Fluorous Chemistry in Biomolecule Synthesis, Purification, and Immobilization

FLUOROUS
Technologies Incorporated

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

Presentation Outline

I. Introduction to Fluorous Chemistry

II. Fluorous Separation Techniques

III. Fluorous Peptide Synthesis
   A. N-terminal tagging
   B. Fluorous capping
   C. Fluorous supported synthesis

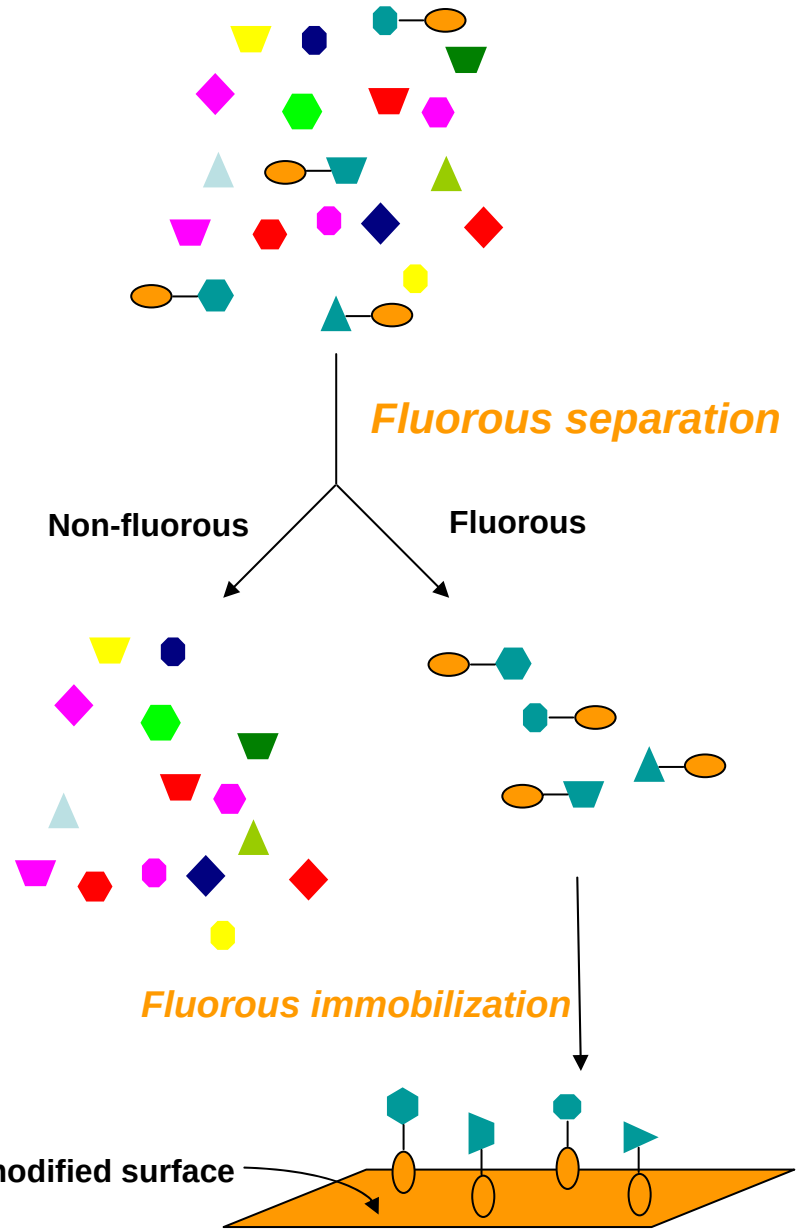
IV. Fluorous Liquid-Liquid Extraction

V. Unpublished Results

VI. Conclusions

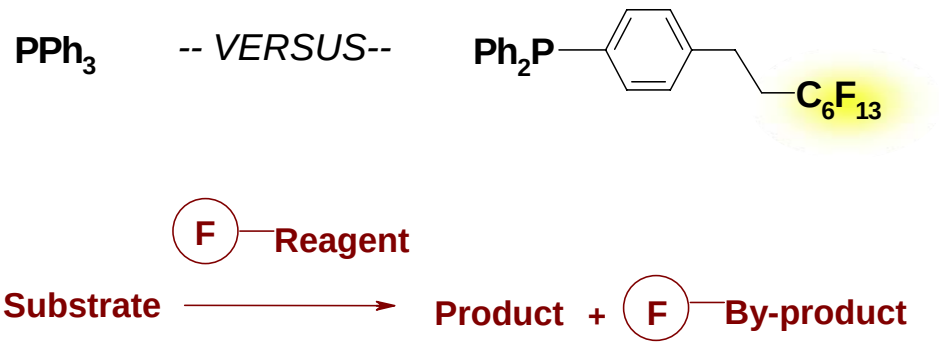
What is Fluorous Technology?

- 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



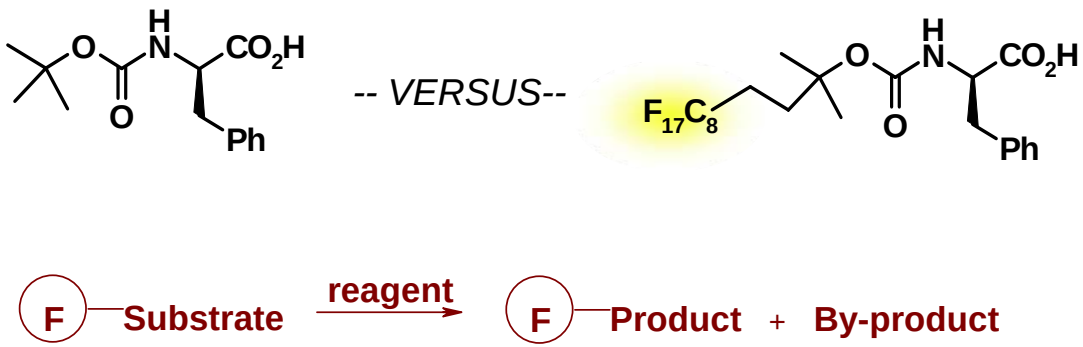
Examples of Fluorous Molecules

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



Peptide context: f-HOBt, f-DCC, f-scavengers

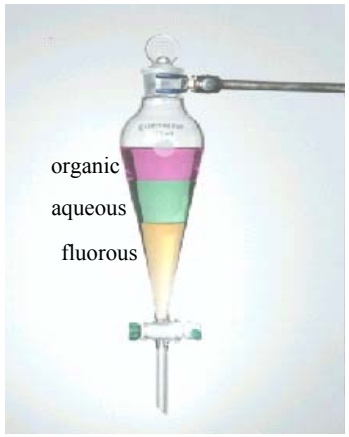
Compounds with temporary fluorous tags (e.g. substrates):



Peptide context: N-terminus protecting groups (tags); side-chain tags; pre-tagged amino acids; fluorous C-terminus supports

