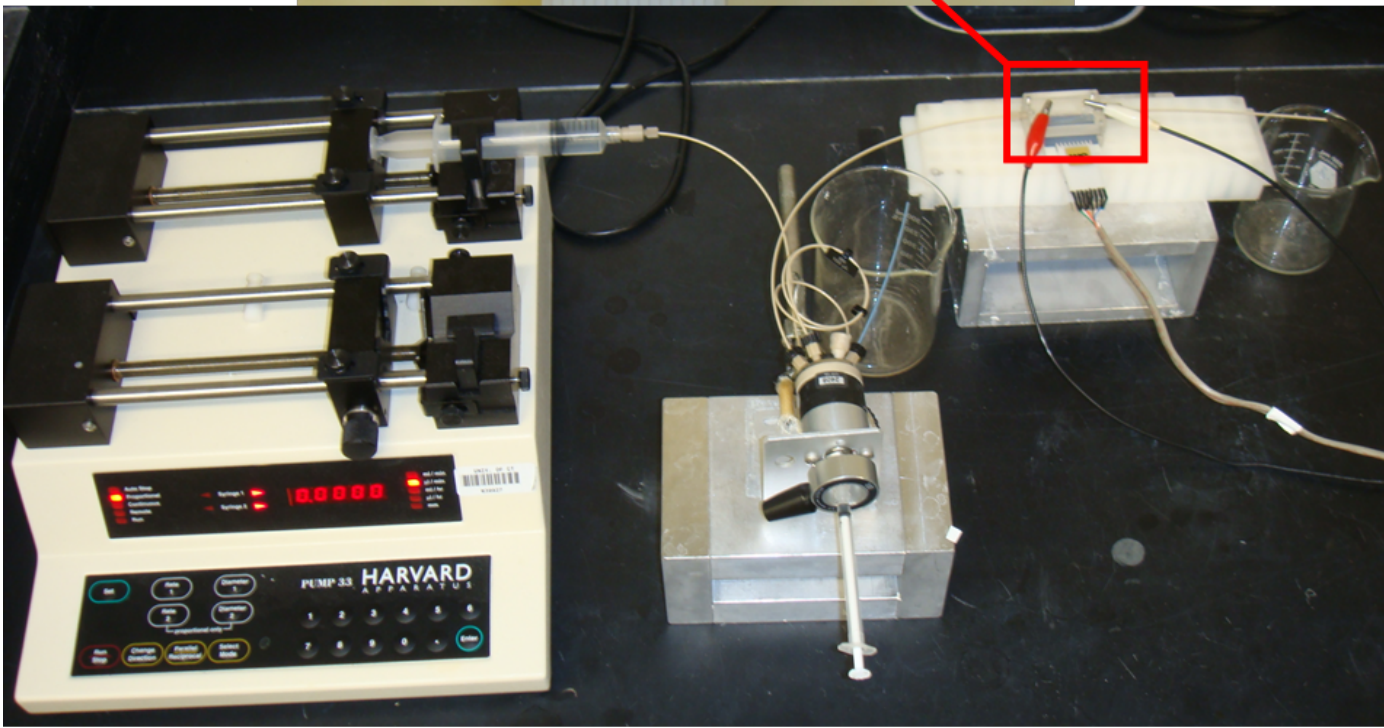
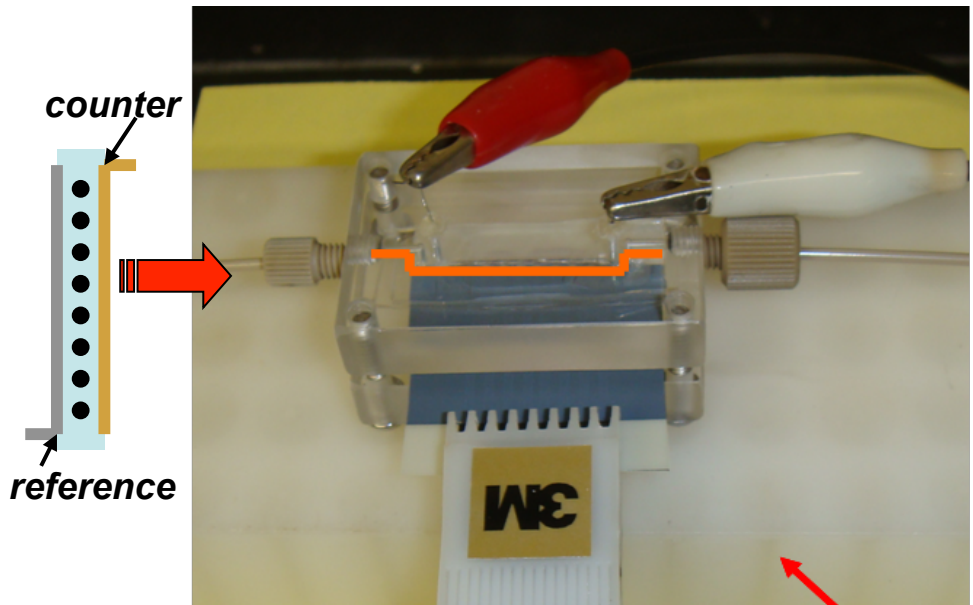
_ prostate and oral cancer biomarker

