Development of Immunoassays for the Detection of Markers of Human and Environmental Exposure

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UC Davis/NIEHS Superfund Hazardous Substances Basic Research Program

“Biomarkers of Exposure to Hazardous Substances”

University of California, Davis
PROJECTS

1. Transport, Transformation and Remediation of Perchlorate and VOCs in the Vadose Zone
2. Aquatic Biomarkers in Site Characterization and Remediation
3. Development and Implementation of Immunoassays for Human and Environmental Monitoring
4. Biomarkers of Exposure to Pulmonary Toxicants
5. Development and Applications of Integrated Cell-Based Bioassays
6. Assessing Adverse Effects of Environmental Hazards on Reproductive Health in Human Populations
7. Thermal Remediation
8. Development of Rapid, Miniaturized Sensors for Use in the Detection of Environmental Toxins
9. Epidemiology Studies
A. Analytical Chemistry Core
B. Statistical Analysis of Toxics Measurements
C. DNA Microarray Core
D. Training Core
F. Administrative Core
<table>
<thead>
<tr>
<th>Topic</th>
<th>Addressed by Projects</th>
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<tr>
<td>Analytical Meth</td>
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<tr>
<td>Biomedical</td>
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<td>Biostatistics</td>
<td>B, C</td>
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<td>6, 9, E</td>
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<td>Engineering</td>
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<td>Epidemiology</td>
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<td>Mechanism</td>
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<td>Remediation</td>
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<td>On Site Projects</td>
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<td>Toxicology</td>
<td>3, 4, 5, 6, A</td>
</tr>
<tr>
<td>Transport</td>
<td>1, 2</td>
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Halogenated Aromatic Hydrocarbons

2,3,7,8-Tetrachlorodibenzop-dioxin
2,3,7,8-Tetrachlorodibenzofuran
3,4,3',4',5'-Pentachlorobiphenyl
Chemical and Biological Techniques to Detect and Quantitate Halogenated Dioxins and Related Chemicals

Biological/Toxic Potency Estimates of a Complex Mixture are Based on TCDD Equivalency Factors (TEFs) for the Specific Chemicals Present in the Mixture.

TEFs are Derived from the Relative Toxic Potency of each HAH and is Directly Related to their Relative Ability to Activate the Ah Receptor (AhR) and AhR Signaling Pathway
ANALYTICAL PROBLEMS

• ANALYTICAL COSTS TOO HIGH
• ANALYSIS TIME TOO LONG
• ANALYSIS NOT FIELD PORTABLE
• ANALYSIS NOT IN ANALYSTS’ HANDS
• NOT ADAPTABLE TO MULTIPLE ANALYTES
<table>
<thead>
<tr>
<th>Compound</th>
<th>Cost Range</th>
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<tbody>
<tr>
<td>ATRAZINE</td>
<td>$50 - $150/Sample</td>
</tr>
<tr>
<td>PARAQUAT</td>
<td>$200 - $500/Sample</td>
</tr>
<tr>
<td>GLYPHOSATE</td>
<td>$500 - $1000/Sample</td>
</tr>
<tr>
<td>TCDD</td>
<td>$1,500 - $5,000/Sample</td>
</tr>
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</table>
ADVANTAGES

• Sensitive
• Selective
• Rapid
• Cost Effective
• Applicable
• Adaptable
DISADVANTAGES

• Sensitivity
• Cross reactivity/Interferences
• Reagent availability
• New technology
• Large sample load required
• Difficult to apply to multianalyte analysis
Absorbance vs Log Concentration

1. Polystyrene Coating Antigen
2. First antibody
3. Analyte
4. Substrate to Product
5. Enzyme coupled to Second Antibody
Immunoassays Developed