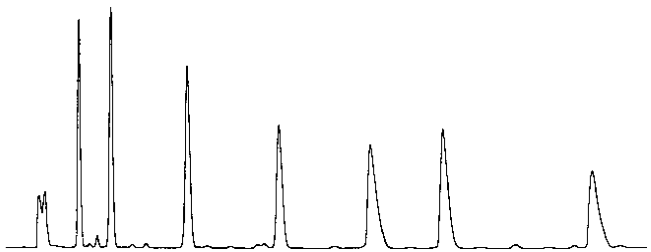
Fluorous Separation Methods

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



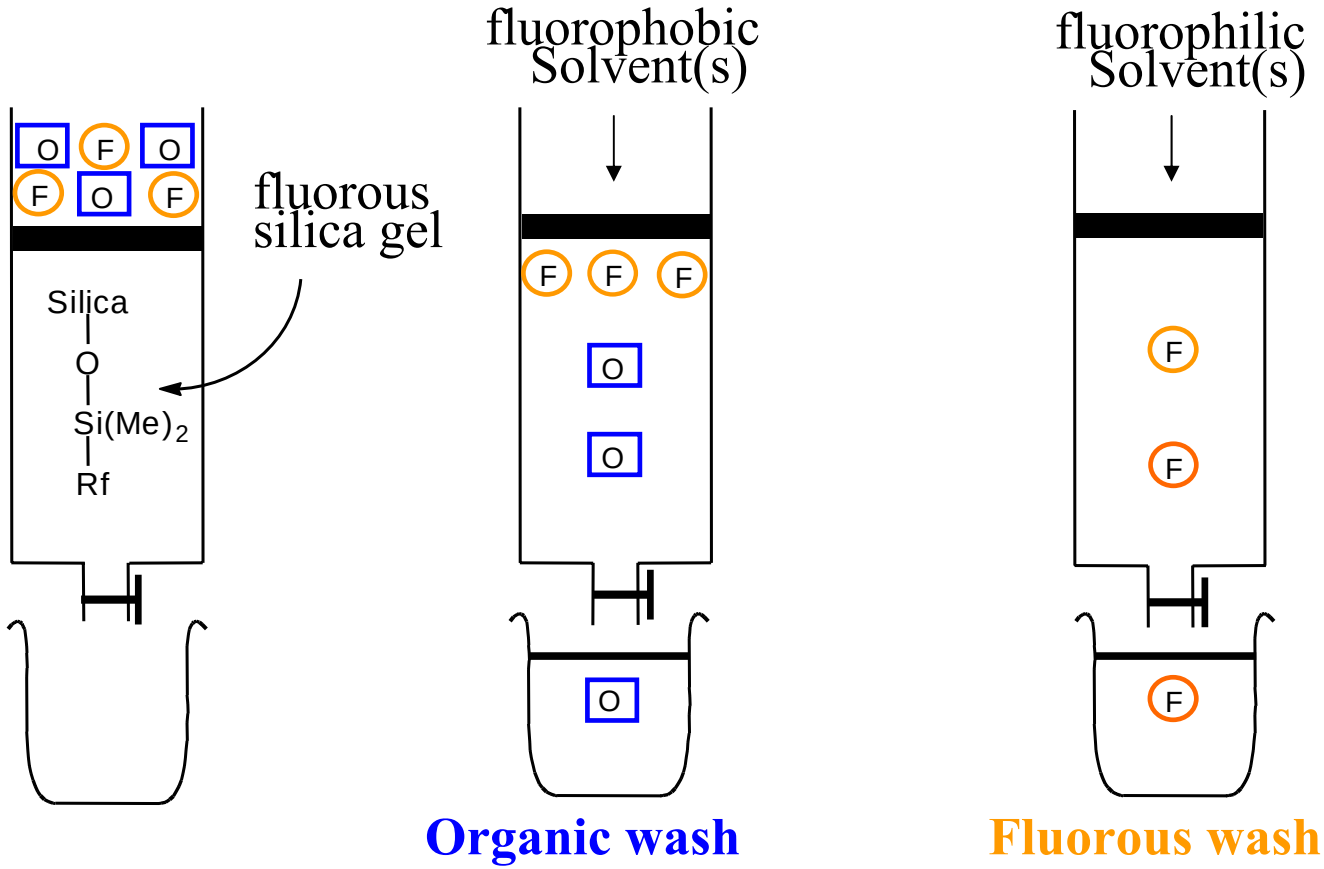
- **Fluorous Solid Phase Extraction (F-SPE)**
  - "Light" fluorous technique
  - Separates fluorous from non-fluorous
  - No fluorous solvents used

- **Fluorous Chromatography (F-HPLC)**
  - Separates fluorous from fluorous
  - More fluorous = Greater retention



Fluorous Solid Phase Extraction

A Light Fluorous Technique

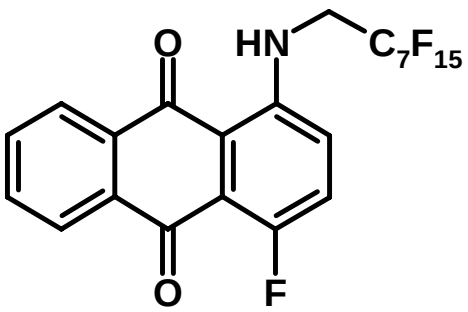


Fluorophobic **Typical Solvents for F-SPE** ***Fluorophilic***

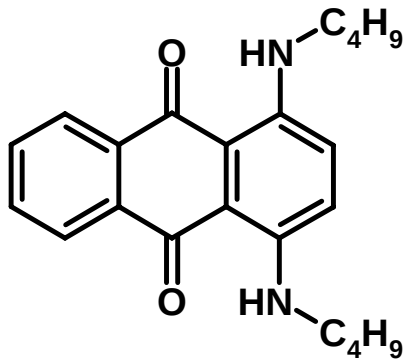
Water DMF DMSO MeOH ACN Acetone Toluene THF

Curran, D. P. *Synlett*. **2001**, *9*, 1488.

FSPE Dye Demonstration



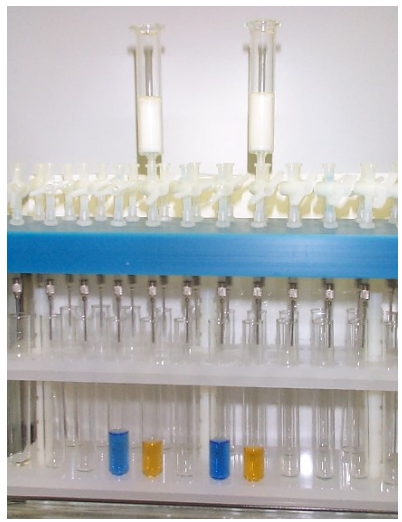
Fluorous Dye
(orange)



Non-fluorous Dye
(blue)

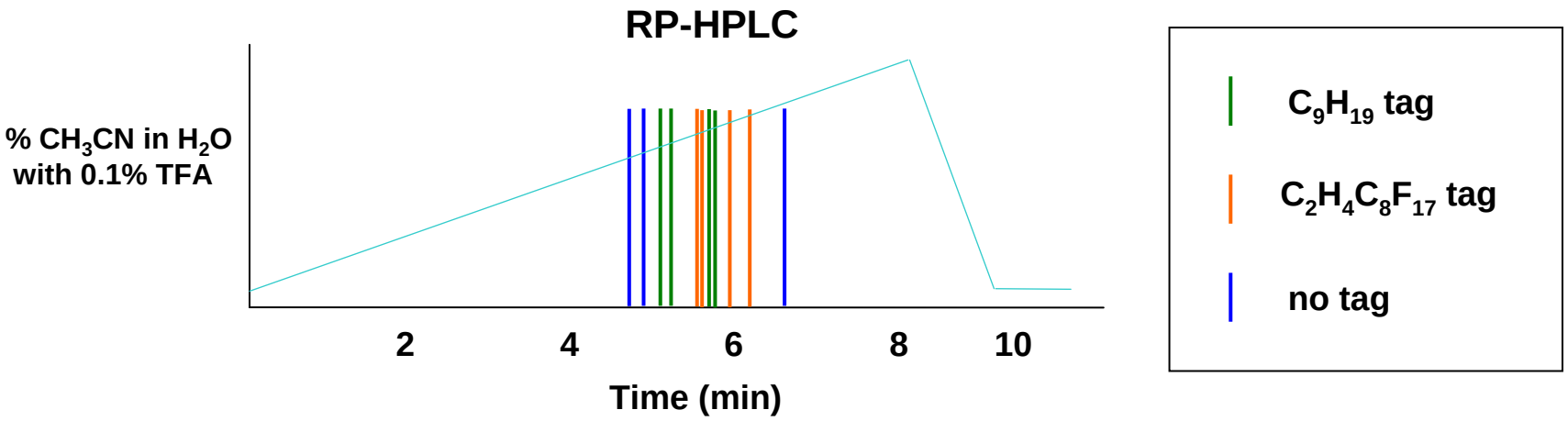
1. Load sample

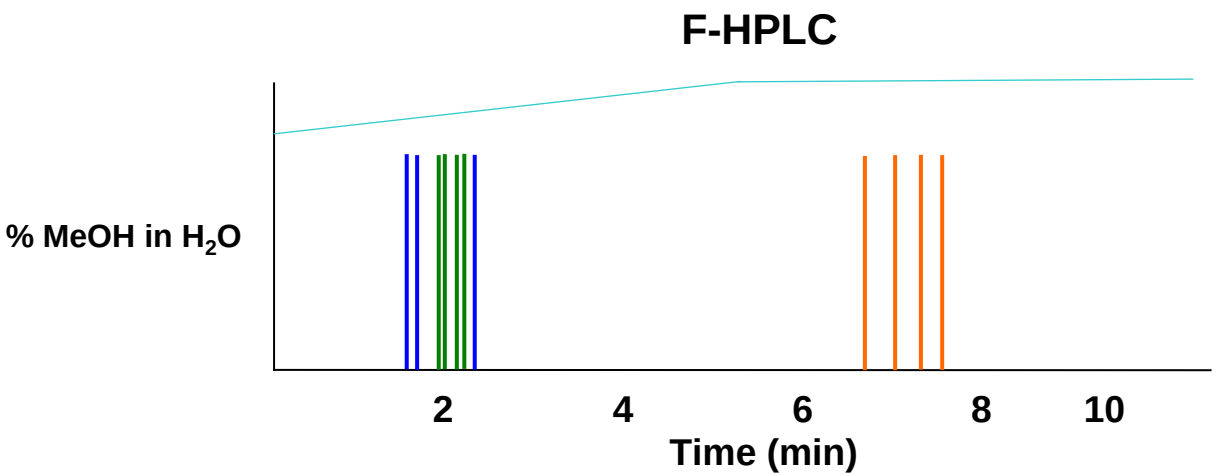
2. Wash non-fluorous dye with MeOH-H_2O (85:15)



