Group Research Interests - an overview

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Example 1. Toxicity Screening

Lipophilic Molecule

\[ \text{Cyt P450, } O_2 \]

Enzyme-activated molecule

+DNA

Damaged DNA

Detect by voltammetry, LC-MS/MS

[Chemical structures: styrene, styrene oxide]
Films for Toxicity Screening

20-40 nm

ds-DNA

Enzyme

PDDA or Ru-PVP (catalyst) (Ru(bpy)$_2^{2+}$-PVP)

Pyrolytic Graphite
Collaboration with Prof. John Schenkman, Pharmacology, Uconn Health Center
Funding from NIH, NIEHS
Enzyme reaction - Incubate:
Reactant + H₂O₂ --> metabolite

Analysis by catalytic SWV or electrochemiluminescence

RuL²⁺ = RuL³⁺ + e-
RuL³⁺ + DNA-G --> RuL²⁺ + DNA-G•
Enzyme/DNA films

1. incubate 37 °C
2. SWV, 50 μM Ru(bpy)$_3^{2+}$, 0.2 mM H$_2$O$_2$
   + 2% styrene
   30 min

Controls - no styrene
   30 min
   15 min
   0 min

Bare PG

Peak increase measures damage of DNA by enzyme-generated metabolite

H$_2$O$_2$ R (e.g. styrene)
RO (e.g. styrene oxide)

Cyt P450
Detection of DNA-styrene oxide adducts after incubations of films + hydrolysis

LC-UV

Nucleobase adducts

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

![Graph showing the comparison of toxicity sensors with LC-MS for DNA damage by methylmethane sulfonate. The graph plots the sensor ratio against the incubation time in MMS (minutes) and shows a comparison between LC-MS/MS and sensor data.](Image)
Alternative way to detect DNA damage with light

Electrochemiluminescence

Ru-PVP

[Ru(bpy)$_2$-(PVP)$_{10}$]$^{2+}$

Pyrolytic Graphite

E=1.15 V

Echem detection

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.
Arrays: Which Liver Cytochrome P450s - generate toxic Benzo[a]pyrene Metabolites
Arrays detect in-vitro DNA damage from metabolites of different enzymes with DNA/enzyme films

**Figure 7.** Influence of incubation time with 50 μM benzo[a]pyrene and 1 mM H$_2$O$_2$ 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.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Rel. turnover rate, 1/min (nmol Enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mb</td>
<td>0.9</td>
</tr>
<tr>
<td>P450cam</td>
<td>3.0</td>
</tr>
<tr>
<td>P450 1A2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Drug development applications**
**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

![Graph showing SWV (10 Hz) of PVP-Ru/PSS/PVP-Os film](image)

**ECL detection in films:**

Single-Walled Carbon Nanotube Forests: Antigen-Antibody Sensing


End COOH groups allow chemical attachment to proteins (antibodies)
AFM of SWNT forest with and without antibody attached

Collaboration with F. Papadimitrakopoulos (ARO funded)

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

(a) SWNT

(b) SWNT + antibody (EDC coupling)
Sandwich Assay for Human Serum Albumin

SWNT forest

Apply E

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

LOD ~ 10 pmol/mL
**Styrene Epoxidation Catalyzed by Myoglobin-Polyion Films in Microemulsions (NSF funded)**

CROSSLINKED PLL/MbX FILM
Fully Covalently Linked Layers
Stable in microemulsions

Amide Links between PLL and Mb
Amide Links between Electrode and PLL
-COOH Groups

Oxidized graphite surface
Catalytic Styrene Epoxidation

Catalytic efficiency controlled by microemulsion composition
Additional Current Projects

• “Green” chiral synthesis using enzyme films in microemulsions, nanoparticle catalysts (NSF)

• Redox chemistry of complex photosynthetic proteins in membrane films (with H. Frank, USDA funded)

• detection of Lyme disease vectors (proteins) in tick saliva (with Uconn Health Center)

• enzyme activity for generating toxic metabolites (with Uconn Health Center)

• EC-Laser fluorescent detection of DNA damage

• arrays for anticancer biomarkers (NIH)

• molecular dynamics of film construction