-human plasma conc. normal < 6 pg/mL; cancer 20-1000 pg/mL

Off-line capture by magnetic particles, microfluidic array

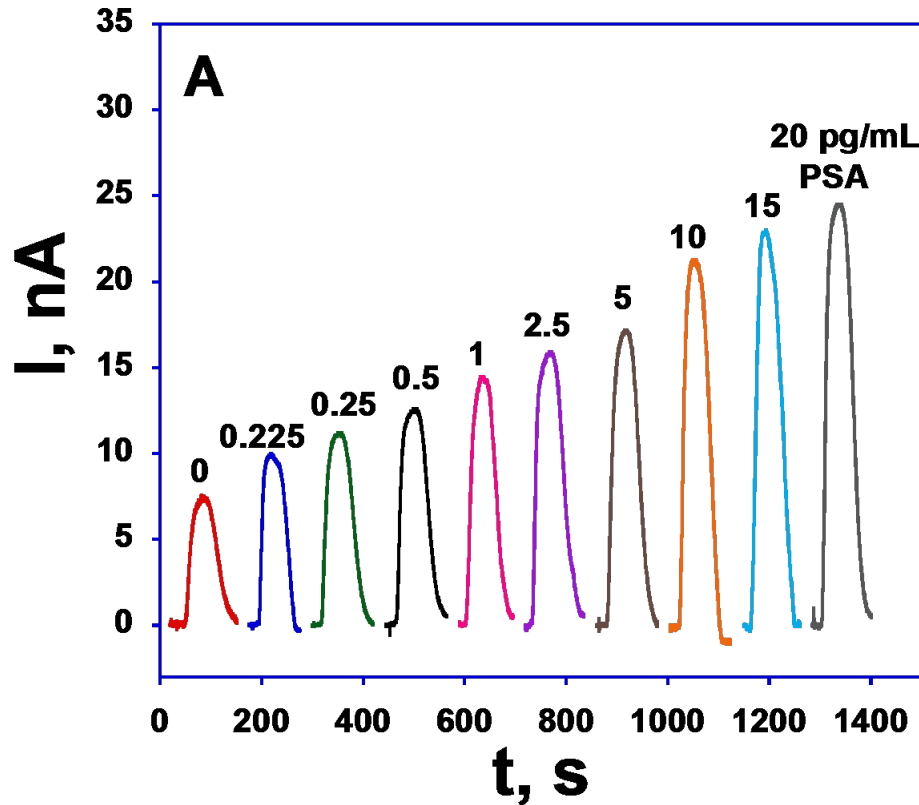


**8-electrode
microfluidic
system**

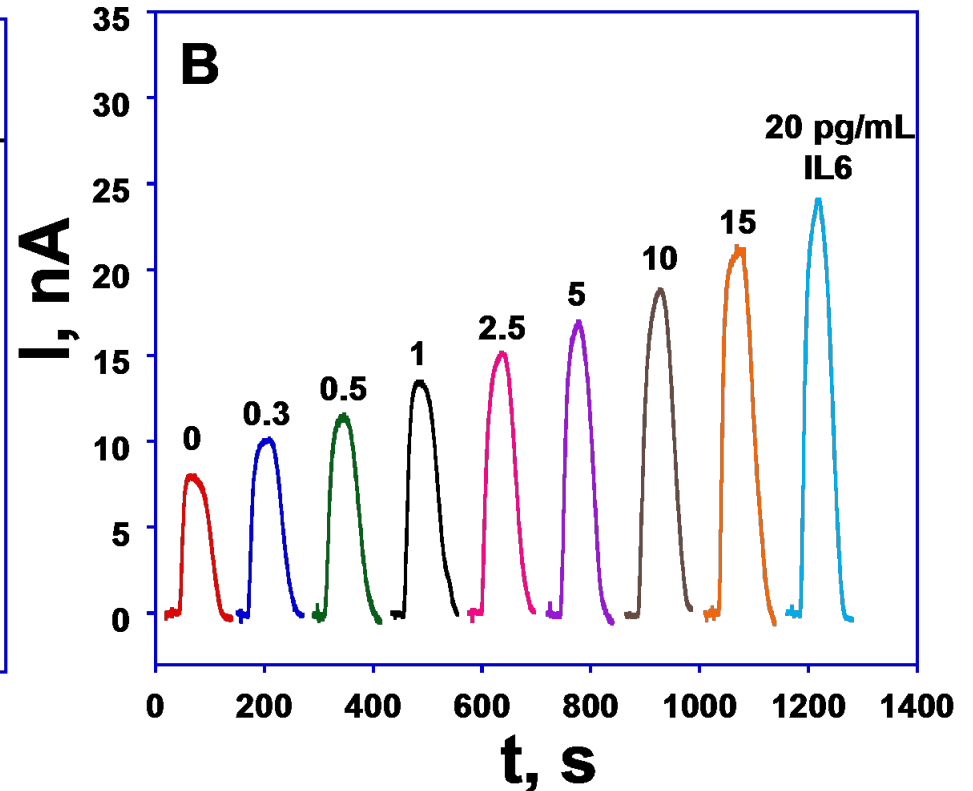


(a) pump, injector and 8-electrode microfluidic array with approximate electrode arrangement at top left

Microfluidic array data with off-line capture of PSA and IL-6 in serum, ~200,000 labels