3. Wash fluorous dye with MeOH

Fluorous Tags vs. Hydrophobic Tags



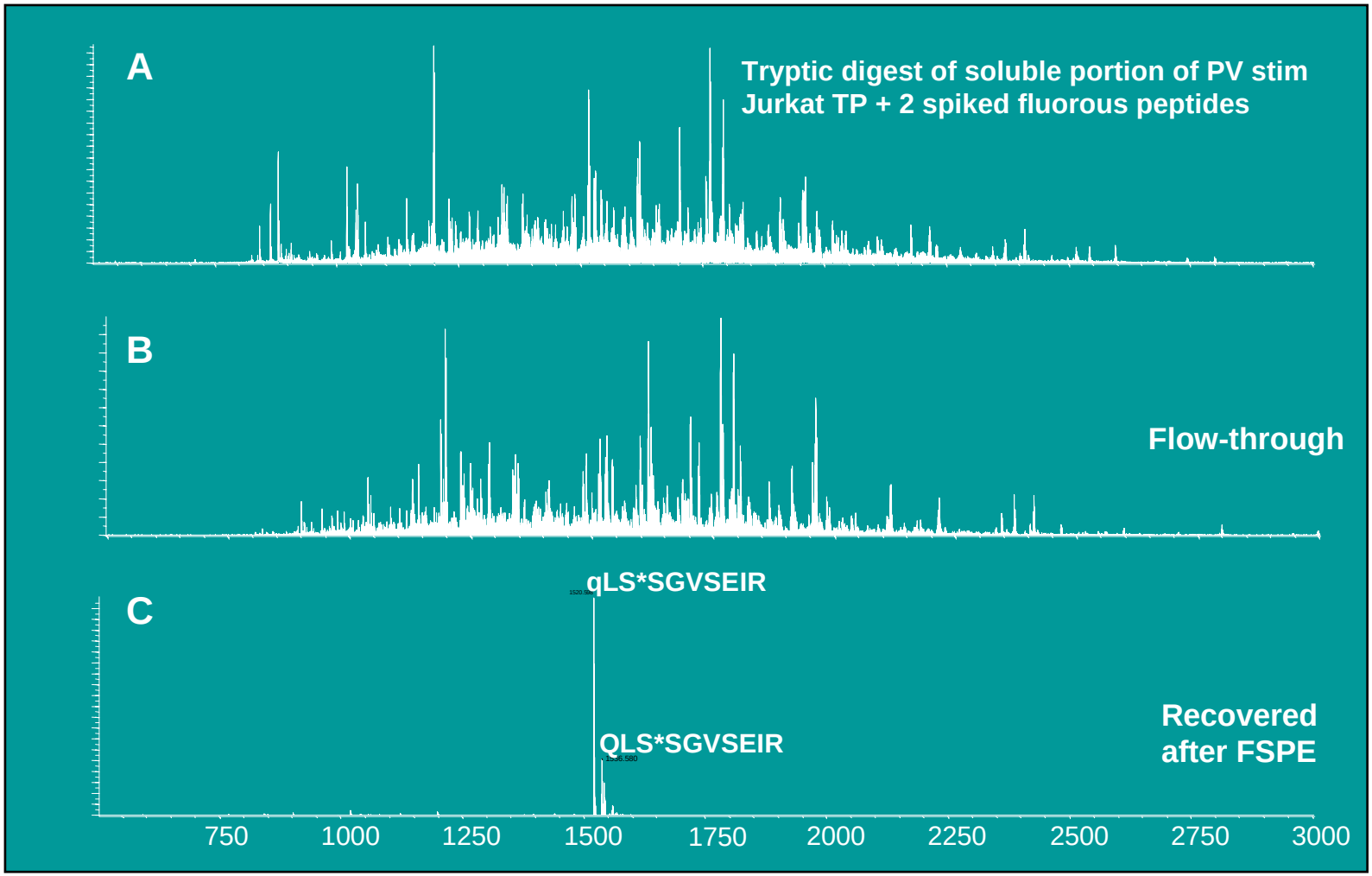


Tagged amino acids are Ser, Glu, Phe, and Trp.

The untagged controls were galactosyl pentaacetate, $(Boc\text{-}Cys\text{-}OH)_2$, and PPh_3.

Fluorous compounds are hydrophobic *and* lipophobic.

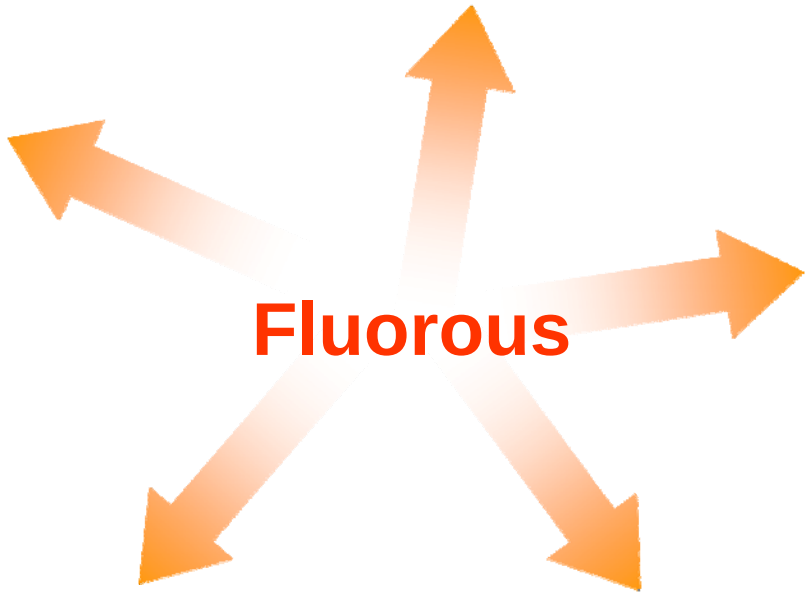
Fluorous SPE Selectivity



Highly selective fluorous purification of complex peptide mixture

Brittain, S.M.; Ficcaro, S.B.; Brock, A.; Peters, E.C. *Nature Biotech*. **2005**, *23*, 463.

