

Fluorous Immobilization for Microarray Formation



ThermoFisher
S C I E N T I F I C

June 19, 2007



Protein structure courtesy Protein Data Bank, PDB ID 152L, J.Zhang,B.Matthews

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**

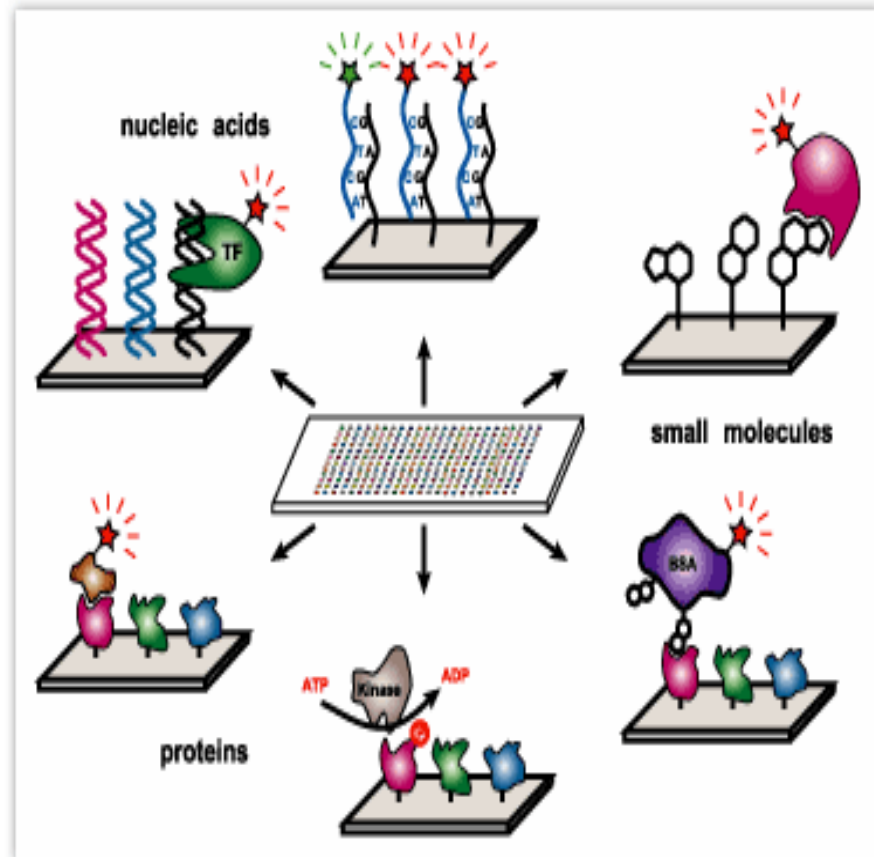


Image courtesy of Dr. Gavin MacBeath,
Bauer Center for Genomics Research,
Harvard University

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**

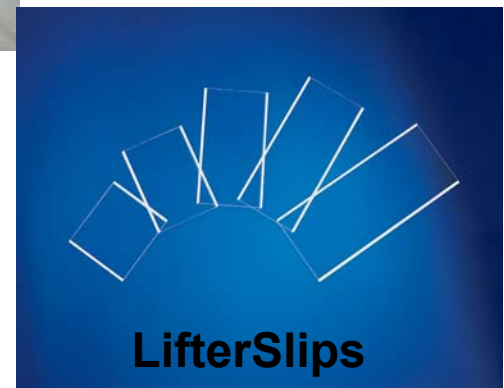


Microarray Products and Accessories from Thermo Scientific

**mBox with handle
for processing**



Slide packaging



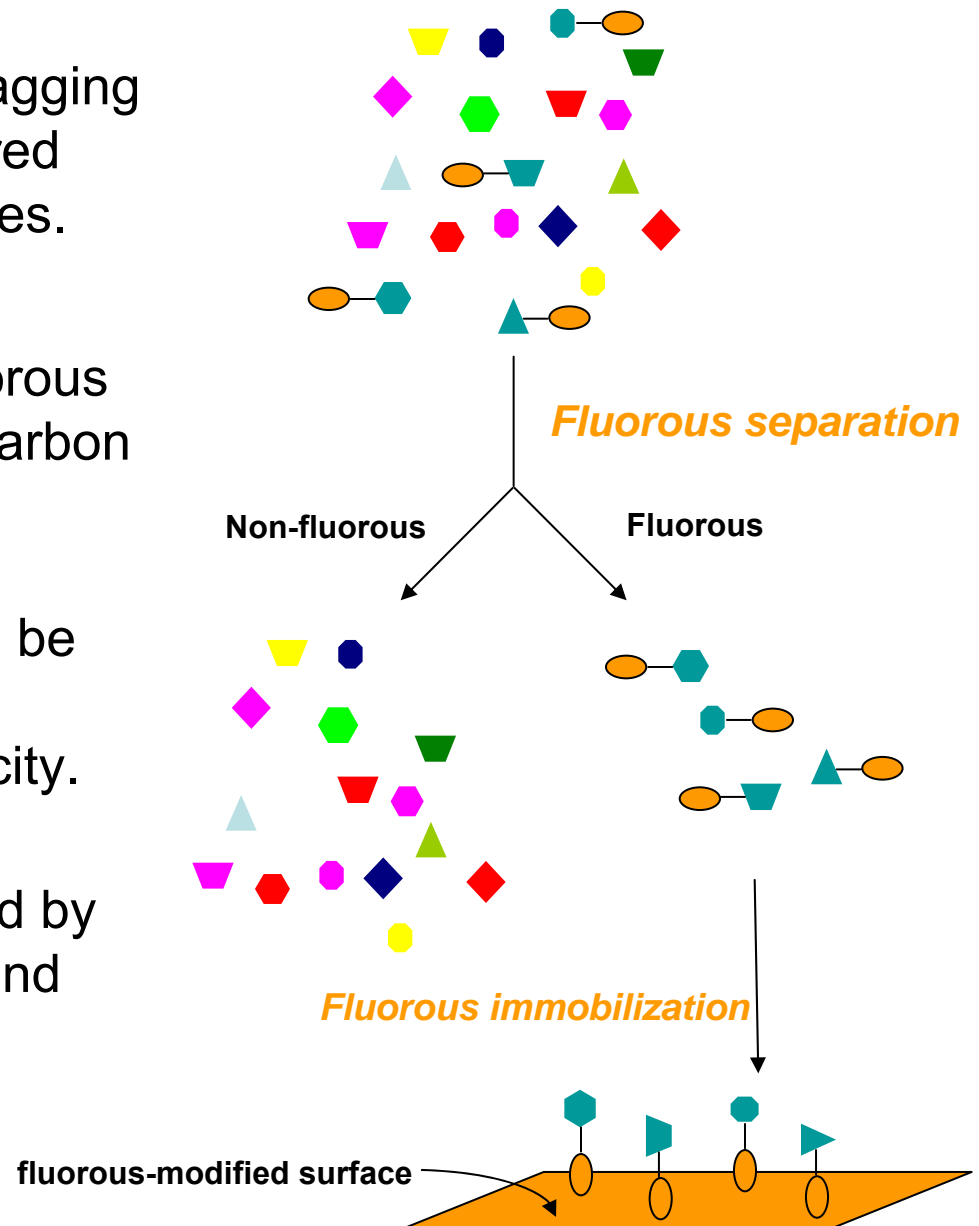
LifterSlips

**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**

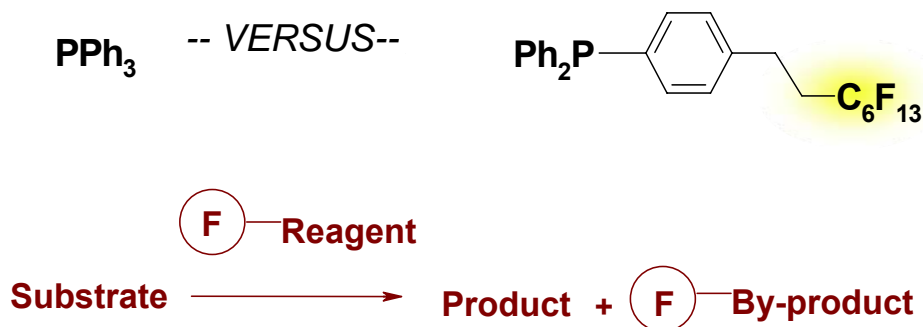
What is Fluorous Technology?