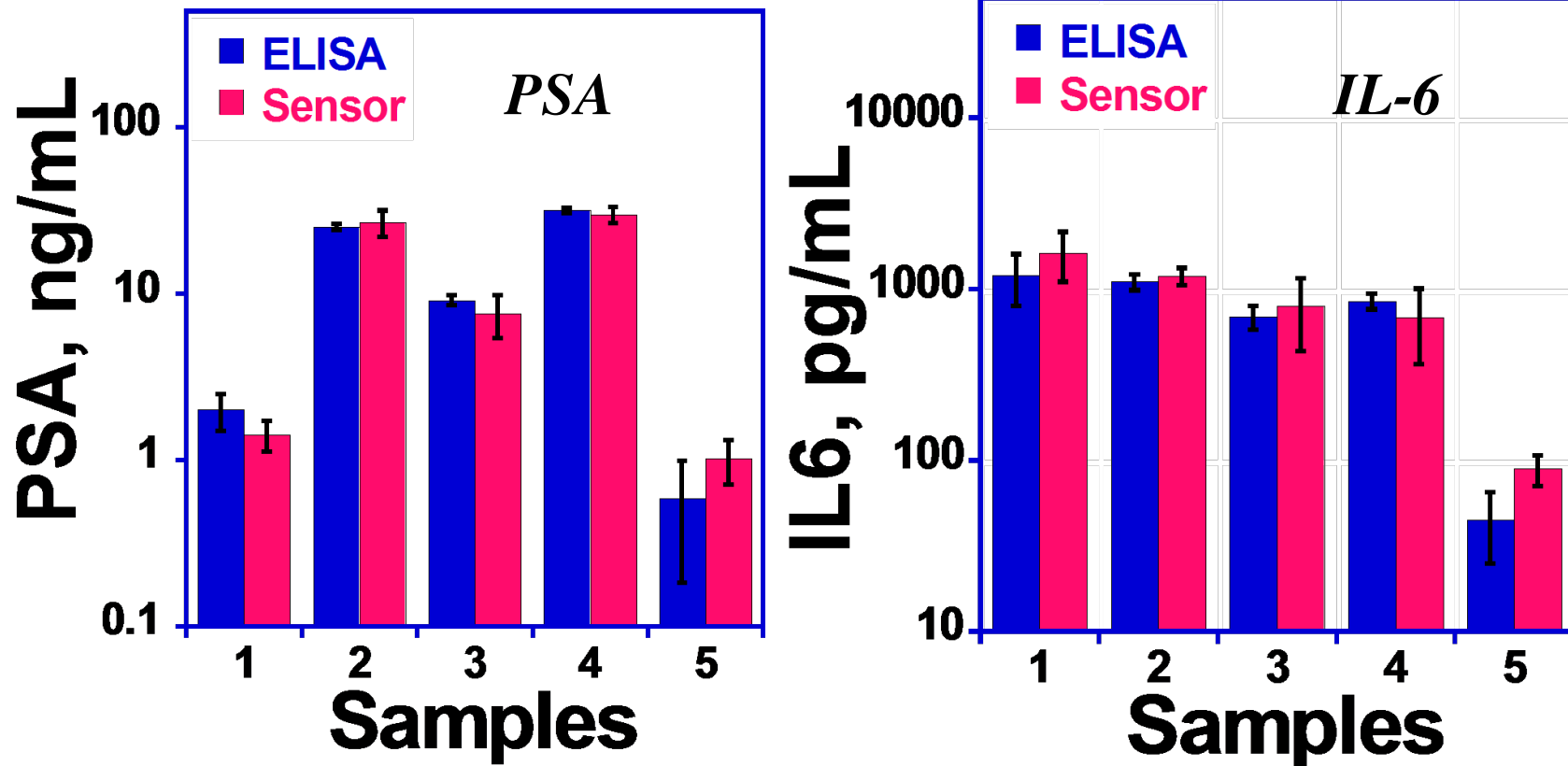


*Flow rate : 100 $\mu\text{L}/\text{min}$
 H_2O_2 : 100 μM
Hydroquinone : 1 mM*