- Triazines
  - Atrazine
  - Simazine
  - Hydroxytriazines
  - N-Dealkylated triazines
  - Atrazine mercapturate
- Thiocarbamates
  - Molinate
  - Thioencarb
- Paraquat
- Amitrole
- Bentazon
- Bromacil
- Carbaryl
- Diflubenzuron
  - Other benzoylphenyl ureas
- Fenoxycarb
- Glyphosate
- Alternaria toxins
- Bacillus thuringiensis δ-endotoxin
- Bacillus thuringiensis β-exotoxin
- 2,4,5-Trichlorophenoxyacetic acid
- Trichlopyr
- Triton Series X and N detergents
- Octyl and nonyl phenol
- Urea Herbicides
  - Monuron
  - Diuron
  - Linuron
- Naphthalene and metabolites
- Nitrophenols (other nitroaromatics)
- Pyrethroids
  - Fenpropathrin
  - Esfenvalerate
  - Permethrin
  - Deltamethrin
  - 3-Phenoxybenzoic acid
  - Metabolites and conjugates
- Dioxins
- General Mercapturates
  - S-benzyl mercapturate
- General Glucuronides
  - Phenyl glucuronide
  - Thiophenyl glucuronide
Major Pyrethroids Used in California

Permethrin (57.5%)

Cypermethrin (22.6%)

Cyfluthrin (7%)

Esfenvalerate (6%)

*In 1998, a total of 650,000 lbs pyrethroids were applied in California
Hapten Design for Compound-specific Assay (I)

Target Compound: Permethrin

Hapten

- Close mimic of analyte
- Distal attachment site
- Four carbon handle optimal
Hapten Design for Class-specific Assay

<table>
<thead>
<tr>
<th>Target Compound</th>
<th>Hapten</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I Pyrethroids (without CN)</td>
<td><img src="image1.png" alt="Hapten Structure" /></td>
</tr>
<tr>
<td>Type II Pyrethroids (with CN)</td>
<td><img src="image2.png" alt="Hapten Structure" /></td>
</tr>
<tr>
<td>All Pyrethroids (with PB)</td>
<td><img src="image3.png" alt="Hapten Structure" /></td>
</tr>
</tbody>
</table>
Standard Curve for Permethrin

permethrin
$IC_{50} = 5$ ppb
LDL = 0.001 ppb

Concentration (ppb)

% Control Absorbance

0 0.001 0.01 0.1 1 10 100 1000 10000
0 20 40 60 80 100
Permethrin-specific Immunoassay (Selectivity)

- **Permethrin**: I50 = 2.5 ppb, LOQ = 0.01 ppb (SPE)
- ni = less than 10 % inhibition at 10 ppm
Relationship between Permethrin Measured by ELISA and GC-MS

\[ Y = 1.211x - 0.068 \]

\[ R^2 = 0.900, n = 27 \]
Role of Immunochemistry in Trace Analysis

- Direct immunochemical analysis
- Prioritization of samples for other methods
- Immunoaffinity cleanup for other methods
- Post separation detection
- HPLC/Microbore LC
- Capillary electrophoresis
- TLC
ELISA: A Complementary Technology

Analytical Chemist

MS  GC  CE  HPLC

IR  Immunoassay  SFC  UV/VIS
IC$_{50}$ = 4 pg/well
Strategies for detection in environmental samples

- Increased throughput
- Increased sensitivity
- Increased specificity

Achieved by

- Small volumes
- Minimized pre-processing of samples
- Minimized autofluorescence

Emission spectra of lanthanides
Development, Validation and Application of Recombinant Cell Bioassay Systems for Rapid Detection of Dioxins and Related Halogenated Aromatic Hydrocarbons

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Chemical and Biological Techniques to Detect and Quantitate Halogenated Dioxins and Related Chemicals

Instrumental  Immunoassay  Bioassays

Biological/Toxic Potency Estimates of a Complex Mixture are Based on TCDD Equivalency Factors (TEFs) for the Specific Chemicals Present in the Mixture.

TEFs are Derived from the Relative Toxic Potency of each HAH and is Directly Related to their Relative Ability to Activate the Ah Receptor (AhR) and AhR Signaling Pathway.
Spectrum of Toxic and Biological Effects Produced by TCDD in Different Species and Tissues

- Immunotoxicity
- Hepatotoxicity
- Wasting Syndrome
- Tumor Promotion
- Dermal Toxicity (Chloracne)
- Teratogenicity
- Lethality
- Uroporphyrin Accumulation (Porphyria)
- Endocrine Disruption
- Modulation of Cell Growth, Proliferation and Differentiation
- Induction of Gene Expression
  - Cytochrome P4501A1/2 and 1B1
  - UDP-Glucuronosyl Transferase1*06
  - Glutathione S-TransferaseYa
  - Quinone Reductase
  - γ-Aminolevulinic Acid Synthase
  - Prostaglandin Endoperoxide H Synthase 2