- 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

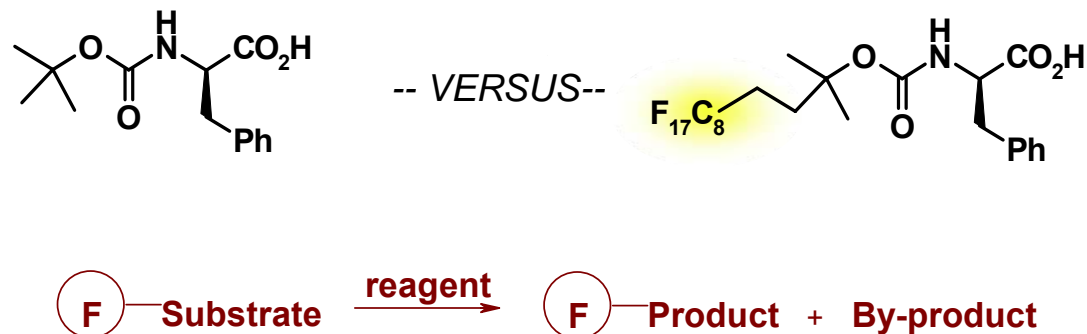


Examples of Fluorous Molecules

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

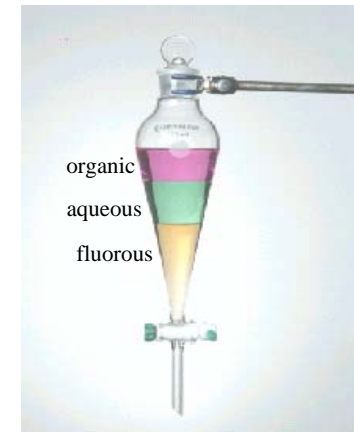


Compounds with temporary fluororous tags (e.g. substrates):



■ Liquid-Liquid Extraction

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



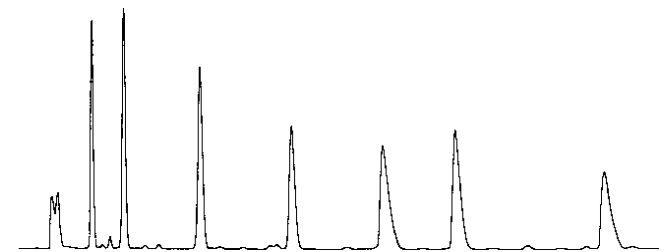
■ Fluorous Solid Phase Extraction (F-SPE)

- “Light” fluorous technique
- Separates fluorous from non-fluorous
- No fluorous solvents used

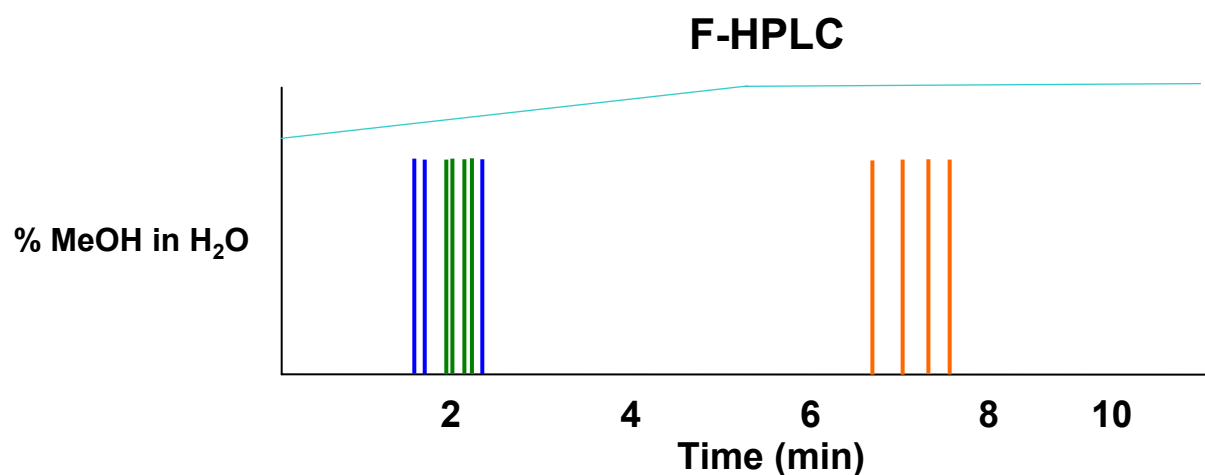
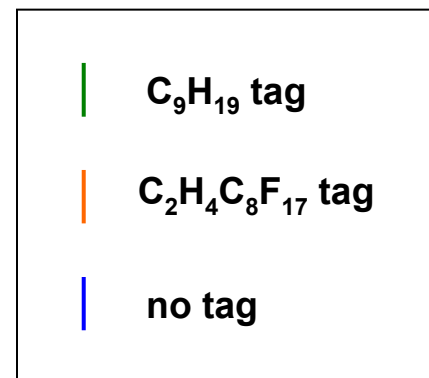
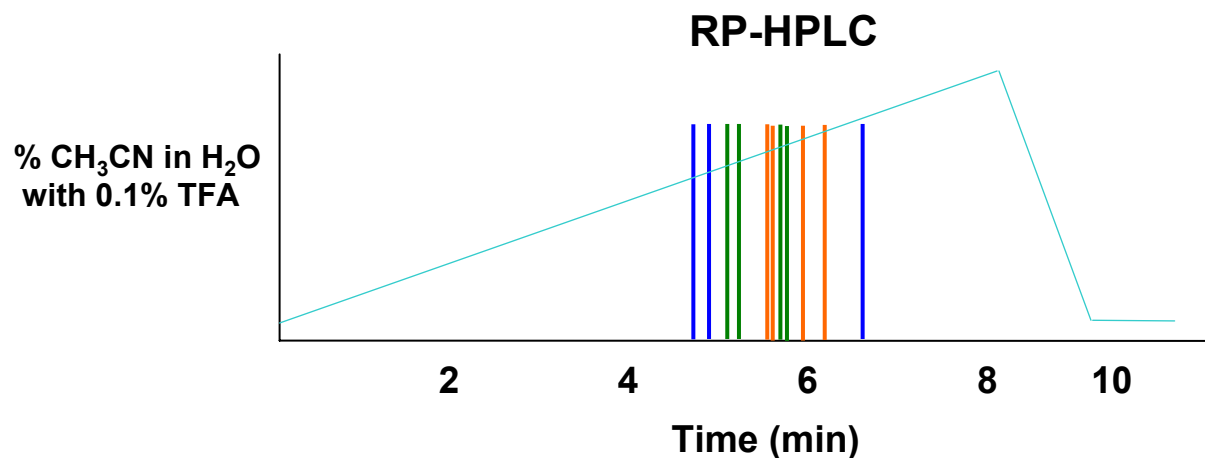


■ Fluorous Chromatography (F-HPLC)

- Separates fluorous from fluorous
- More fluorous = Greater retention



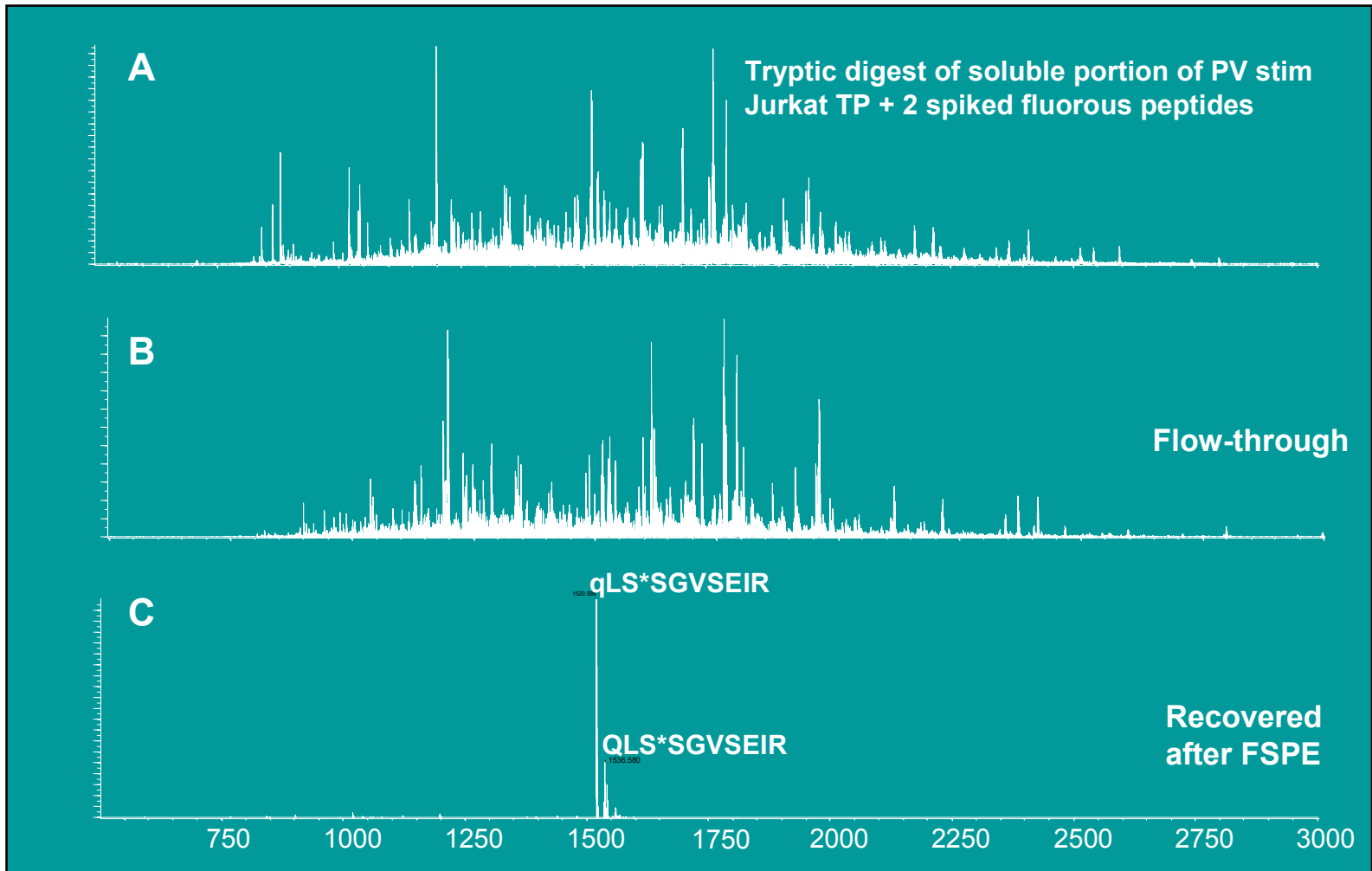
Fluorous Tags vs. Hydrophobic Tags



Tagged amino acids are Ser, Glu, Phe, and Trp.