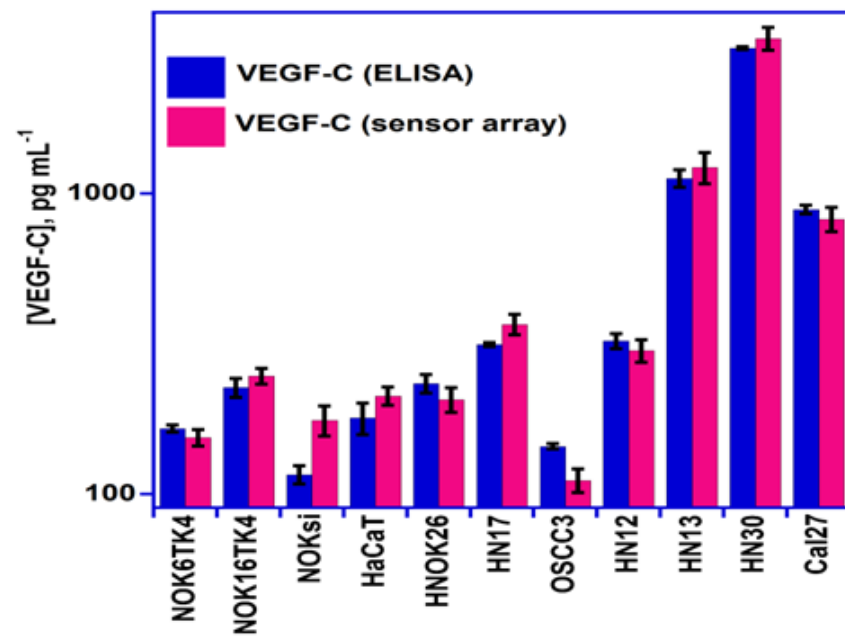
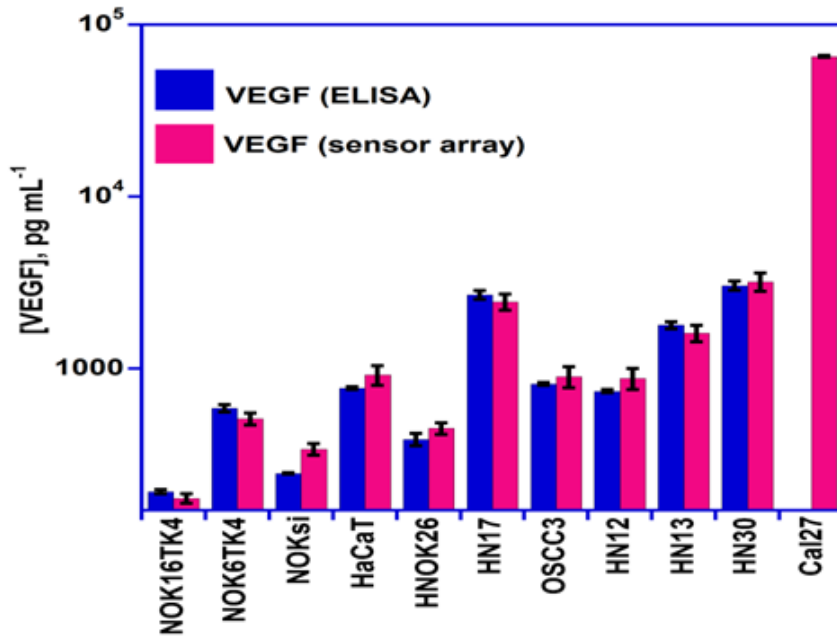
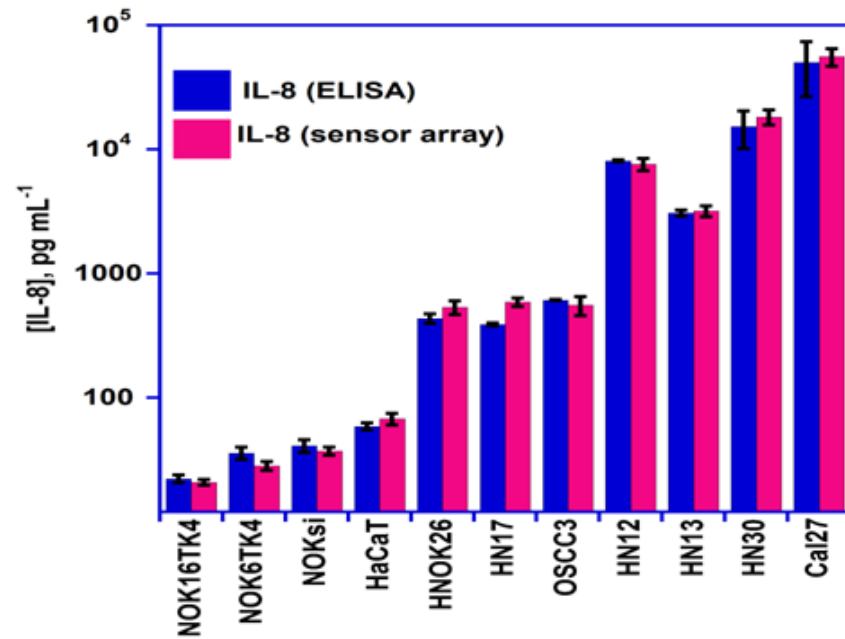