Reaction compatibility



Lewis acidic

- Friedel-Crafts
- BBr_3

Metal catalysis

- Suzuki
- Heck
- Buchwald
- Stille
- Co, Rh

Redox

- LAH
- hydrogenation
- H_2O_2
- Swern

Ionic

- Enolate
- Grignard,
- lithiate
- cationic

Free radical

- cyclization
- dehalogenation
- deoxygenation

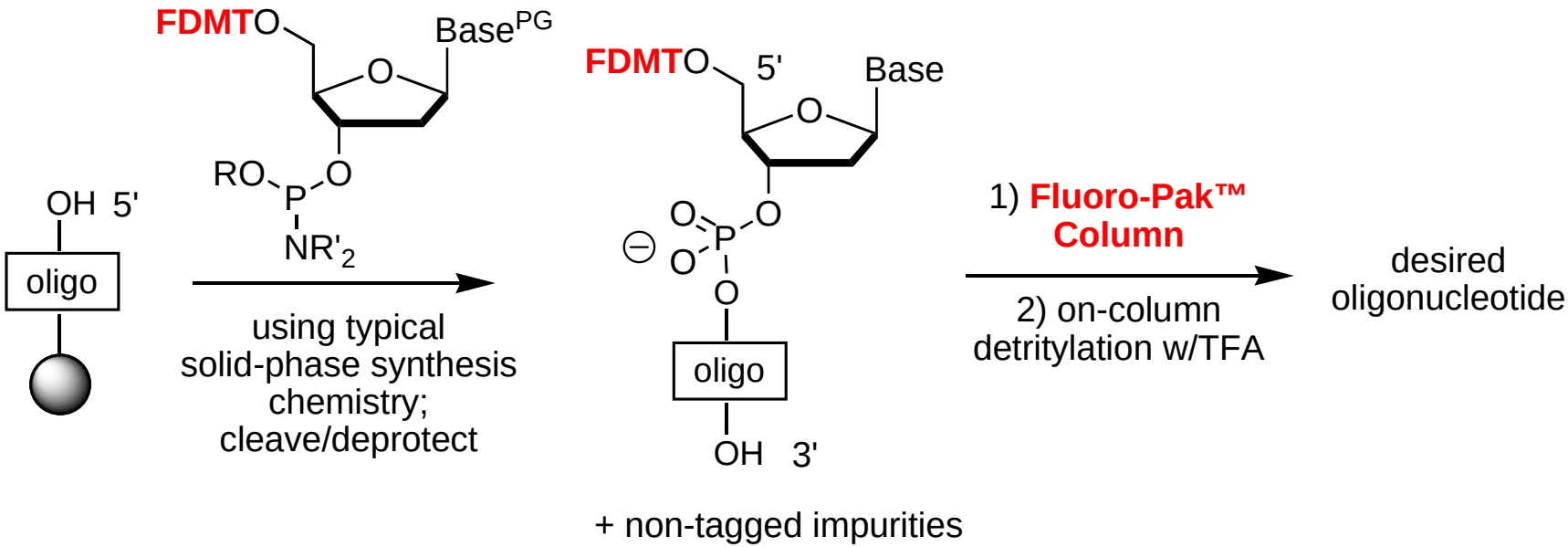
Fluorous oligomer synthesis strategies

- **Solid-supported synthesis with fluorous *tagging***
  - Conventional solid phase synthesis with terminal fluorous tagged monomer.
  - General method to purify oligonucleotides and peptides over a broad range of polarities.
  - FSPE or FHPLC used for simple pre-purification prior to final HPLC, increasing throughput.
- **Solid-supported synthesis with fluorous *capping***
  - Conventional solid phase synthesis with fluorous capping of deletion sequences
  - Purification by precipitation or FSPE
- **Solution phase synthesis with fluorous supports / tags**
  - Totally homogeneous chemistry
  - Suitable for shorter sequences
  - Potential strategies for condensations and ligations

In conventional organic synthesis, fluorous is often an attractive alternative to solid-phase. In biomolecule synthesis, it *complements* both solution- & solid-phase protocols

Fluorous Oligonucleotide Synthesis

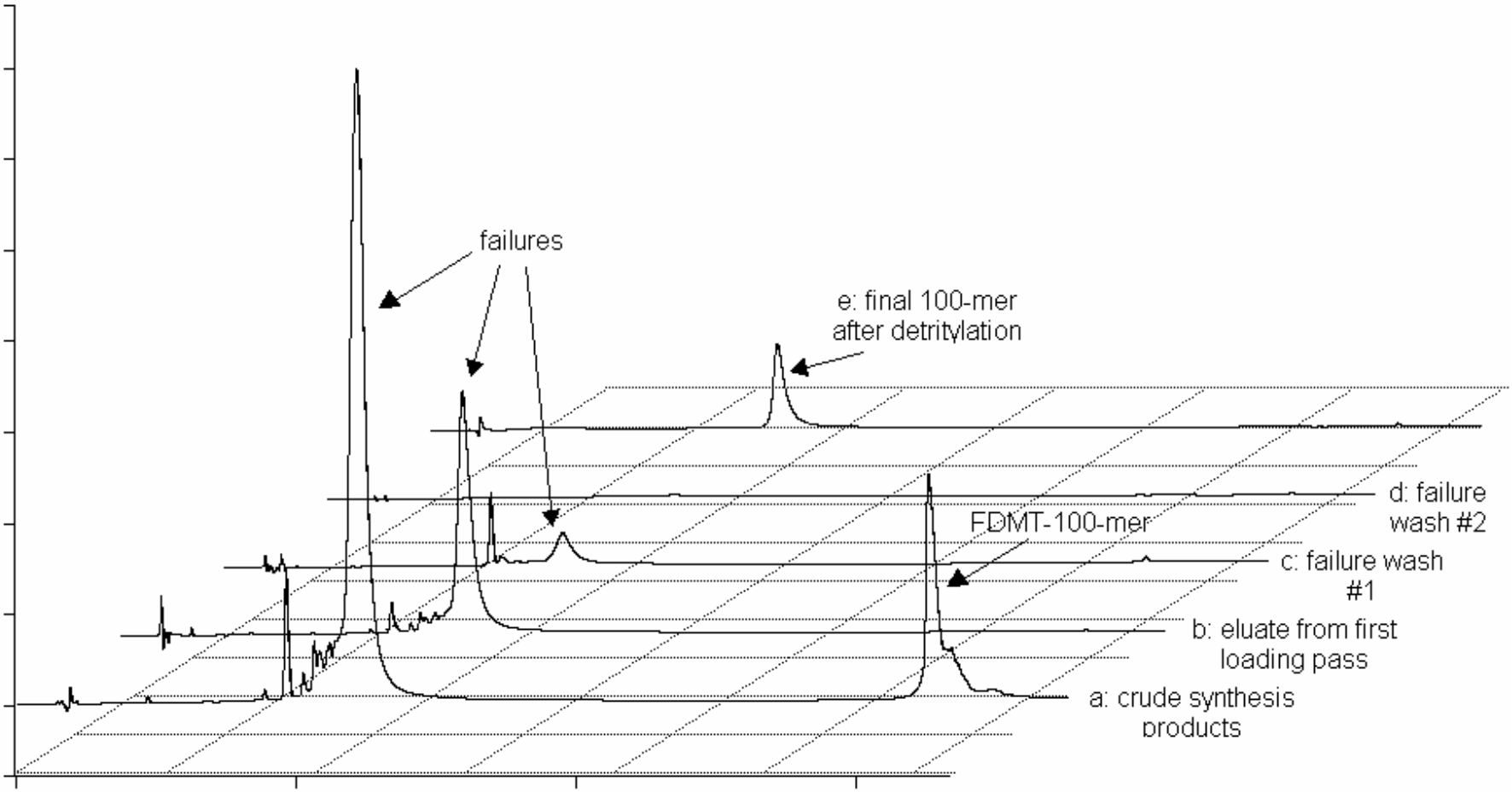
1) ***A fluorous phosphoramidite is used in the final coupling.***
2) ***Capture the fluorous-tagged oligonucleotide on a Fluoro-Pak™ column.***
3) ***Remove the fluorous tag by on-column detritylation.***
4) ***Elute the desired oligonucleotide.***



Sequences as long as 100-mers have been synthesized and isolated in excellent ODU and recoveries.