The untagged controls were galactosyl pentaacetate, (Boc-Cys-OH)₂, and PPh₃.

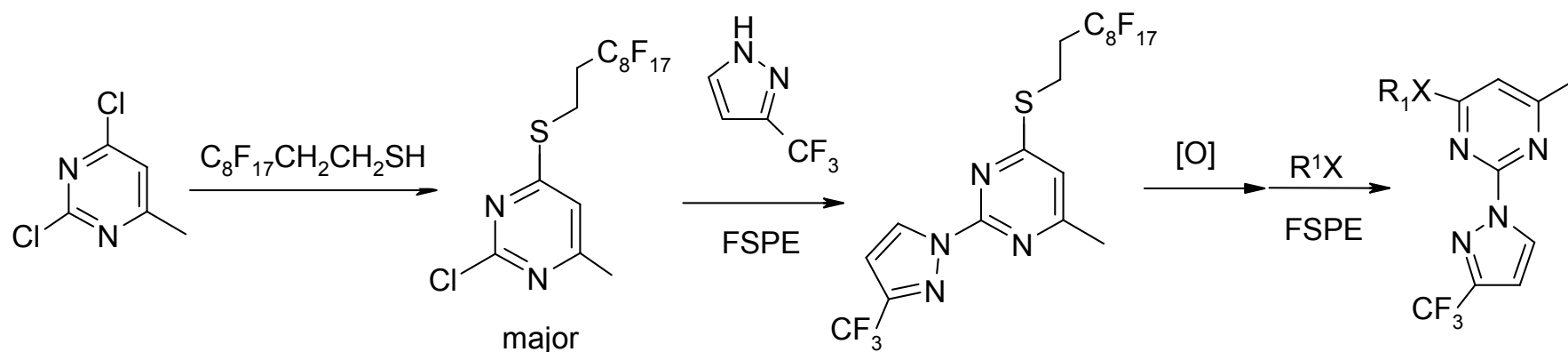
Fluorous compounds are hydrophobic *and* lipophobic.



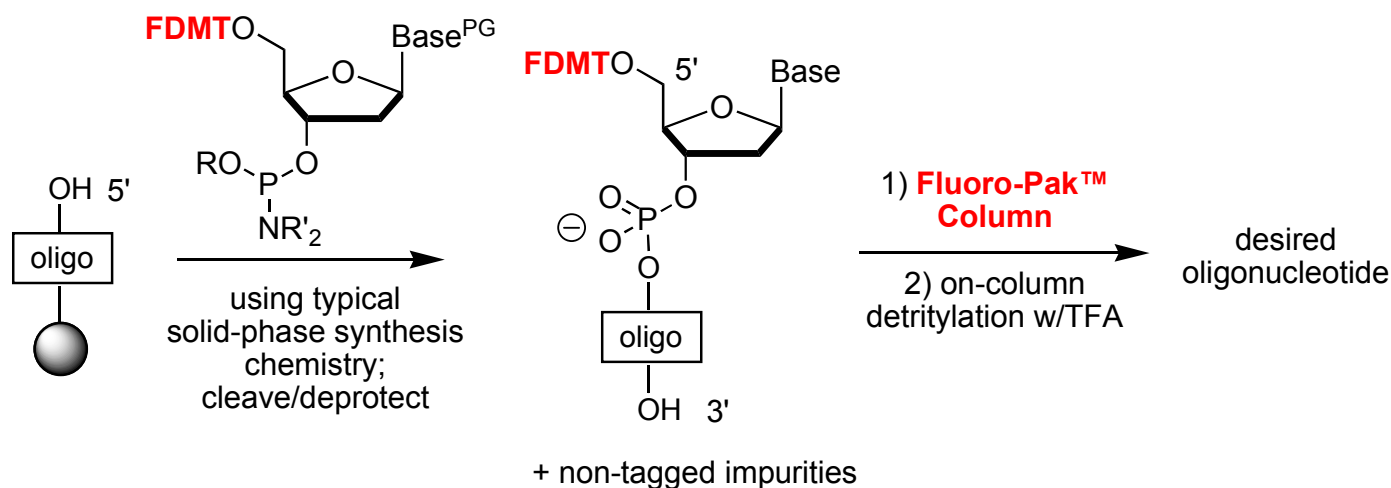
Highly selective fluorinated purification of complex peptide mixture

Fluorous-Based Synthesis and Purification

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

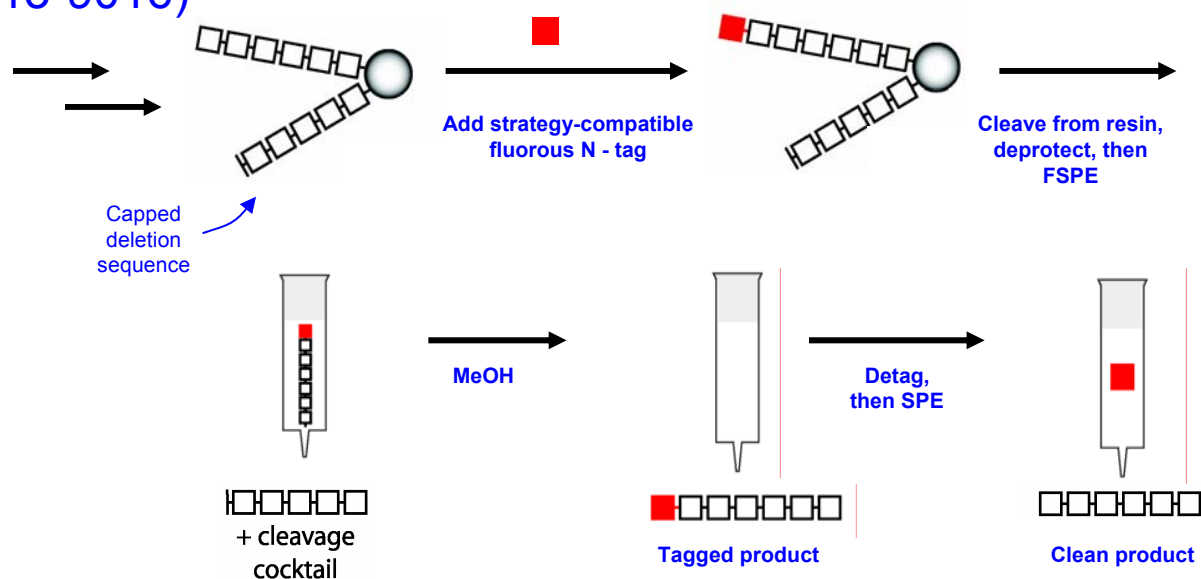


- Synthetic Oligonucleotides (Pearson, W.H. *et al*, *J. Org. Chem.* **2005**, 70, 7114.)

