Fluorous Immobilization for Microarray Formation

June 19, 2007
Microarray Applications . . . A General Overview

Genomic Arrays
- Comparative Genomic Hybridization
- Gene Expression Profiling

Protein Arrays
- Protein - protein interactions
- DNA - protein interaction
- Small molecule screening
- Enzyme-substrate analysis
- Reverse Phase Arrays
- Cell lysates

Antibody Arrays
- Protein profiling
- Antibody characterization
- Quantitative Multiplexed ELISA
- Sandwich arrays

Non Biological
- Chemical pedestals
- Microfluidics
- Precise sub-nanoliter deposition

Image courtesy of Dr. Gavin MacBeath, Bauer Center for Genomics Research, Harvard University
Surface Chemistries Available Through Thermo Scientific or Fluorous Technologies:

- Poly-L-Lysine, Aminosilane, Aldehyde Silane, Epoxysilane
- 3-D Gels (for protein work) available for beta-testing
- Fluorous-coated slides for small molecules, peptides, carbohydrate, protein arrays, etc
- High sensitivity BioBright™ Slides for improved detection of low expression molecules (produces 10-20X increased signal:noise) available for beta-testing
Microarray Dept.

3150 Sqft
With 800 Sqft Cleanroom
Microarray Products and Accessories from Thermo Scientific

mBox with handle for processing

Slide packaging

Novel and Custom Surfaces Available Upon Request
Complimentary Samples provided
I. Introduction to Fluorous Techniques and Chemistry

II. Early Fluorous Based Immobilization

III. Fluorous Microarray: Initial Report

IV. Properties of Fluorous Slides

V. Applications of Fluorous Microarrays

VI. Benefits
What is Fluorous Technology?

- Fluorous chemistry is a novel tagging technology that separates desired molecules from complex mixtures.

- Molecules can be rendered fluorous by the attachment of perfluorocarbon domains.

- Fluorous tagged molecules can be separated from non-fluorous molecules exploiting fluorophilicity.

- Fluorous techniques are marked by high selectivity, low reactivity, and exceptional breadth.
Examples of Fluorous Molecules

Compounds with permanent fluorinated domains (e.g. reagents):

\[
PPh_3 \quad -- \text{VERSUS--} \quad \text{Ph}_2\text{P}-\text{Ph}-\text{C}_6\text{F}_{13}
\]

Substrate → Product + F

Compounds with temporary fluorous tags (e.g. substrates):

\[
\text{O}\overset{\text{N}}{\text{H}}\text{CO}_2\text{H} \quad -- \text{VERSUS--} \quad \text{O}_{\text{Ph}}\overset{\text{N}}{\text{H}}\text{CO}_2\text{H}
\]

F Substrate → reagent → F Product + By-product
Fluorous Separation Methods

- **Liquid-Liquid Extraction**
  - “Heavy” fluorous technique
  - Generally requires large F content, ~60%

- **Fluorous Solid Phase Extraction (F-SPE)**
  - “Light” fluorous technique
  - Separates fluorous from non-fluorous
  - No fluorous solvents used

- **Fluorous Chromatography (F-HPLC)**
  - Separates fluorous from fluorous
  - More fluorous = Greater retention
Fluorous Tags vs. Hydrophobic Tags

Fluorous compounds are hydrophobic and lipophobic.
Fluorous Specificity and Selectivity

Highly selective fluorous purification of complex peptide mixture

Fluorous-Based Synthesis and Purification

- **Small Molecule Libraries** (Zhang, W. *Tetrahedron*, 2003, 59, 4475)


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**Diagram Details**

1. Fluoro-Pak™ Column
2. on-column detritylation w/TFA

+ non-tagged impurities
Fluorous-Based Synthesis and Purification


• Leverages fluorine’s unique physical properties

• Novel mechanism bioorthogonal to other separation/immobilization technologies

• Allows diverse chemistry and versatile separation options

• Strong IP coverage around the process & materials
Fluorous Protein Immobilization

Amine modification

Adsorption

Solid fluorocarbon

Immobilized protein suitable for affinity chromatography

<table>
<thead>
<tr>
<th>Enzyme</th>
<th># of fluorous groups</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>4-5</td>
<td>70-80%</td>
</tr>
<tr>
<td>Cathepsin C</td>
<td>6-12</td>
<td>75-95%</td>
</tr>
<tr>
<td>Polynucleotide phosphorylase</td>
<td>13-17</td>
<td>76-82%</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>3-5</td>
<td>71-93%</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>6-11</td>
<td>66-82%</td>
</tr>
</tbody>
</table>

Fluorous Based Synthesis and Immobilization

Fluorous supported oligosaccharide synthesis

First report of:
- direct microarraying onto homemade fluorous derivatized glass slides
- Use of same tag for synthesis, separation and immobilization

Fluorescence images of arrayed carbohydrates probed with FITC labeled lectins
- Highly Specific
- Preservation of Activity
- Concentration dependent response