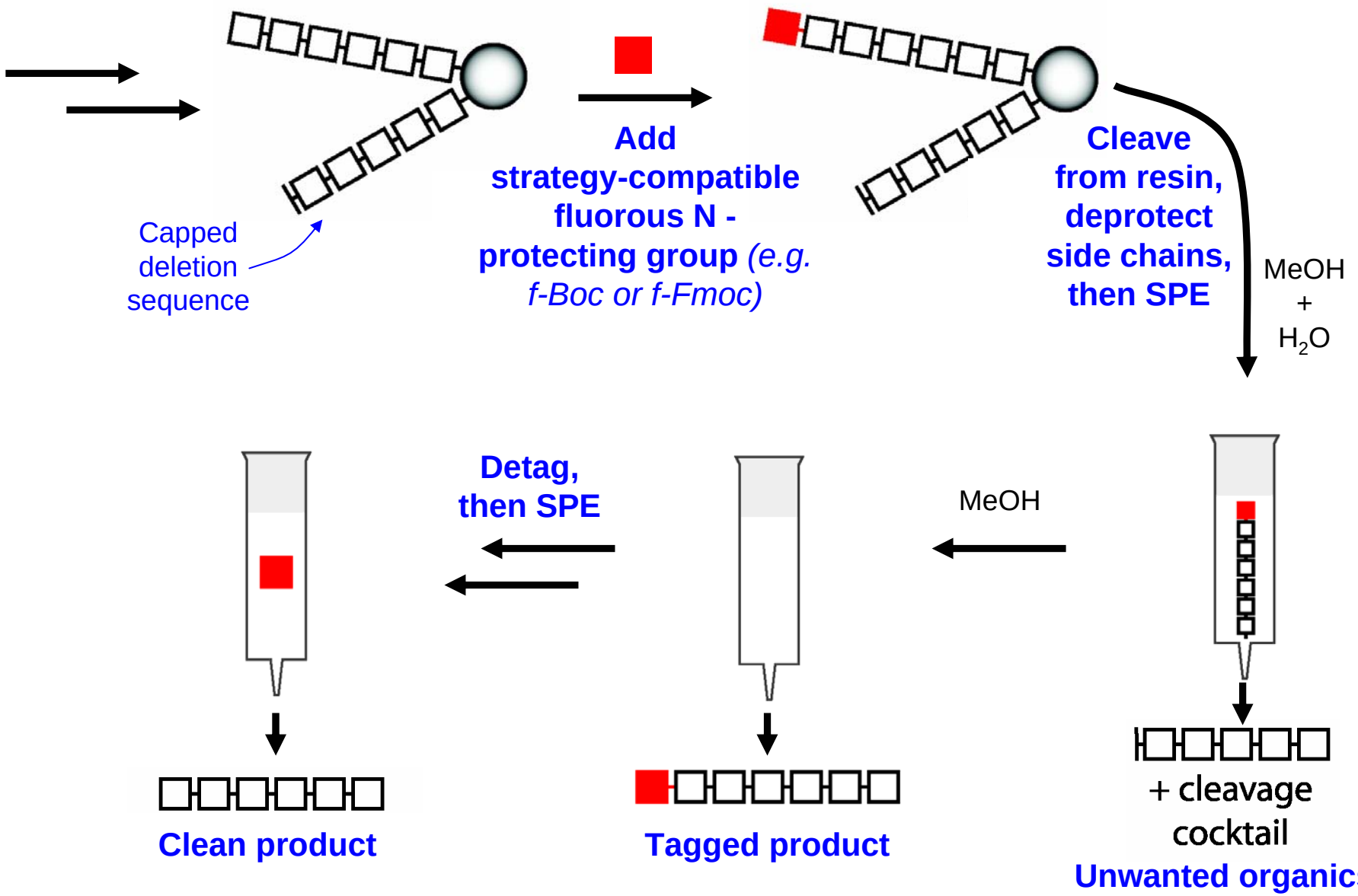
Slide Courtesy of Berry and Associates

RP-HPLC Progression of 100-mer



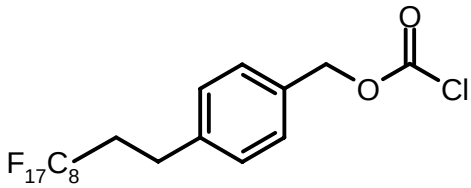
Pearson, W.H. *et al*, *J. Org. Chem*. **2005**, *70*, 7114.

SPPS with fluorous N-terminus tagging

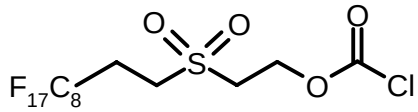


Overkleeft, van Boom, *et al*. *Tetrahedron Letters* **2003**, *44*, 9013-9016

N-terminal Fluorous SPPS



f-Z-Cl



f-MscCl

Peptide	N-tag	Purification method	*Yield(%)*	*Purity(%)*
GVWPLFLLLLALPPKAYAG	f-Z	column	35	
GCCSLPPCALNNPDYC	F-Msc	FHPLC	37	98
	F-Msc	FSPE	59	91
RQIKIWFQNRRMKWKK	F-Msc	FHPLC	10	94
	F-Msc	FSPE	7	72
SELDDRADALQAGFSPFES SAAKLKRKYWWKNLK	F-Msc	FHPLC	21	99

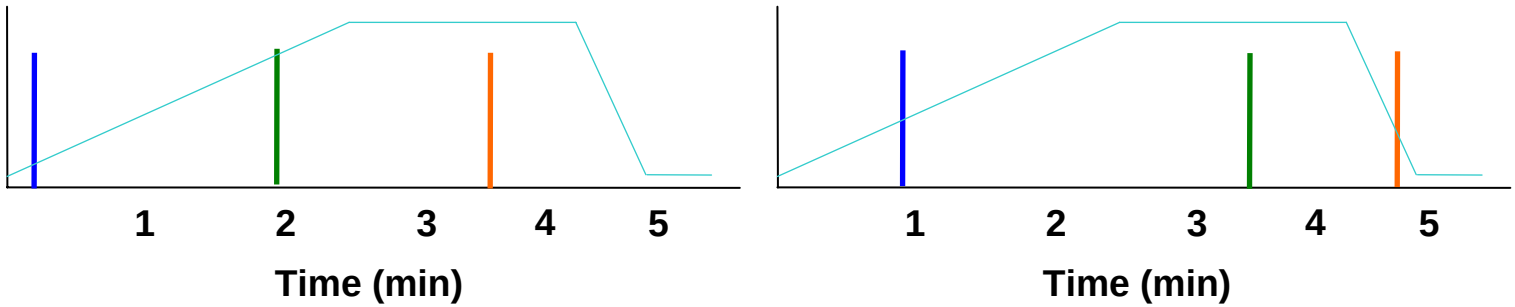
Overkleeft, van Boom, *et al. Tetrahedron Letters* **2003**, *44*, 9013-9016

f-Msc is base labile and compatible with conventional Fmoc strategies. F-Boc and f-Z are acid labile and compatible with conventional Boc strategies.