The biological and toxicological effects of TCDD and related halogenated aromatic hydrocarbons (HAHs) are mediated by the Ah Receptor (AhR)
AhR Signal Transduction Pathway

Endogenous Ligands

New Polypeptides

Increased Cytochrome P-4501A1

Other Gene Products

Toxicity
AhR Signal Transduction Pathway

- Endogenous and Exogenous Ligands
- Translation of New Polypeptides
- Increased Cytochrome P4501A1
- ARNT
- Exogenous and Endogenous Ligands
- Other Gene Products
- mRNA
- DREs
- Other Factors
- Translocation
- Proteosome
- Degradation
- New Polypeptides
- Other Gene Products
- Toxicity
Development of a Ah Receptor-Based Bioassay Bioassay System for Detection and Relative Quantitation of Dioxin and Related HAhs

TCDD + AhR

Ligand Binding

TCDD:AhR

Transformation

TCDD:AhR*

DNA Binding

TCDD:AhR:DRE

Gene Expression*

Transcriptional Activation
CALUX (Chemically-Activated Luciferase Expression) Cell Bioassay

- TRANSLATION
- New Polypeptides
- Increased Cytochrome P-450A1
- Luciferase Activity
- Luciferase
- Other Factors?

TCDD
- HAHs
- PAHs

AhR
- hsp90
- XAP2

ARNT

DREs

CYP1A1

mRNA
H1L6.1c3 Mouse Hepatoma Cells Plated into 96-Well Microplates

Chemicals Added to Each Well and Incubated for 24 hours

Wells are Washed, Cells Lysed, and Luciferase Activity Measured in a Microplate Luminometer
TCDD Dose Dependent Induction of Luciferase Activity in Stably Transfected Mouse Hepatoma (Hepa1c1c7) Cells

EC50 = 10 pM
MDL = 1 pM
Current Microplate Assay
MDL = 0.03pg/assay
Activation of the CALUX Cell Bioassay by PCDDs, PCDFs and PCBs
## WHO Toxic Equivalency Factors (TEFs) and CALUX Relative Potency (REP) Factors for Chlorinated Dibenzo-p-Dioxins, Dibenzofurans and Biphenyls.

<table>
<thead>
<tr>
<th>Compound</th>
<th>WHO-TEF</th>
<th>CALUX REP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2378-TCDD</td>
<td>1</td>
<td>1.00 ± 0.01</td>
</tr>
<tr>
<td>12378-PoCDD</td>
<td>1</td>
<td>0.73 ± 0.1</td>
</tr>
<tr>
<td>123478-HxCDD</td>
<td>0.1</td>
<td>0.075 ± 0.014</td>
</tr>
<tr>
<td>123678-HxCDD</td>
<td>0.1</td>
<td>0.098 ± 0.017</td>
</tr>
<tr>
<td>123789-HxCDD</td>
<td>0.1</td>
<td>0.061 ± 0.012</td>
</tr>
<tr>
<td>1234678-HpCDD</td>
<td>0.01</td>
<td>0.01 ± 0.008</td>
</tr>
<tr>
<td>OCDD</td>
<td>0.0001</td>
<td>0.00034 ±0.00008</td>
</tr>
<tr>
<td>2378-TCDF</td>
<td>0.1</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td>12378-PoCDF</td>
<td>0.5</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>22478-PoCDF</td>
<td>0.5</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>123478-HxCDF</td>
<td>0.1</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>123678-HxCDF</td>
<td>0.1</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>123789-HxCDF</td>
<td>0.1</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>224678-HxCDF</td>
<td>0.1</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>1234678-HpCDF</td>
<td>0.01</td>
<td>0.024 ± 0.007</td>
</tr>
<tr>
<td>1234789-HpCDF</td>
<td>0.01</td>
<td>0.044 ± 0.010</td>
</tr>
<tr>
<td>OCDF</td>
<td>0.0001</td>
<td>0.0016 ± 0.0005</td>
</tr>
<tr>
<td>PCB 77</td>
<td>0.0005</td>
<td>0.0014 ± 0.0004</td>
</tr>
<tr>
<td>PCB 81</td>
<td>0.0001</td>
<td>0.00045 ± 0.00012</td>
</tr>
<tr>
<td>PCB 114</td>
<td>0.0005</td>
<td>0.00014 ± 0.00002</td>
</tr>
<tr>
<td>PCB 126</td>
<td>0.1</td>
<td>0.038 ± 0.007</td>
</tr>
<tr>
<td>PCB 156</td>
<td>0.0005</td>
<td>0.00014 ± 0.00002</td>
</tr>
<tr>
<td>PCB169</td>
<td>0.01</td>
<td>0.0011 ± 0.0003</td>
</tr>
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Bioassay Systems - Considerations