Features of Commercial Fluorous Modified Slides

Joint development of FTI and Thermo Fisher Scientific

Key Features

- Low, stable background fluorescence
  - Less prone to change vs. APS
- High contact angle, extremely hydrophobic surface
  - 2x vs. APS or Epoxy
- Compact, consistent spot size
  - ~35% smaller vs. APS

100° average contact angle (goniometer)

![Graph showing pre-scan background and average spot size](image)
Fluorous Small Molecule Microarrays

Work of Schreiber Group, Broad Institute

Initial evaluations using Fluorescent-tagged Streptavidin:

- Fluorous slides spotted with:
  - F-tagged biotin-PEG and F-tagged biotin cadaverine
  - Untagged biotin-PEG and biotin cadaverine (negative control)
  - DMSO-only (negative control)

Results:

- Excellent, consistent binding and signal to noise
- No binding with negative controls
- Biotin-PEG performed better than biotin cadaverine (expected)
- 5-10 mM concentration gave excellent results

Fluorous Small Molecule Microarrays

FK Binding Protein (FKBP) evaluations:

• Fluorous slides spotted with:
  – F-tagged Holt ligand, known binder to FKBP
  – Untagged Holt ligand (negative control)
  – DMSO-only (negative control)
  – Assayed using labeled antibody with purified FKBP or FKBP lysate

Results:

– Excellent, consistent binding and signal to noise
– No binding with negative controls
– 1.25 - 5 mM concentration gave excellent results
– FKBP lysate gave excellent results with minimal non-specific binding

Examining a real world application

- Histone deacetylases (HDAC) are well-known to cause tumor growth and therefore attractive targets for chemotherapy agents.

- Small molecule libraries of SAHA analogs have been produced and screened for HDAC inhibition.

- Broad Institute researchers are using an integrated fluorous approach to synthesis, immobilize, and analyze HDAC inhibitor libraries.

Detection of Histone Deacetylase Binding

Fluorous Microarrays Demonstrate:

• Low, Uniform Background

• Excellent Signal to Noise
  • Superior to any other surface they have used, including APS

• Tight Features (~150 μm diameter)

• Simplified workflows

• Low non-specific binding

• Single fluorous chain can immobilize a small molecule-protein complex and present a specific orientation

Potential Applications

Many additional classes of compounds now under investigation

- Oligonucleotides
- Protein arrays
- Peptides
- Antibodies
Fluorous Microarray Benefits

1. No blocking step
2. Excellent spot morphology (40% reduction in spot diameter)
3. Less washing steps
4. Low non-specific binding allows use of cell lysates
5. High sensitivity
6. Low and uniform background fluorescence
7. High signal-to-noise

Fluorous immobilization → fluorous-modified surface → Incubation
Fluorous Microarrays of Non F-Tagged Content

Taking Advantage of Fluorous Benefits Using Existing Content

- Would allow microarray producers to use existing content to produce fluorous microarrays
- Requires spot-on-spot spotting
  - Acoustic drop ejection vs. pin spotting
Fluorous Microarrays - A Simpler Alternative

- Fluorous immobilization
- Fluorous-modified surface
- Plating
- Incubation

- Mother plate with existing content
- Daughter plate with fluorous tagging reagent in each well

- Would allow microarray producers to use existing content to produce fluorous microarrays and leverage workflow benefits
Other Surfaces: Fluorous Affinity Based MS

- Direct deposition, enrichment, and MS analysis on a fluorous modified porous Si chip.
- Enrichment and detection of fluorous tagged amino acids, peptides and carbohydrates demonstrated.
- Potential applications in proteomics, metabolomics and diagnostics

Go, E.P.; et al, J. Proteome Res. 2007, 6, 1492.
The FTI Total Advantage Product Offering

No other technology offers all three steps (synthesis, purification, immobilization) in one continuous work flow process!
Future Directions

• Immobilization of other molecular classes
  – Oligonucleotides
  – Peptides
  – Proteins and Antibodies
  – Aptamers

• Hybridization / Incubation Protocols

• Fluorous reverse phase arrays

• Fluorous Modification of Other Surfaces
  – 3D-surfaces, polymers, etc.

• Utilization of other Spotting Technologies
  – Inkjet
  – Acoustic Droplet Ejection (ADE)
Potential Applications

• Genomics
  – Gene expression profiling
  – Genotyping

• Proteomics
  – Profiling of biomarkers
  – Analysis of protein activities
  – Autoimmune and inflammatory disease detection
  – Allergy screening

• Drug Discovery and Vaccine Development
  – ADME/Tox applications
  – Small molecule and fragment screening

• Diagnostics
  – Cancer detection / profiling
  – Viral diagnostics
Fluorous Modified Slides

Key Features

- Low, stable background fluorescence
- High contact angle, extremely hydrophobic
- Compact, consistent spot size
- Uniform surface: slide to slide, batch to batch
- Robust handleability

Now available from FTI!

- Part Number 850-9100
- 10 slides/box
- $110/box