Selectivity of Fluorous Peptide Separations

H_2N-AFAAFA-$CONH_2$ | F_{13}-Z-HN-AFAAFA-$CONH_2$ | uracil

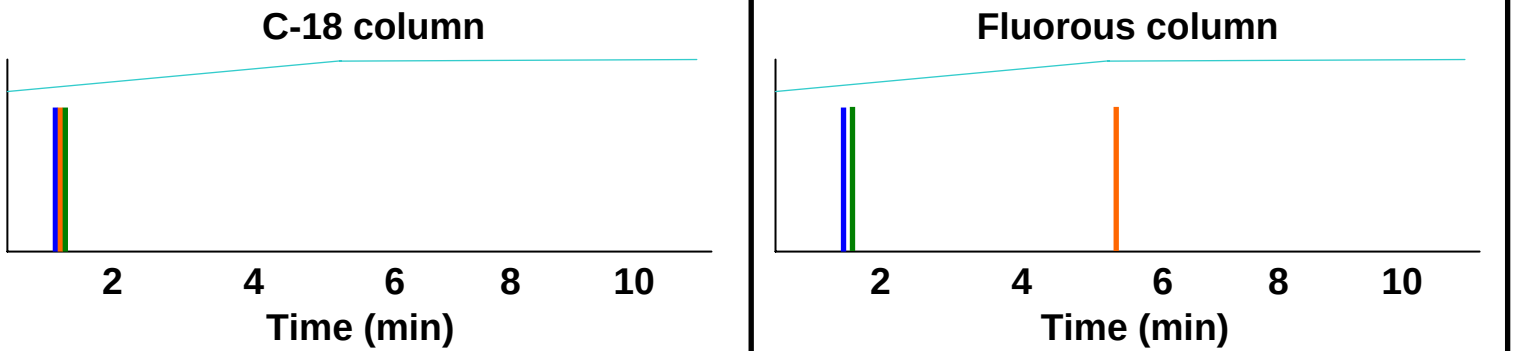
% CH_3CN in H_2O with 0.1% TFA



$\alpha_{F/non\text{-}capped} = 1.6$

$\alpha_{F/non\text{-}capped} = 1.7$

% MeOH in H_2O



$\alpha_{F/non\text{-}capped} = 1$

$\alpha_{F/non\text{-}capped} = 30$

Fluorous HPLC of hydrophobic peptide is >10x better than RP-HPLC

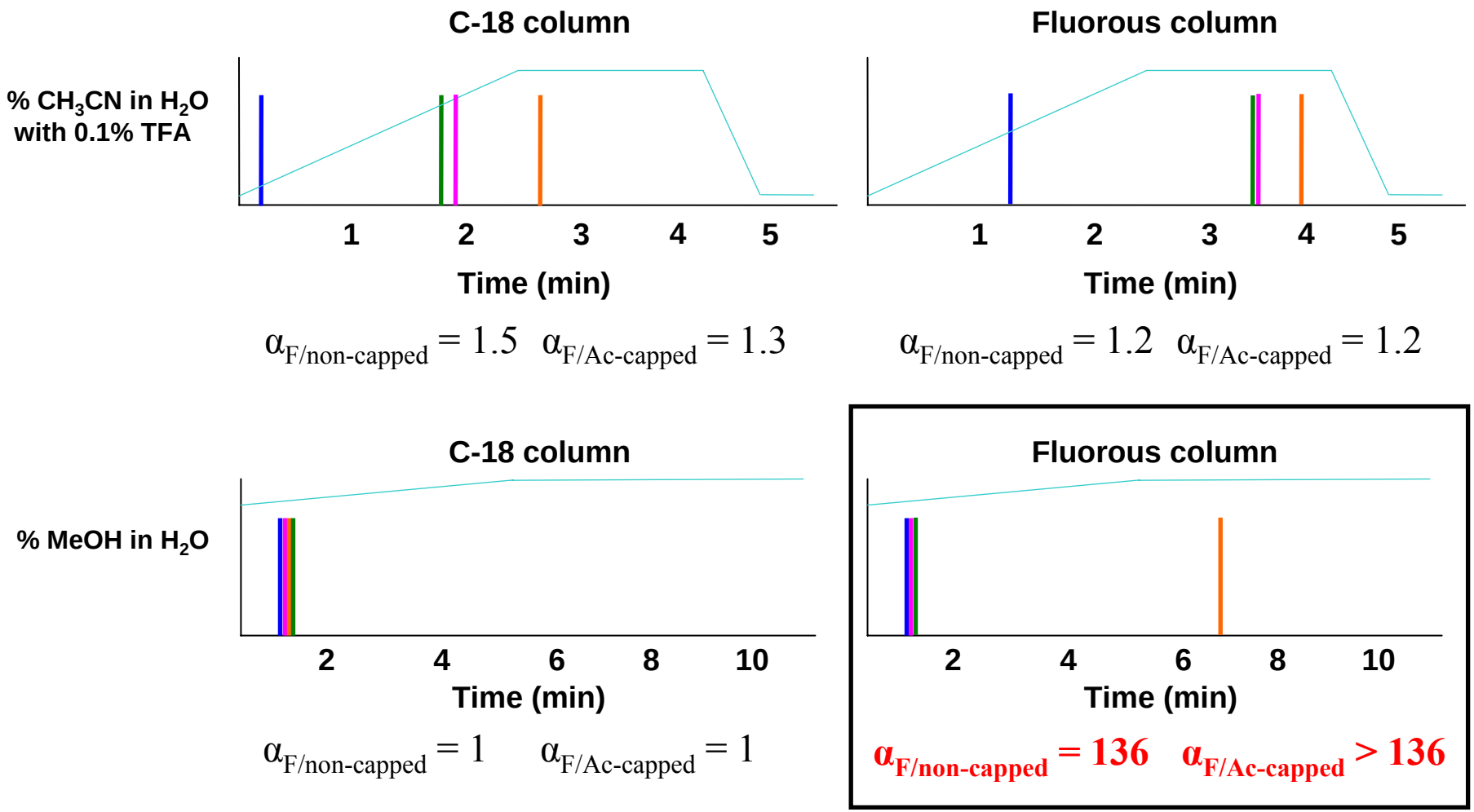
Selectivity of Fluorous Peptide Separations

H_2N-KLVFFAE-$CONH_2$

Ac-HN-KLVFFAE-$CONH_2$

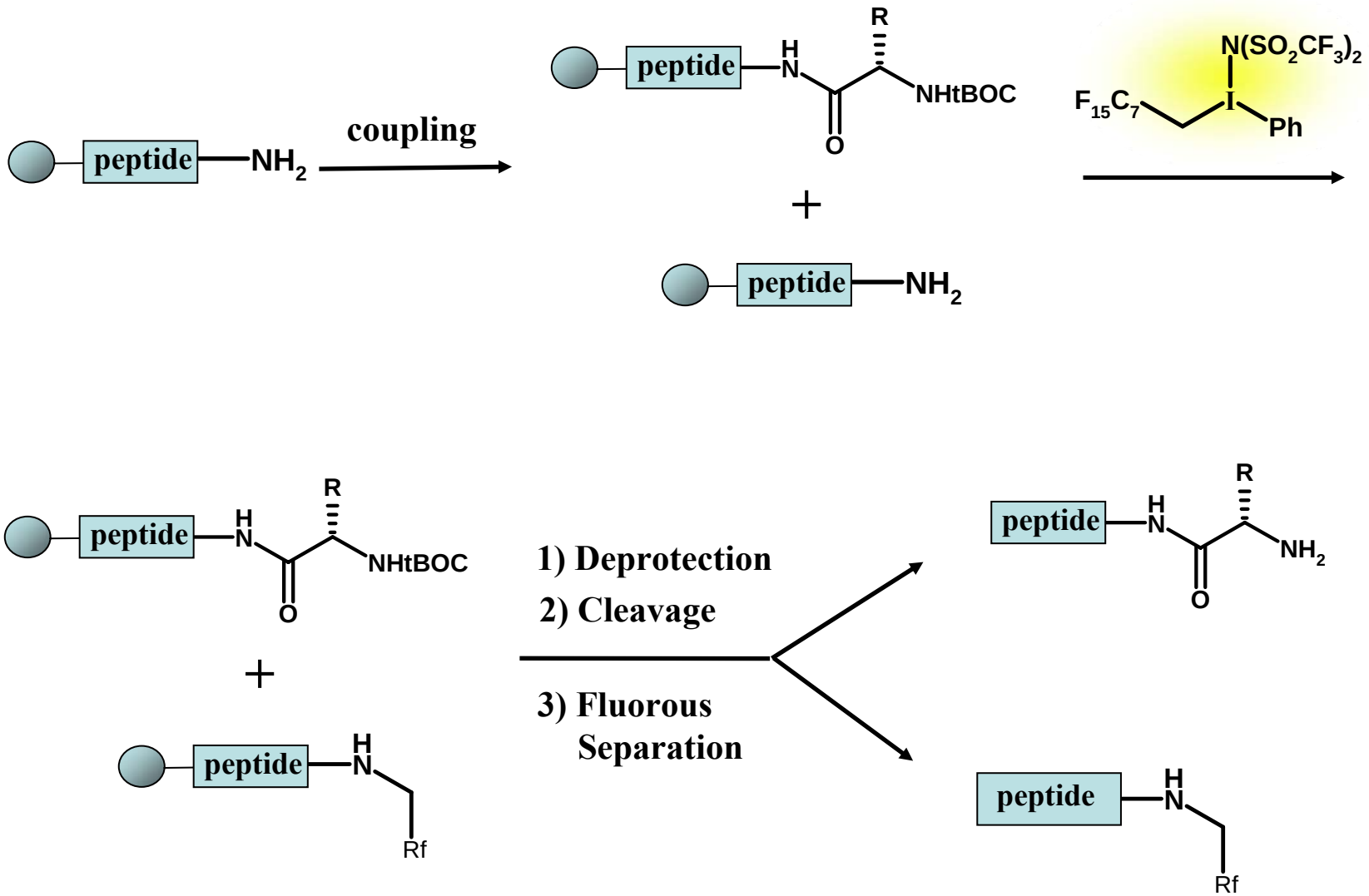
uracil

F_{17}Msc-HN-KLVFFAE-$CONH_2$



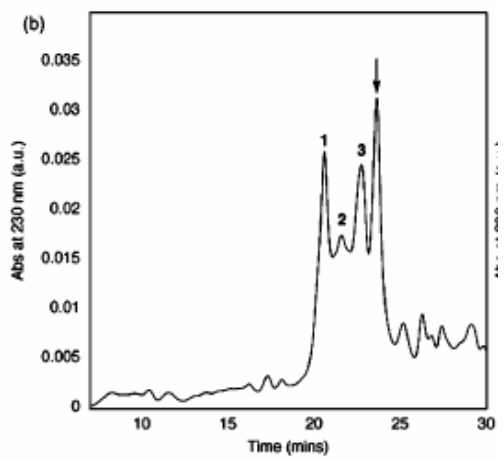
Fluorous HPLC of hydrophobic peptide is 10^2 better than RP-HPLC!

SPPS with fluorous capping...