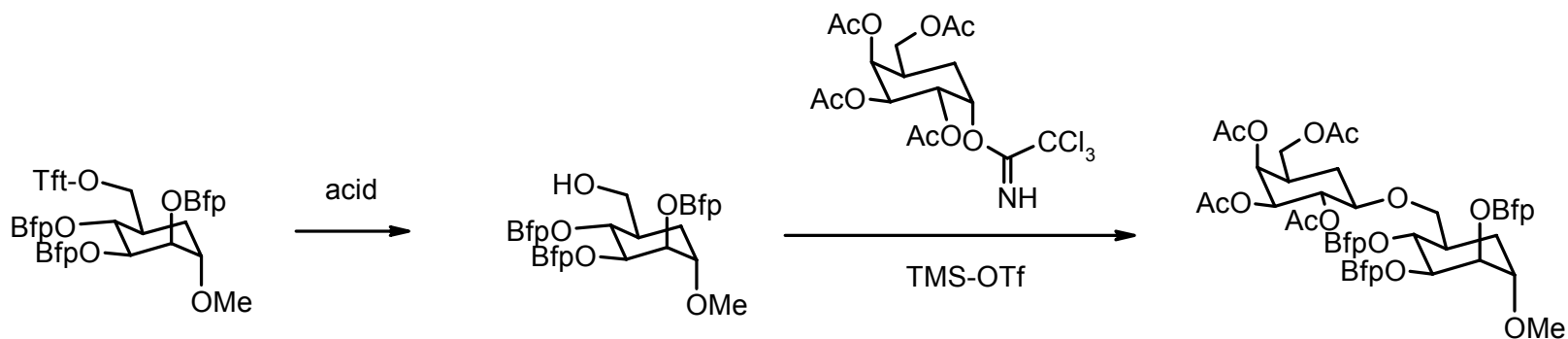


Fluorous-Based Synthesis and Purification

- Peptide Synthesis (Overkleeft, van Boom, *et al. Tetrahedron Letters* **2003**, 44, 9013-9016)

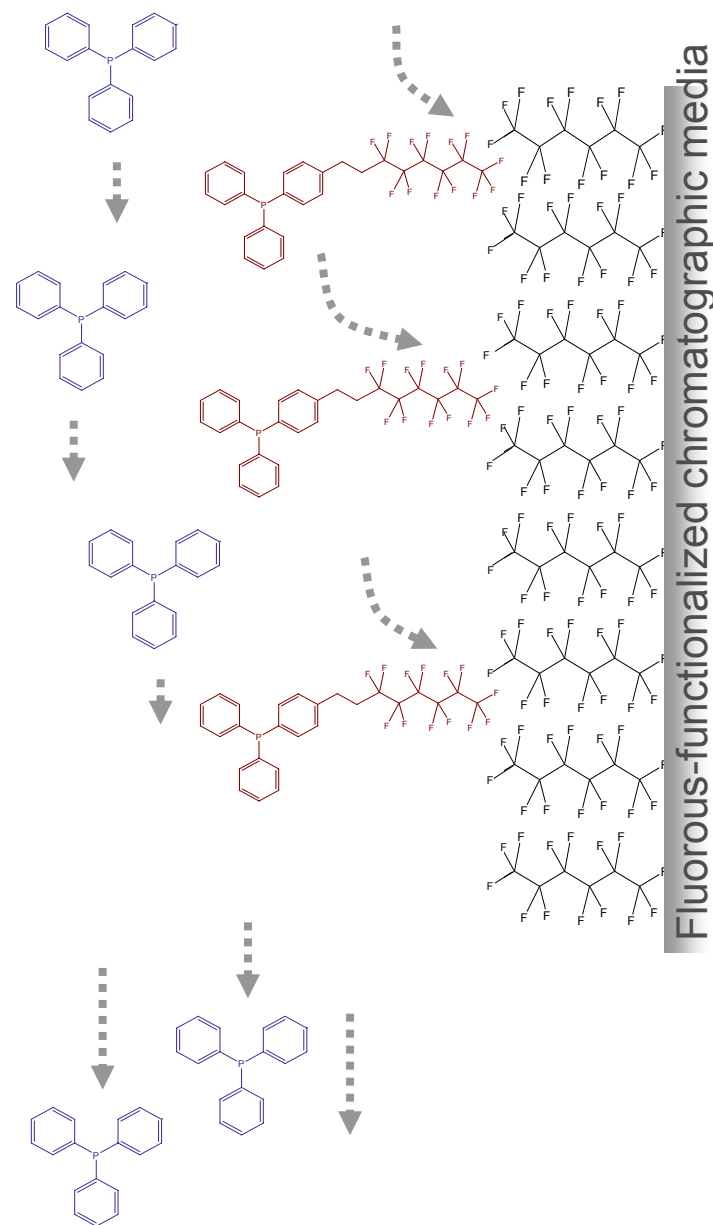


- Oligosaccharides (Miura, T.; Inazu, T. *et al. J. Org. Chem.* **2004**, 69, 5348)

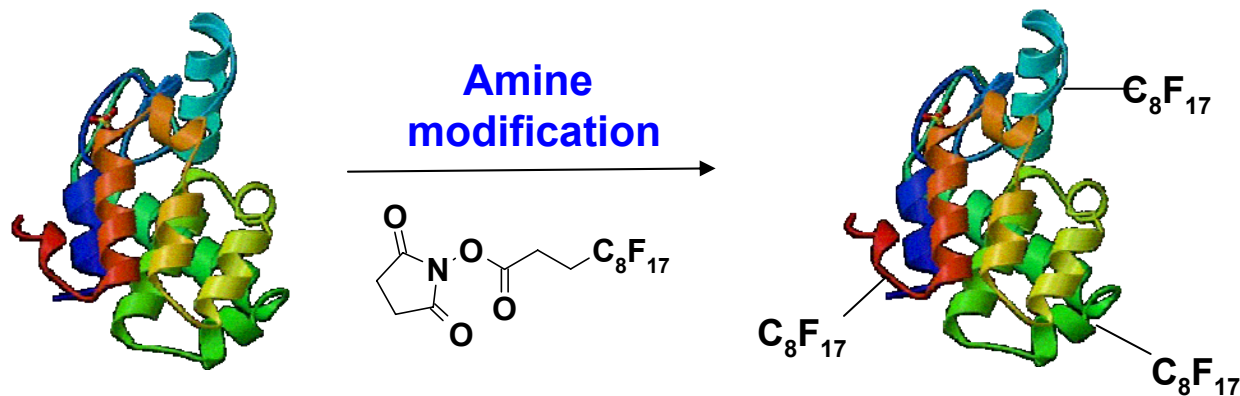


Fluorous Separation and Immobilization

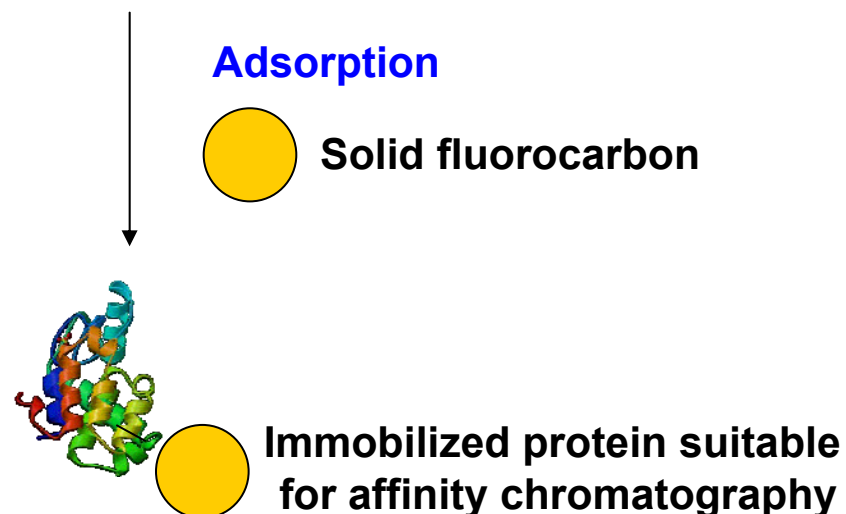
- Leverages fluorine's unique physical properties
- Novel mechanism bioorthogonal to other separation/ immobilization technologies
- Allows diverse chemistry and versatile separation options
- Strong IP coverage around the process & materials



Fluorous Protein Immobilization

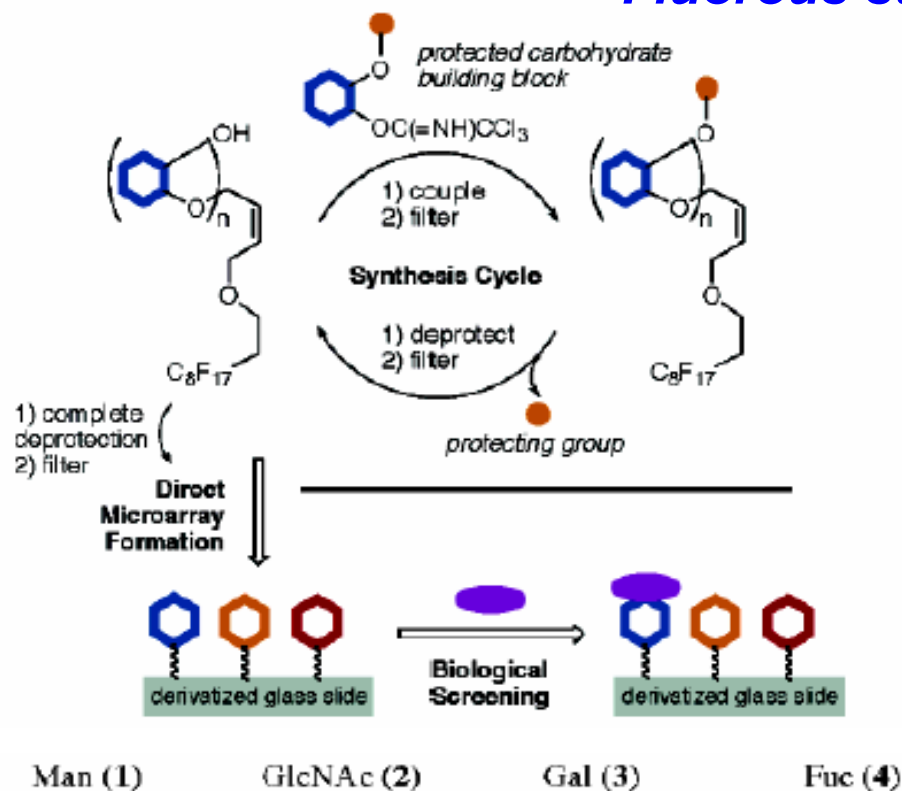


<u>Enzyme</u>	<u># of fluorous groups</u>	<u>Activity</u>
Thrombin	4-5	70-80%
Cathepsin C	6-12	75-95%
Polynucleotide phosphorylase	13-17	76-82%
α -Chymotrypsin	3-5	71-93%
Glucose oxidase	6-11	66-82%