Structural Diversity of AhR Agonists and/or Antagonists: Potential for False Positives in HAH Detection

- Promiscuous ligand binding by the AhR requires appropriate extraction and chemical clean-up methods to remove unwanted compounds.
Flow Diagram for Analysis of Samples by CALUX and GC/MS

Sample

Extraction and Chemical Clean-up

Total HAHs

PCDD/PCDF & PCB

CALUX Bioassay Analysis

Positive

Analysis by HRGC/MS

No Compounds that Activate the Ah Receptor and/or Contains Compounds that Block Activation (i.e. Antagonists)

Negative

Estimate of relative activity
Bioassay Systems - Considerations

- Calculation of relative biological potency of an unknown sample extract

EC50 = 20pM
Thus the original extract contains the equivalent (bioassay-TEQ) of 20 nM TCDD
Applications of the CALUX Dioxin Cell Bioassay

1. Biological Samples
   - Blood (whole serum and extracts)
   - Breast Milk
   - Tissue Extracts

2. Environmental Samples
   - Soil and Sediment
   - Ash
   - Emission (PUF)
   - Pulp and Paper

3. Food Samples
   - Animal Fats (oil and fats)
   - Milk and Butter
   - Animal Feeds
Comparative GCMS/CALUX Results from the Umea Round Robin Analysis of Environmental Samples for Chlorinated Dibenzo-p-Dioxins, Dibenzofurans and Biphenyls.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TEQ (ng/g sample)</th>
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<tbody>
<tr>
<td></td>
<td>GC/MS</td>
</tr>
<tr>
<td>Soil A</td>
<td>0.3</td>
</tr>
<tr>
<td>Soil B</td>
<td>0.17</td>
</tr>
<tr>
<td>Soil C</td>
<td>0.19</td>
</tr>
<tr>
<td>Soil D</td>
<td>0.30</td>
</tr>
<tr>
<td>Ash A</td>
<td>0.40</td>
</tr>
<tr>
<td>Ash B</td>
<td>0.04</td>
</tr>
<tr>
<td>Ash C</td>
<td>0.42</td>
</tr>
<tr>
<td>Solution F</td>
<td>222</td>
</tr>
<tr>
<td>Solution H</td>
<td>3.42</td>
</tr>
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Double-Blind Validation Results
Comparison of CALUX and HR GC/MS Analysis of Ash and Exhaust Gas Samples

$R^2 = 0.947$

$R^2 = 0.918$
Double-Blind Validation Results
Comparison of CALUX and HR GC/MS
Analysis of Soil and Human Fat Samples

**Soil**

- R² = 0.854

**Human Fat**

- R² = 0.8051
Environmental Samples Contain Additional Ah Receptor Active HAHs?

- **Ash**
  - \( R^2 = 0.947 \)

- **Soil**
  - \( R^2 = 0.854 \)

- **Exhaust Gas**
  - \( R^2 = 0.918 \)

- **Human Fat**
  - \( R = 0.8051 \)

\[
\begin{array}{c}
\text{CALUX, pg TEQ/gram} \\
0.01 & 0.1 & 1 & 10 & 100 & 1000 & 10000 \\
0.1 & 1 & 10 & 100 & 1000 & 10000 & 100000 \\
\end{array}
\]
**Bioassay Systems - Further Considerations**

- Calculation of relative biological potency of an unknown sample extract
- Promiscuous ligand binding by the AhR requires appropriate extraction and chemical clean-up methods for each bioassay