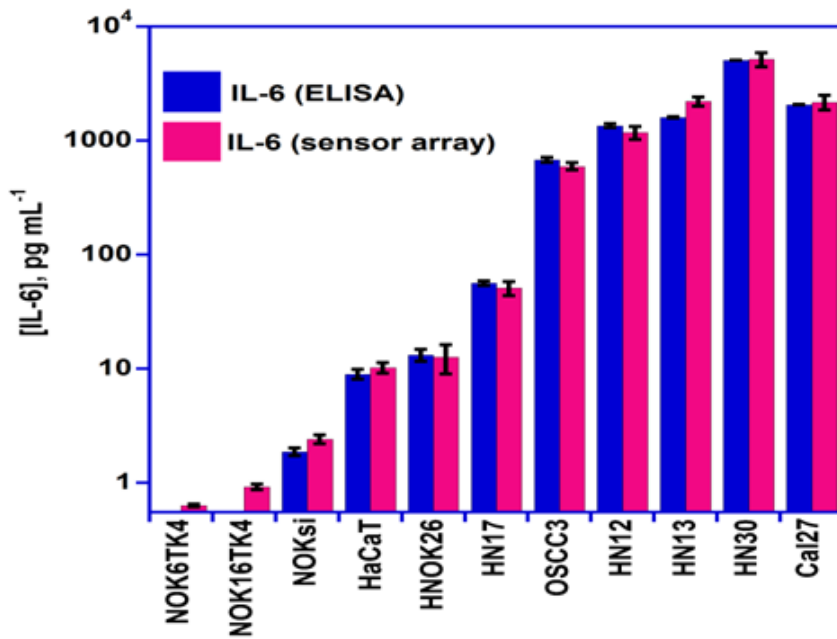
*Limit of detection of PSA: 0.23 pg/mL
Limit of detection of IL6 : 0.30 pg/mL*