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

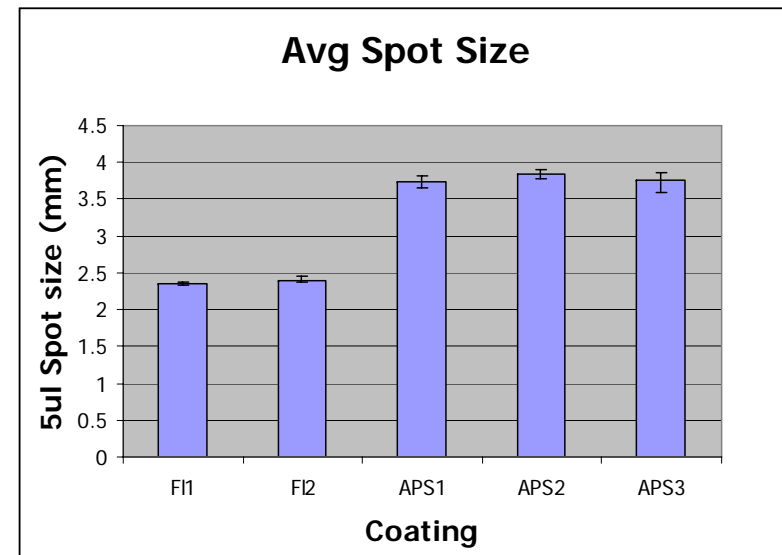
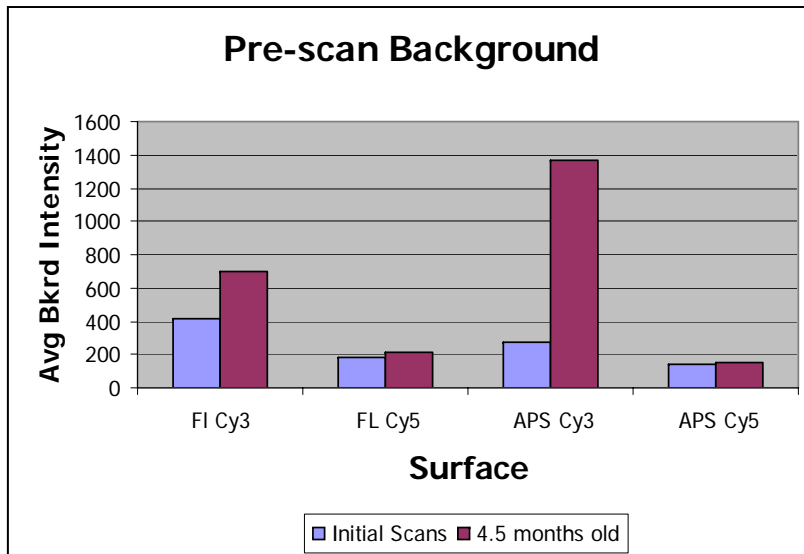
Joint development of FTI and Thermo Fisher Scientific

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



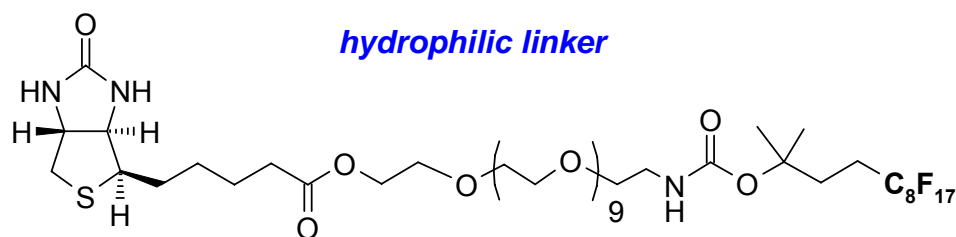
100° average contact angle (goniometer)



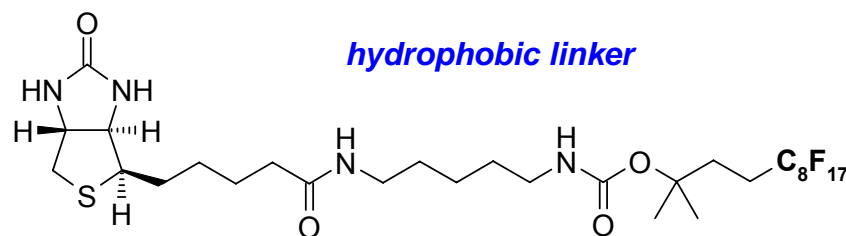
Work of Schreiber Group, Broad Institute

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)



Fluorous Biotin-PEG



Fluorous Biotin-Cadaverine

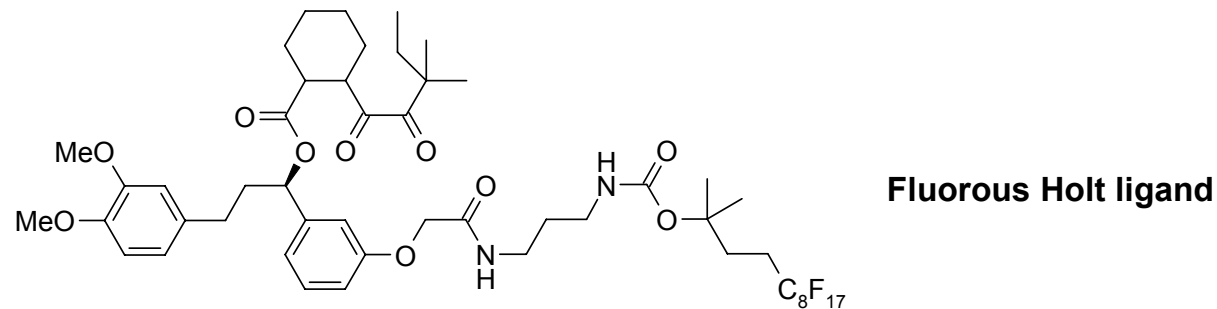
Results:

- 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

Vegas, A.J.; Bradner, J.E.; Tang, W.; McPherson, O.M.; Greenberg, E.F.; Koehler, A.N.; Schreiber, S.L. Manuscript in preparation.

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*



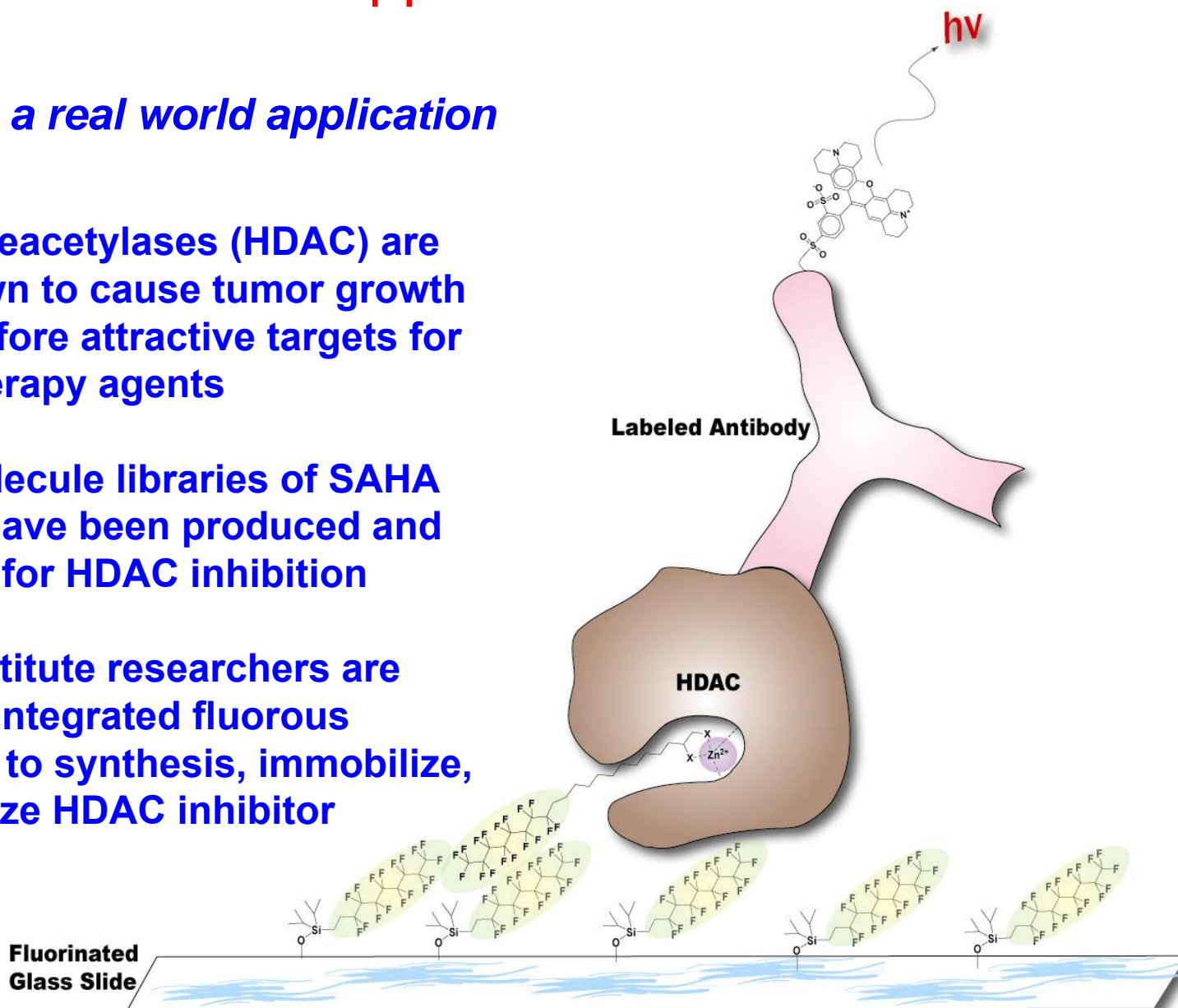
Results:

- 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*

Vegas, A.J.; Bradner, J.E.; Tang, W.; McPherson, O.M.; Greenberg, E.F.;
 Koehler, A.N.; Schreiber, S.L. Manuscript in preparation.

Examining a real world application

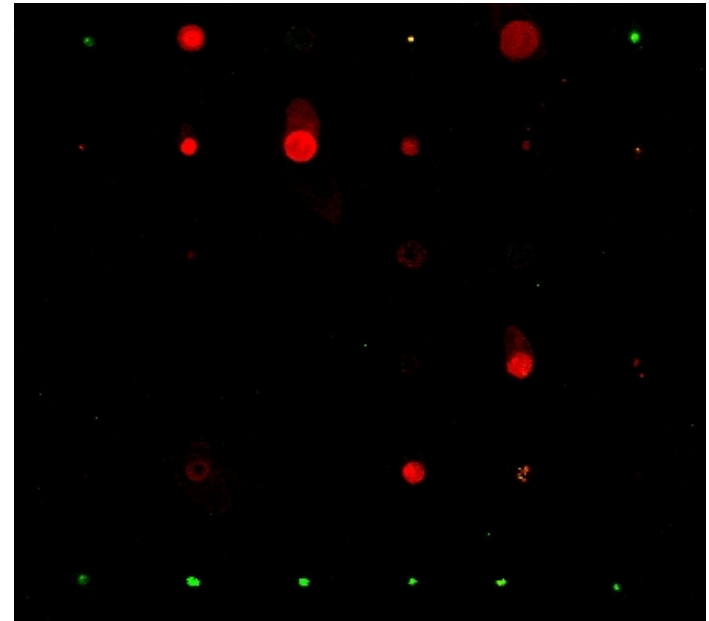
- Histone deacetylases (HDAC) are well-known to cause tumor growth and therefore attractive targets for chemotherapy agents
- Small molecule libraries of SAHA analogs have been produced and screened for HDAC inhibition
- Broad Institute researchers are using an integrated fluorous approach to synthesis, immobilize, and analyze HDAC inhibitor libraries.



Vegas, A.J.; Bradner, J.E.; Tang, W.; McPherson, O.M.; Greenberg, E.F.; Koehler, A.N.; Schreiber, S.L. Unpublished results.

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**

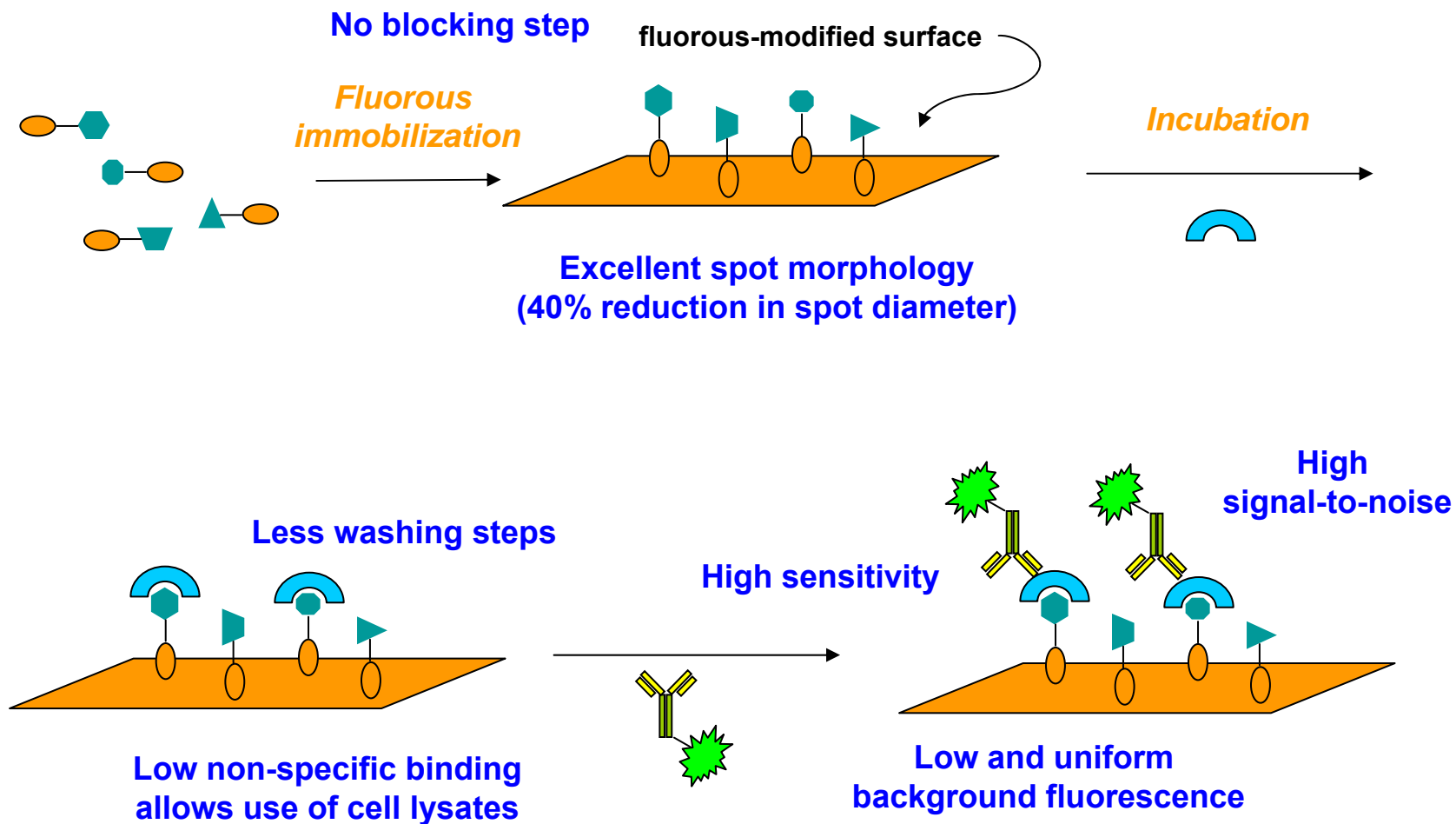


Vegas, A.J.; Bradner, J.E.; Tang, W.; McPherson, O.M.; Greenberg, E.F.; Koehler, A.N.; Schreiber, S.L. Unpublished results.