- Discrepancy between bioassay and instrumental analysis needs to be resolved
  a. Use of bioassay-specific REPs for TEQ estimates from instrumental analysis
  b. False positives or other AhR-active HAHs
Comparison of GC/MS TEF and CALUX REP Values

![Comparison of GC/MS TEF and CALUX REP Values](chart)

**Chart Description:**
- The chart compares the CALUX REP values for dioxin-like HAhs with the WHO TEF values for dioxin-like HAhs.
- The x-axis represents the WHO TEF values, while the y-axis shows the CALUX REP values.
- The data points indicate a correlation between the two sets of values, with most points clustering around the 45-degree line, suggesting a close agreement.
- The chart visually demonstrates the comparison, allowing for an easy assessment of the alignment between the two sets of values.
Tiered Flow Diagram for Sample Screening

CALUX

- **POSITIVE** Run immunosassay

- **NEGATIVE** No compounds that bind productively to Ah receptor

- **POSITIVE** Run GC/MS for confirmation
  - PCB ELISA
  - TCDD ELISA

  - **NEGATIVE** No TCDD-like compounds
    - **NEGATIVE** No TCDD-like compounds
    - **BOTH NEGATIVE** Use CALUX to drive chemical purification

  - **POSITIVE** Run GC/MS for confirmation

  *ELISA Also suggests that there are more 2,3,7-PCDD/Fs than GC/MS indicates.*

  *What could it be?*
Halogenated Aromatic Hydrocarbons

2,3,7,8-Tetrachlorodibenzo-p-dioxin
2,3,7,8-Tetrachlorodibenzofuran
Polybrominated Diphenyl Ether

Sources:
- Formed during PDBE Synthesis
- Burning of PBDE Containing Materials
- Photochemical Reactions of PBDEs

2,3,7,8-PBDD/F found in PBDE workers @ n.d - 500pg/g blood lipid.

Mo-OctBDF produced from combustion of DBDE-polypropylene matrices

Polybrominated Dibenzofurans (PBDFs)
Polybrominated Dibenzo-p-dioxins (PBDDs)
Brominated Dioxins and Related HAHs are Relatively Potent Activators

![Graph](image-url)
In Vivo ED_{50} Values for Toxic and Induction Effects of Select PHDDs in the Rat

<table>
<thead>
<tr>
<th>Congener</th>
<th>In Vivo ED_{50} (μmol/kg body weight)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body Weight Loss</td>
<td>Thymic Atrophy</td>
</tr>
<tr>
<td>2,3,7,8-TeCDD</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>2,3,7,8-TeBDD</td>
<td>0.068</td>
<td>0.034</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDD</td>
<td>0.62</td>
<td>0.17</td>
</tr>
<tr>
<td>1,2,3,7,8-PeBDD</td>
<td>0.87</td>
<td>0.39</td>
</tr>
<tr>
<td>2,3-DiB-7,8-DiCDD</td>
<td>0.012</td>
<td>0.0073*</td>
</tr>
<tr>
<td>2-B-3,7,8-TriCDD</td>
<td>0.12</td>
<td>0.035</td>
</tr>
<tr>
<td>1,2,4,7,8-PeCDD</td>
<td>34.0</td>
<td>11.2</td>
</tr>
<tr>
<td>1,2,4,7,8-PeBDD</td>
<td>12.9</td>
<td>6.17</td>
</tr>
</tbody>
</table>

Immature male Wistar rats (n=4), 14 days after a single intraperitoneal dose. Adapted from IPCS Env. Health Criteria #205 - data from Mason et al. (1987); Safe et al. (1989).
AhR Gene Expression Cell Bioassay Systems

**Advantages**
- Sensitive, relatively rapid and easy to carry out
- Amenable to high throughput analysis
- Relatively inexpensive compared to instrumental analysis
- A significant amount of validation data is available for the CALUX bioassay
- Overestimate TCDD equivalents in environmental samples (new dioxin-like HAHs?)