Immunosensor assay validation on human serum
Good agreement with ELISA (std assay, one protein/run)



Bhaskara V. Chikkaveeraiah, Vigneshwaran Mani, Vyomesh Patel, J. Silvio Gutkind, and James F. Rusling, Microfluidic electrochemical immunoarray for ultrasensitive detection of two cancer biomarker proteins in serum, *Biosensors & Bioelectron.* 2011, 26, 4477– 4483.

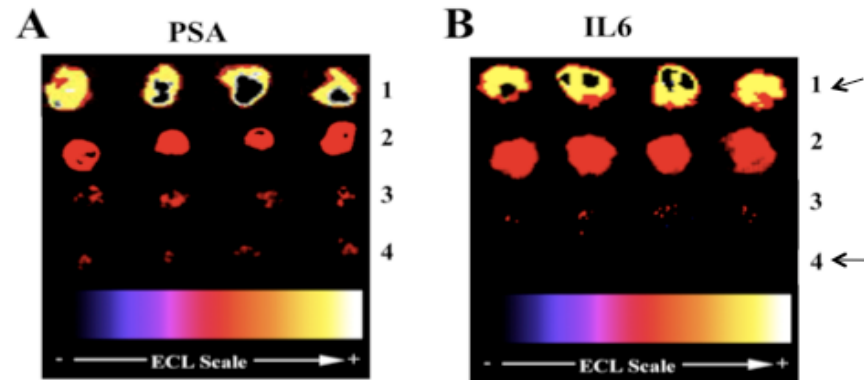
Oral cancer and normal cell conditioned media



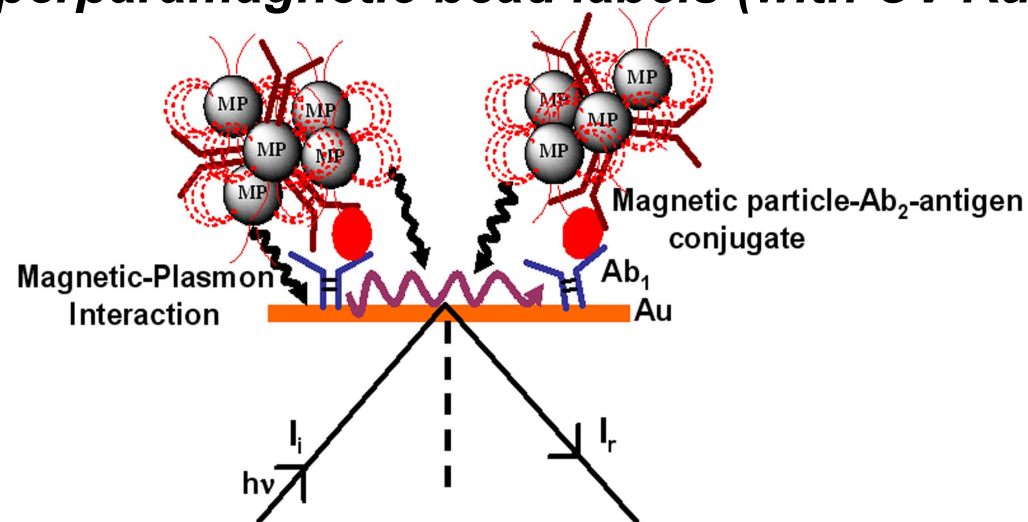
Other methods of protein detection

ECL arrays for detection of PSA and IL-6

DL ~ 0.1 pg/mL



Surface plasmon resonance (SPR) using superparamagnetic bead labels (with CV Kumar)



Our Univ. of Connecticut group, Dec. 2011