Many additional classes of compounds now under investigation

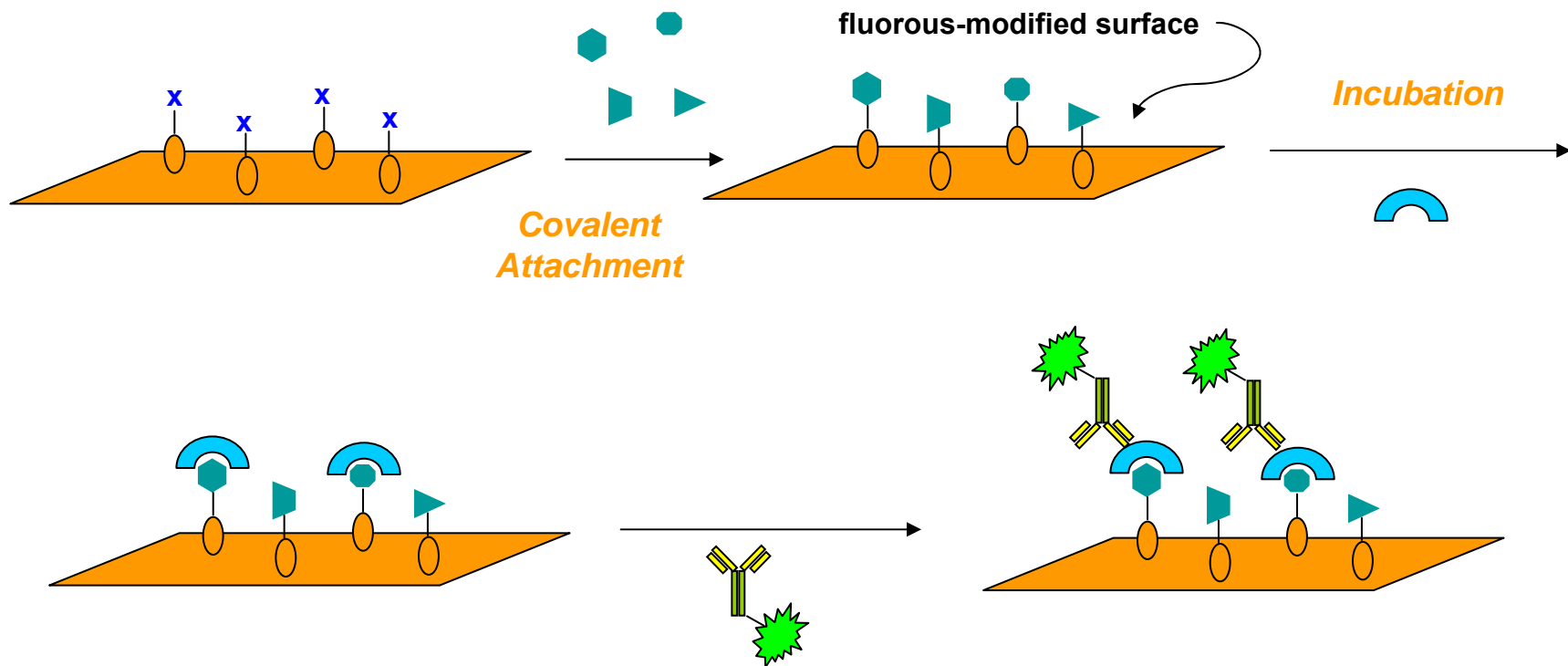
- Oligonucleotides
- Protein arrays
- Peptides
- Antibodies

Fluorous Microarray Benefits



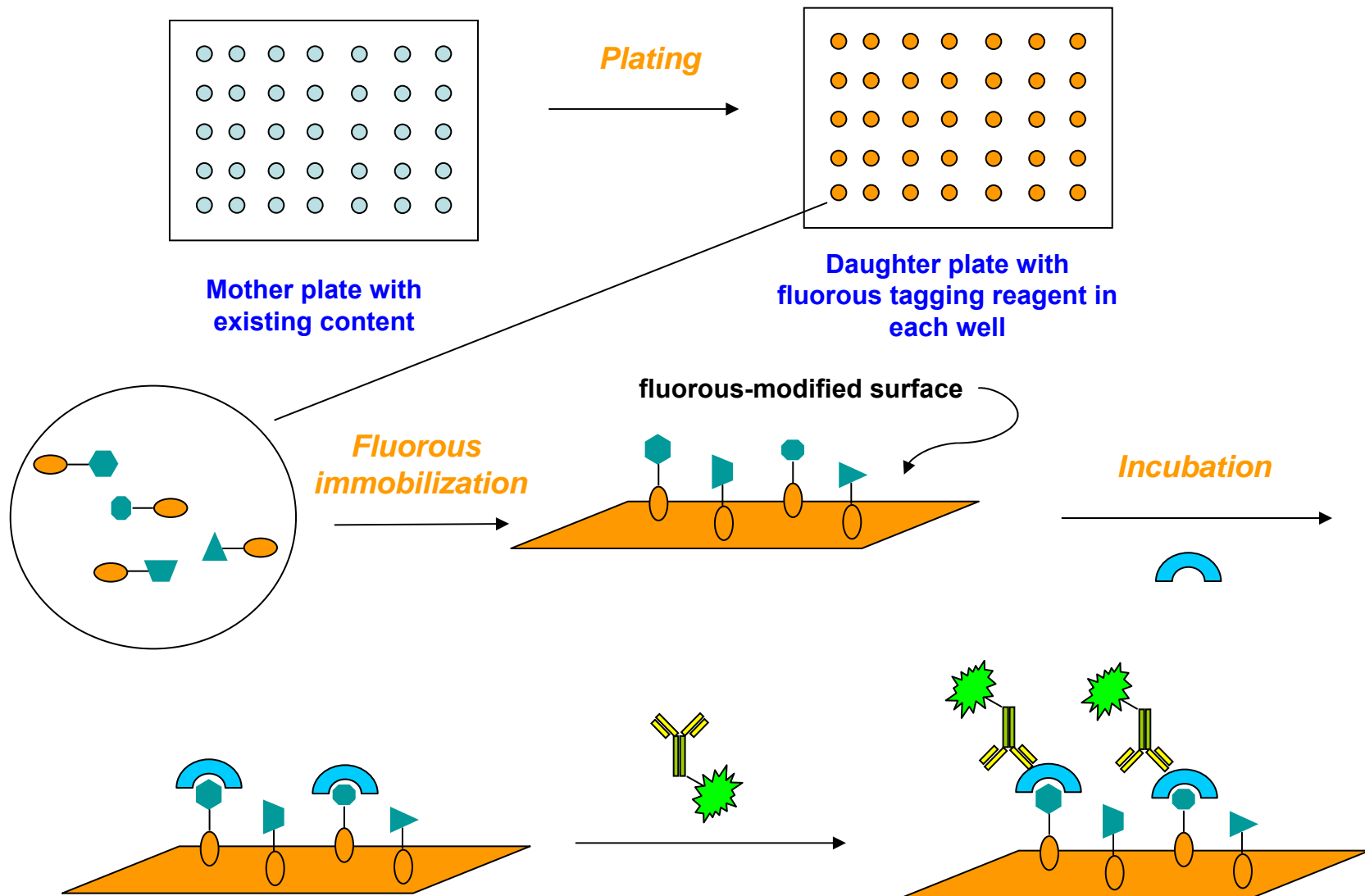
Fluorous Microarrays of Non F-Tagged Content

Taking Advantage of Fluorous Benefits Using Existing Content



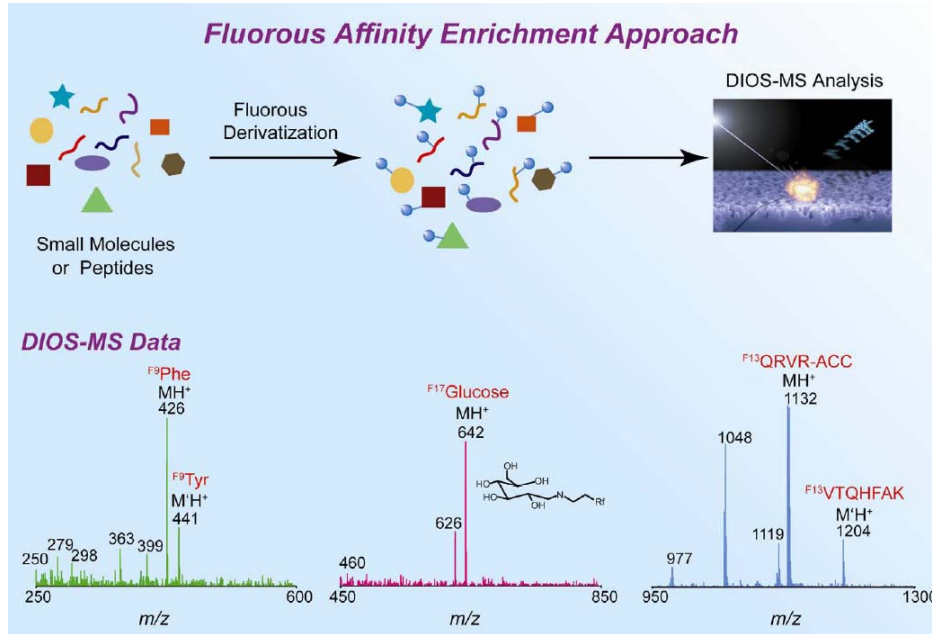
- Would allow microarray producers to use existing content to produce fluorous microarrays
- Requires spot-on-spot spotting
 - Acoustic drop ejection vs. pin spotting

Fluorous Microarrays- A Simpler Alternative



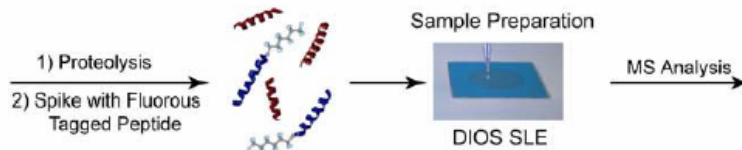
- Would allow microarray producers to use existing content to produce fluorinated microarrays and leverage workflow benefits