**Disadvantages/Limitations**
- Experience in cell culture techniques necessary
- Proper sample cleanup methods needed
- Instrumentation - Luminescent microplate readers, cell culture facilities
- Overestimate TCDD equivalents in environmental samples (false positives?)
- Extracts containing chemicals that are directly toxic to cells can not be analyzed
- Synergistic/antagonistic effects (over/underestimate potency)
Bioassay Systems - Further Considerations

• Calculation of relative biological potency of an unknown sample extract

• Promiscuous ligand binding by the AhR requires appropriate extraction and chemical clean-up methods for each bioassay (in vitro assays require more extensive clean-up methodologies)

• Discrepancy between bioassay and instrumental analysis needs to be resolved
  a. false positives or other AhR-active HAHs
  b. Use of bioassay-specific REPs for TEQ estimates from instrumental analysis

• Toxic Equivalency Factors (TEFs) versus Relative Potency (REPs) Values
  TEQs (instrumental) versus Bioassay-TEQs

• Need method(s) to correct for extraction and clean-up efficiency and recovery since samples can’t simply be spike with AhR-active HAHs like 2,3,7,8-TCDD

• Establish quality control criteria for bioassay methodologies

• Require full validation studies (versus instrumental analysis) for different matrices
Cell Bioassay Systems - Development

Microplates containing cells can be sealed and stored at room temperature for up to 14 days with little loss of TCDD-inducible luciferase activity.

Viewplate microplate with well sealer

This development now allows plates to be prepared off-site and mailed to the site for extract treatment and analysis.
## Application and Utilization of Bioanalytical Methods for Dioxin Analysis

- Detection, quantitation and chemical identification of dioxin-like chemicals in a variety of matrices including:
  - Environmental samples (soil, water, air)
  - Biological (blood, milk, tissues)
  - Food and feed
  - Commercial and consumer products

- Determination of the effectiveness of bioremediation, biodegradation and contamination clean-up procedures for dioxin-like chemicals.

- Identification and characterization of other classes of dioxin-like chemicals.
Acknowledgements and Support

University of California @ Davis
Michael Ziccardi, Joe Rogers, Patricia Garrison,
Bruce Hammock, Shirley Gee, Guomin Shan

Xenobiotic Detection Systems
George Clark, David Brown, Mick Chu

Hiyoshi Corporation (Japan)
Hiroshi Murata

Scientific Institute of Public Health, Brussels, Belgium
Leo Goeyens, Ilse van Overmeire

National Institutes of Environmental Health Sciences (NIEHS)
Superfund Basic Research Program - ESO4699, ES04911
Nanotechnology and Biosensors

Ian Kennedy

Department of Mechanical and Aeronautical Engineering and
Biomedical and Electrical Computer Engineering Graduate Groups

University of California Davis

Supported by the NIEHS Superfund Basic Research Program and NSF NNI
Strategies for detection in environmental samples

Increased throughput
Increased sensitivity
Increased specificity

Achieved by
Small volumes
Minimized pre-processing of samples
Minimized autofluorescence
New fluorescent labels
Novel technologies

- Competitive immunoassays in microdroplets using fluorescence quenching
- Microdroplet cavity resonances
- Application of the microdroplet approach to analysis on a chip
- Development of new labels using novel materials
  - Quantum dots
  - Encapsulated lanthanide oxides
  - Absorbing labels such as $C_{60}$
Quenching fluoroimmunoassay in microdroplets

Competitive Quenching Fluoro ImmunoAssay (QFIA)

Antibody quenching

▲ analyte

▲ Labeled analyte
Assay for TCP

Calibration curve for 2,4,6-TCP in PBS and in urine 1/50; 10 nM Af; 2.5 mg/ml Ab43; incubation time 45 min at room temperature

<table>
<thead>
<tr>
<th>µg/L</th>
<th>ELISA</th>
<th>QFIA 200 µL</th>
<th>QFIA microdrop</th>
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<tr>
<td>IC50</td>
<td>2.74</td>
<td>4.2</td>
<td>0.45</td>
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<tr>
<td>LOD</td>
<td>0.2</td>
<td>0.36</td>
<td>0.45</td>
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<table>
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<tr>
<th>µg/L urine</th>
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</thead>
<tbody>
<tr>
<td>IC50</td>
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<tr>
<td>LOD</td>
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</table>
Micro cavity resonances