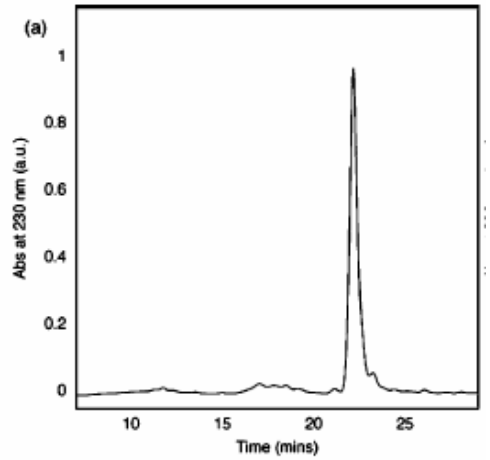


Kumar, K.; Montanari, V. *J. Am. Chem. Soc.* **2004**, *126*, 9528

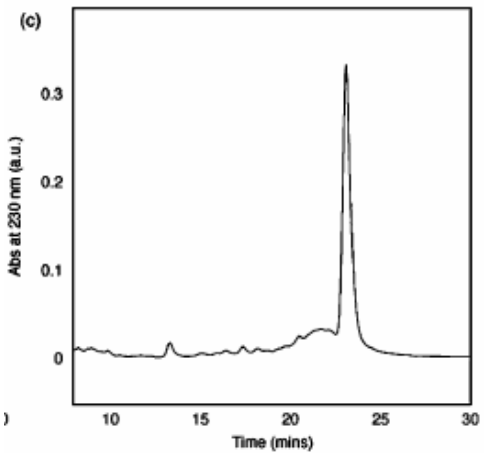
Capping (continued)



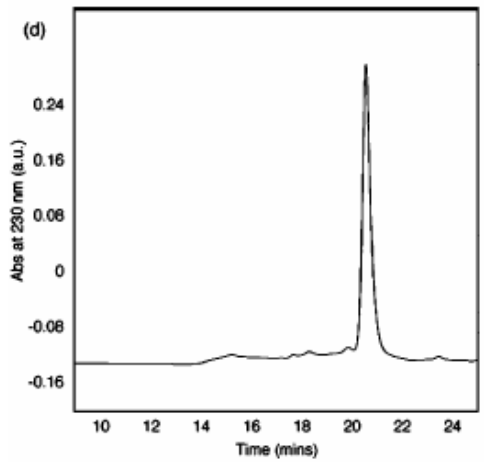
Ac-NH-RAV*KVY*ADAA*EDESAEAFAEF-CONH$_2$
(no capping)



Ac-NH-VEA*AID*YI*DA-CONH$_2$



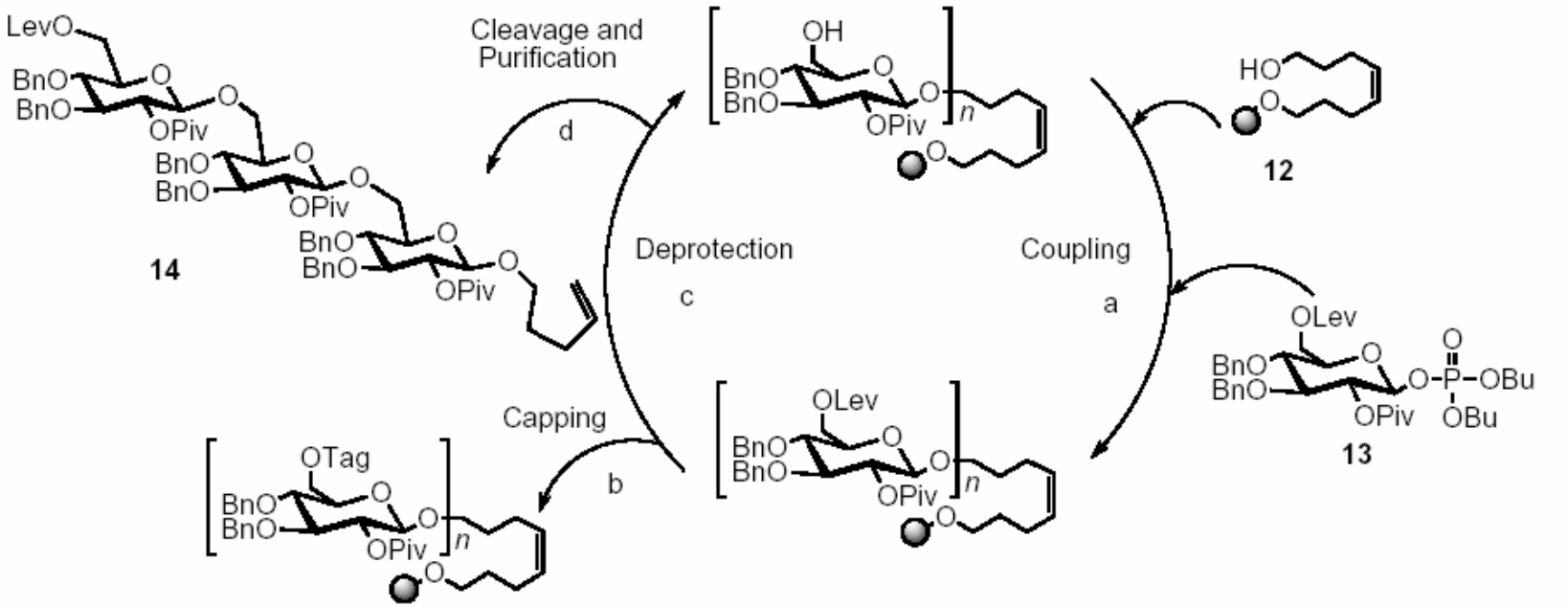
Ac-NH-RAV*KVY*ADAA*EDESAEAFAEF-CONH$_2$



Ac-NH-PT*GYGS*SSRRAPET-CONH$_2$

Kumar, K.; Montanari, V. *J. Am. Chem. Soc.* **2004**, *126*, 9528

Oligosaccharide synthesis with fluorous capping



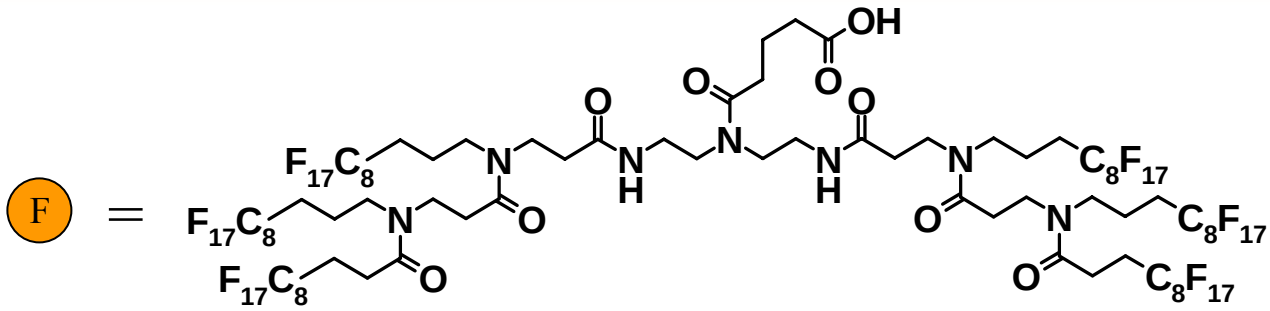
- Tagging conducted after each coupling

Tag = **Oligosaccharide** — **Si** — C_8F_{17}

- Tagged deletion sequences removed by FSPE *(quick intermediate purification in solution phase synthesis)*

Seeberger, P.H. et al, *Angew. Chem. Intl. Ed*. **2001**, *40*, 4433

Fluorous supports



- **Fluorous Supported Peptide Synthesis**
  (Inazu, T. *et al,* Chem. Comm. 2003, 972.)