Other Surfaces: Fluorous Affinity Based MS

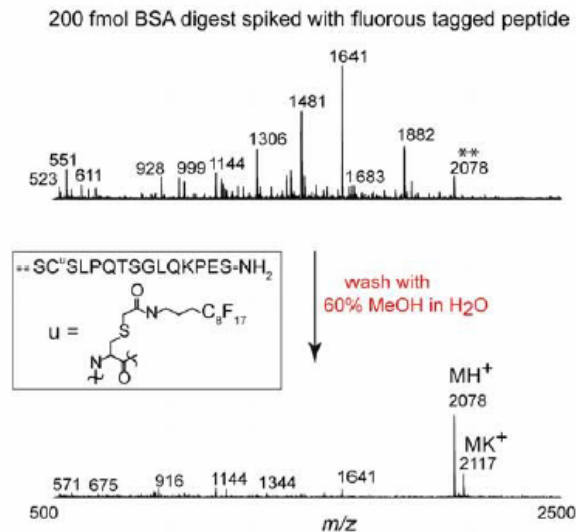


- Direct deposition, enrichment, and MS analysis on a fluorous modified porous Si chip.
- Enrichment and detection of fluorous tagged amino acids, peptides and carbohydrates demonstrated.
- Potential applications in proteomics, metabolomics and diagnostics

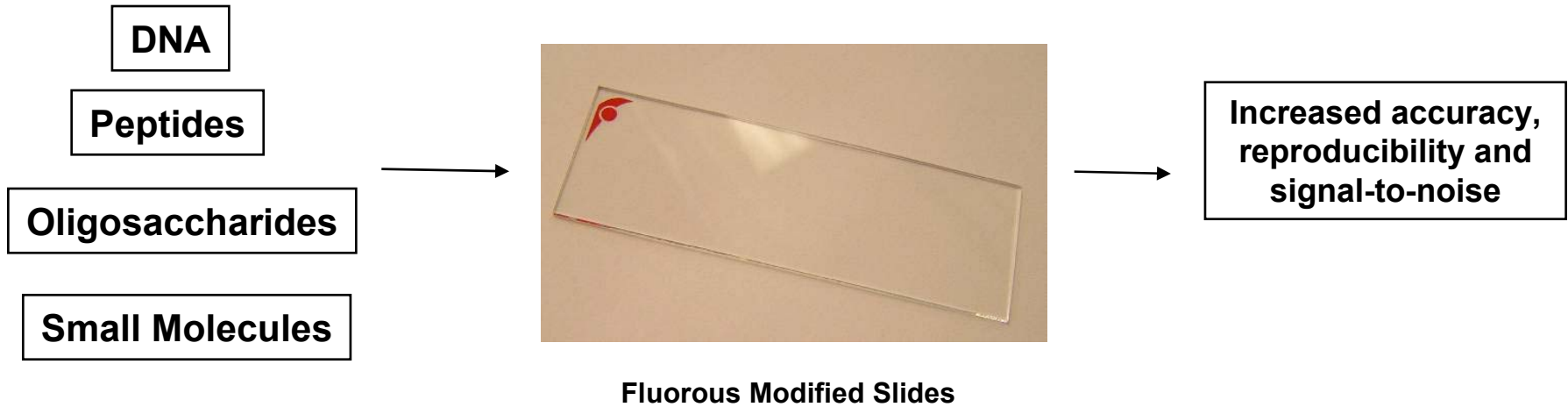
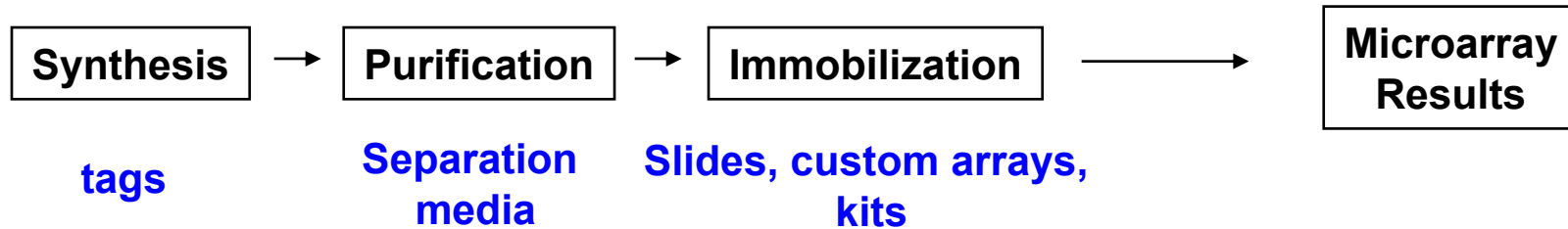
Fluorous Proteomics with DIOS-MS



A solution containing a mixture of a peptide tagged with C_8F_{17} group and a BSA tryptic digest was deposited on a DIOS chip bearing fluoroalkyl chains. DIOS-MS spectrum of the mixture shows peptide peaks from BSA digest and the fluorous tagged peptide. The spot containing the peptides was washed with 60% methanol. DIOS-MS analysis of the spot shows the selective adsorption of the fluorous tagged peptide.



The FTI Total Advantage Product Offering



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

- 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)



**Actively
Seeking
Partnerships
and
Collaborations!**

- 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

Now available from FTI!

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

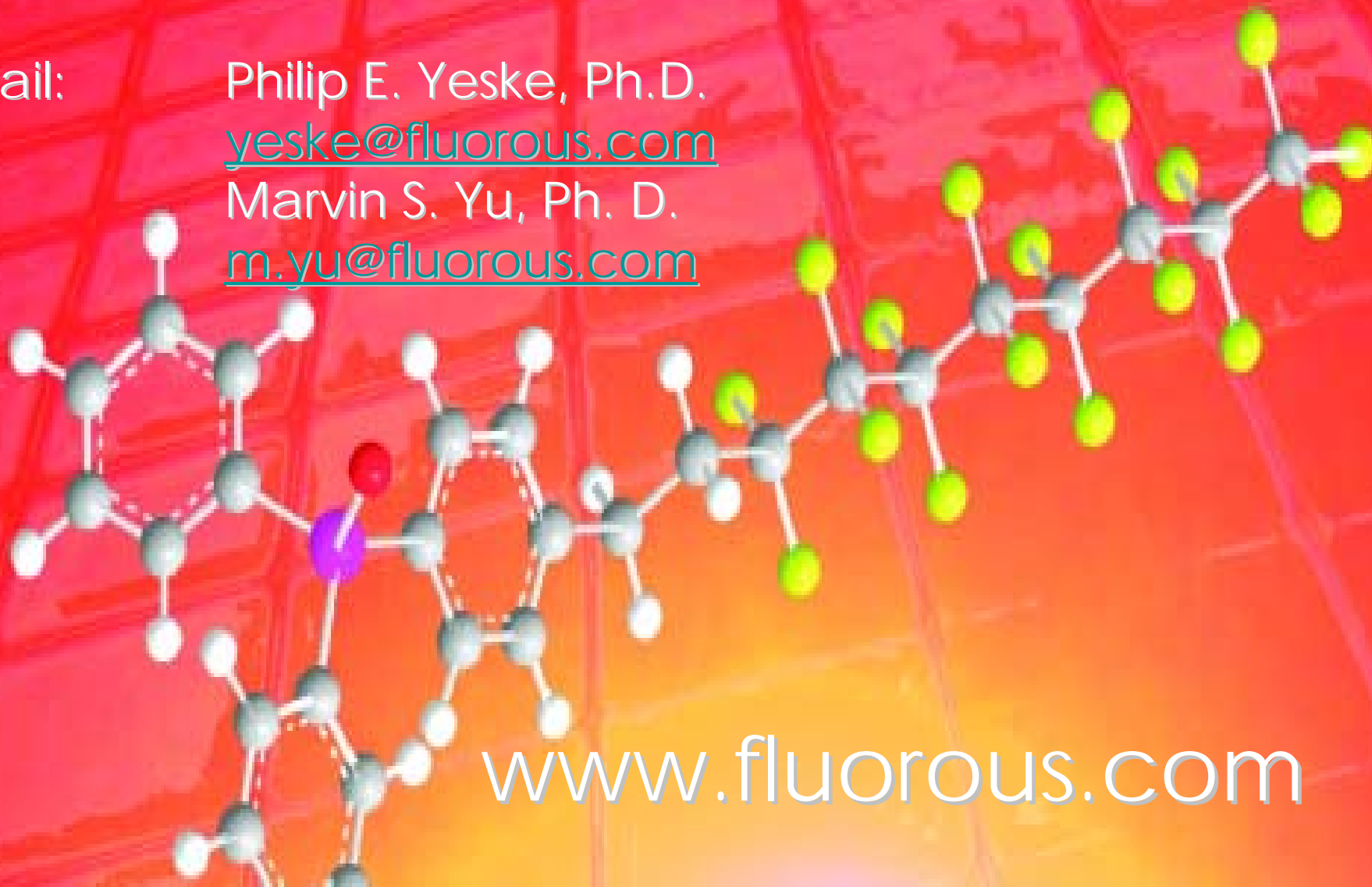
Key Features

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

Phone: 412-826-3050
877-FLUOROFLASH

Fax: 412-826-3053

Email: Philip E. Yeske, Ph.D.
yeske@fluorous.com
Marvin S. Yu, Ph. D.
m.yu@fluorous.com



www.fluorous.com