- Intense optical field created by internally reflected fluorescence from Rhodamine
Intensities within droplets greatly reduced when $C_{60}$ is added to droplet.
Detecting bacteria

- Single E. coli detected using optical resonances without labeling
- Couple to immunoassay for specificity or use mode structure for information
Microdroplets on a chip

Biosensor chip

Microdrops of aqueous sample

Microchannel filled with low refractive index silicon oil
Trapped microdroplet with long interrogation time
Lanthanide oxide nanoparticles

- Sharp emission spectrum increases spectral sensitivity
- Long lifetime emission permits gated detection
- Surface treatment prevents quenching
- No need for chelation
- Magnetic moments useful for separation and for imaging contrast agents
- No photobleaching
Lanthanide oxides as reporters for bioassays

<table>
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<td>La</td>
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<td>Gd</td>
<td>Tb</td>
<td>Dy</td>
<td>Ho</td>
<td>Er</td>
<td>T</td>
<td>Yb</td>
<td>Lu</td>
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Diagram: Visual Light Region of the Electromagnetic Spectrum

Infrared: 0.5 µm to 3 µm
Visible: 0.4 µm to 0.7 µm
Ultraviolet: 0.3 µm to 0.4 µm
Europium oxide spectra

**Europium**

Excitation spectrum observed at 610 nm

Emission spectrum excited at 466 nm

**Rhodamine**

Excitation spectrum observed at 548 nm

Emission spectrum excited at 524 nm
Gas phase synthesis and functionalization

A functionalized nanoparticle containing lanthanide oxide can be used in an immunoassay that takes advantage of the specific binding between an antigen and a homologous antibody.
Pure Eu$_2$O$_3$ particles

Photoluminescence Spectrum:
Dominant Peaks at 615 and 623 nm;
Short Lifetime Due to Concentration Quenching Combined with Small Size.

Pure Eu$_2$O$_3$; Monoclinic Phase; Gas-phase Synthesis.
Composite Eu$_2$O$_3$/SiO$_2$ particles

Eu$_2$O$_3$/SiO$_2$ Composite Nanoparticles; Higher-density Core with Lower-density Shell; Gas-phase Synthesis.

Photoluminescence Spectrum: Dominant Peak at ~615 nm; Lifetime ~1 ms
Europium oxide lifetime

- Lifetimes of 1 ms or more
- Biological background typically less than 10 ns

Eu:Y$_2$O$_3$
Functionalized nanoparticles

- Microwave chemistry and gas phase chemistry used to add protective SiO layer and NH₂ functional group
Optical properties of functionalized particles

- Optical properties of Eu$_2$O$_3$ unchanged by surface treatment
- Additional phosphors obtained by doping lanthanides into hosts
- Used in immunoassay for atrazine
Nanophosphors separated with microchip electrophoresis

- 80 μm channel electrophoresis system
- separated two size classes of nanoparticles
- can be used for assays
Europium oxide label in a competitive immunoassay

Competition

Labeled analyte

Ab
Europium oxide label in a competitive immunoassay

Separation

Magnetic bead and 2 Ab
Europium oxide in a pyrethroid metabolite assay

- Competitive assay for 3-phenoxybenzoic acid (PBA) with magnetic separation
- Europium oxide particle conjugated to the hapten

$IC_{50} = 2 \times 10^{-4} \text{ ng mL}^{-1}$
New nanoparticle assay formats

- Multi wavelength labels can be used to provide an internal standard for sandwich immunoassays
Time gated detection

[Diagram showing intensity vs. time with peaks for Organic dye, Background, Gated detection time, and Eu nanoparticle]
Eu phosphors in micro channels
Fluorescence lifetimes

- Eu lifetime can be greater than 1ms
- Fluorescein lifetime of the order of 10 ns
MEMS sensors

Quartz transmits UV excitation
Glass blocks excitation light, transmits visible signal
Summary

Nanoscale materials, engineered from the “bottom up”, can be functionalized for use in biology

Optical properties promise more sensitive detection in complex matrix, possibly reagentless

Eu labels demonstrated in immunoassays with up to $10^4$ improvement in detection limits

Other properties of nanoscale materials can be useful (magnetic, thermophoretic, electrophoretic) for separation

Brilliant dust?
Thank You

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Thank You

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