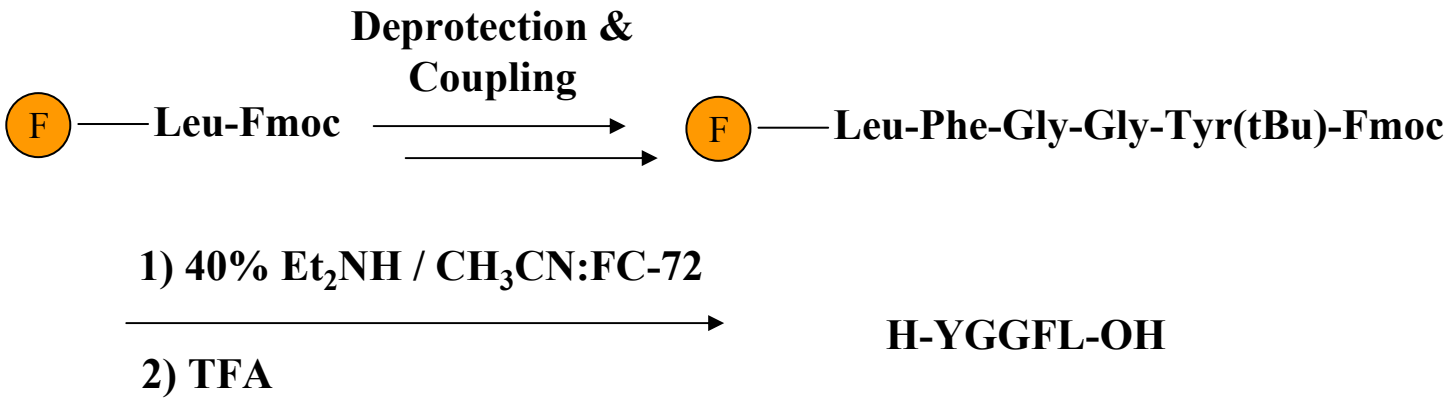
  Tripeptide produced in 67% yield in excellent purity using liquid-liquid extraction and final HPLC purification.

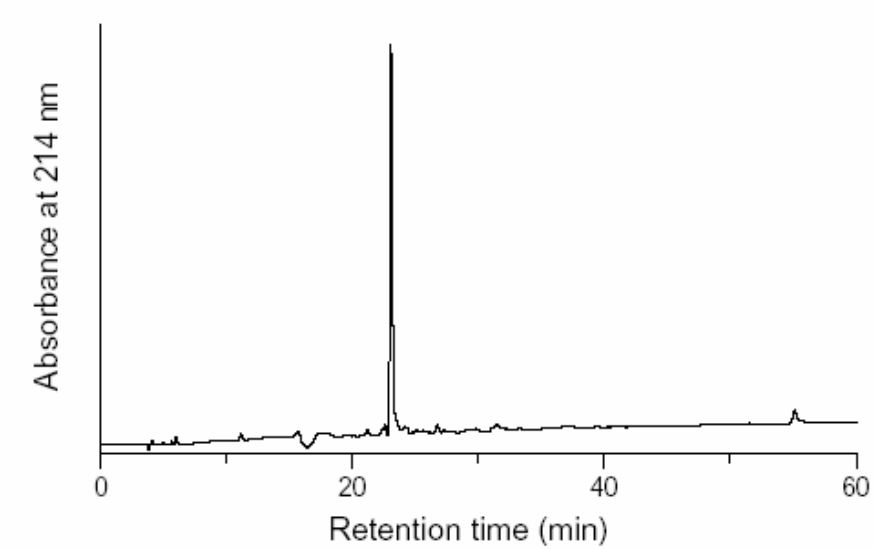
- **Fluorous Supported Oligosaccharide Synthesis**
  (Inazu, T. *et al,* Angew. Chem. Int. Ed. 2003, *42*, 2047.)

  Trisaccharide produced in 42% yield. Final purification by column chromatography after detachment from fluorous support.

Offers potential for total solution-phase synthesis with general purification protocols, and possibly for exploitation of the fluorous phase in segment condensation. These applications are very early in development, however.

Fluorous Supported Synthesis





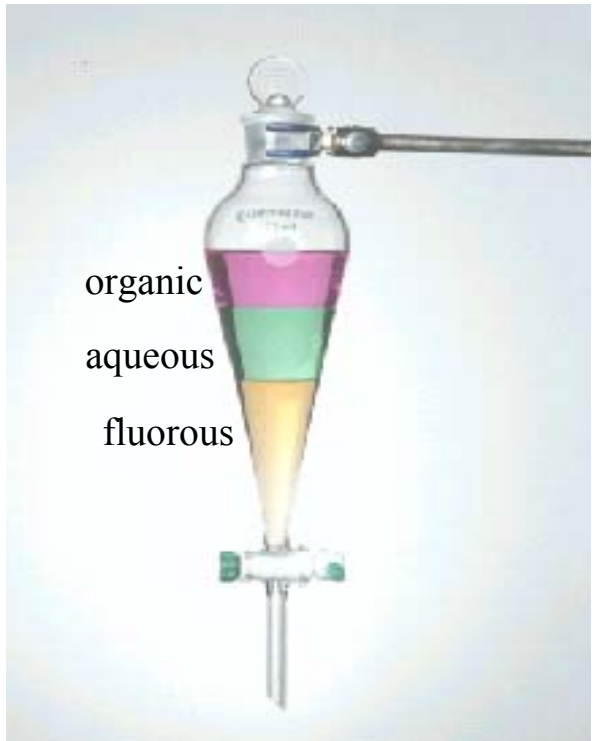
- **Overall 70% yield over nine steps**
- **Final purification by RP-HPLC**
- **All reactions easily monitored**

Mizuno, M. *et al*, *Tetrahedron Lett*. **2004**, *45*, 3425.

Fluorous Liquid-Liquid Extraction (FLLE)

Advantages:

- Highly selective for fluorous tagged components
- Ease of scale-up
- Fluorous solvents readily available in bulk



Disadvantages:

- Partition coefficients for single fluorous tagged molecules are low.
  - Multiple fluorous chains necessary
  - Potential environmental persistence issues
  - Cost and complexity of synthesis

Fluorous Liquid-Liquid Extraction

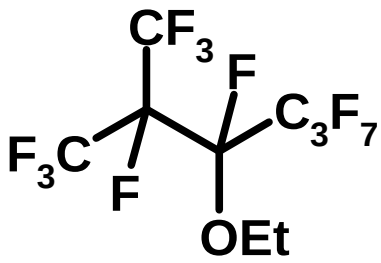
Current Strategy: Increase number of fluorous ponytails until desired fluorous partitioning is obtained. In other words, a substrate tuning model.

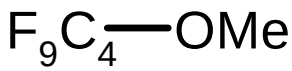
Result: Fluorous reagents, tags, and scavengers have very high MWs increasing cost and complexity and limiting the use of fluorous LLE separations.

Hypothesis: Solvent tuning can be used to influence fluorous partition coefficients.

Desired Result: Useful partition coefficients leading to fluorous components with lower MWs, lower consumption of fluorous solvents, use of more benign solvents.

Alternative Fluorous Solvents

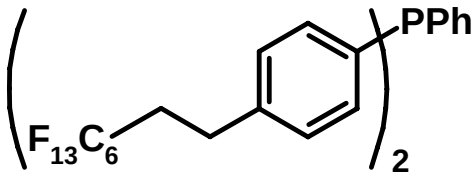




	HFE-7500	HFE-7100
Atmospheric Lifetime	2.8 years	4.1 years
Ozone depletion potential	0	0
Global Warming Potential	210	450
Acute toxicity(LD_{50} rats)	>2000 mg/kg	>5000 mg/kg
28 day tox	None at 1000mg/kg	Increased liver activity at 300 mg/kg
Mutagenicity	None detected	None detected
Ecotoxicity	Very low	Very low
VOC	No	No
Bioaccumlative	No	No

Overall assessment is that these solvents have low environmental impact

Solvent Tuning Studies

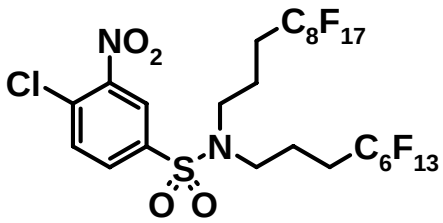


A

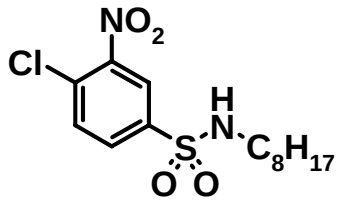
Entry	Compound	Solvent system	% Fluorous	% Organic
1	A	FC-72 / THF	<0.5	>99.5
2	A	FC-72 / CH_3CN	71	29
3	A	FC-72 / DMF	11	89
4	A	FC-72 / 5% H_2O in CH_3CN	90	10
5	A	FC-72 / 5% H_2O in DMF	94	6
6	A	FC-72:HFE-7100/5% H_2O in DMF	>99.5	<0.5
7	PPh3	FC-72 / DMF	<0.5	>99.5
8	PPh3	FC-72 / 5% H_2O in DMF	<0.5	>99.5
9	PPh3	HFE-7100 / 5% H_2O in DMF	11	89

FC-72 = C_6F_{14} HFE-7100 = $MeOC_4F_9$

Solvent Tuning Studies



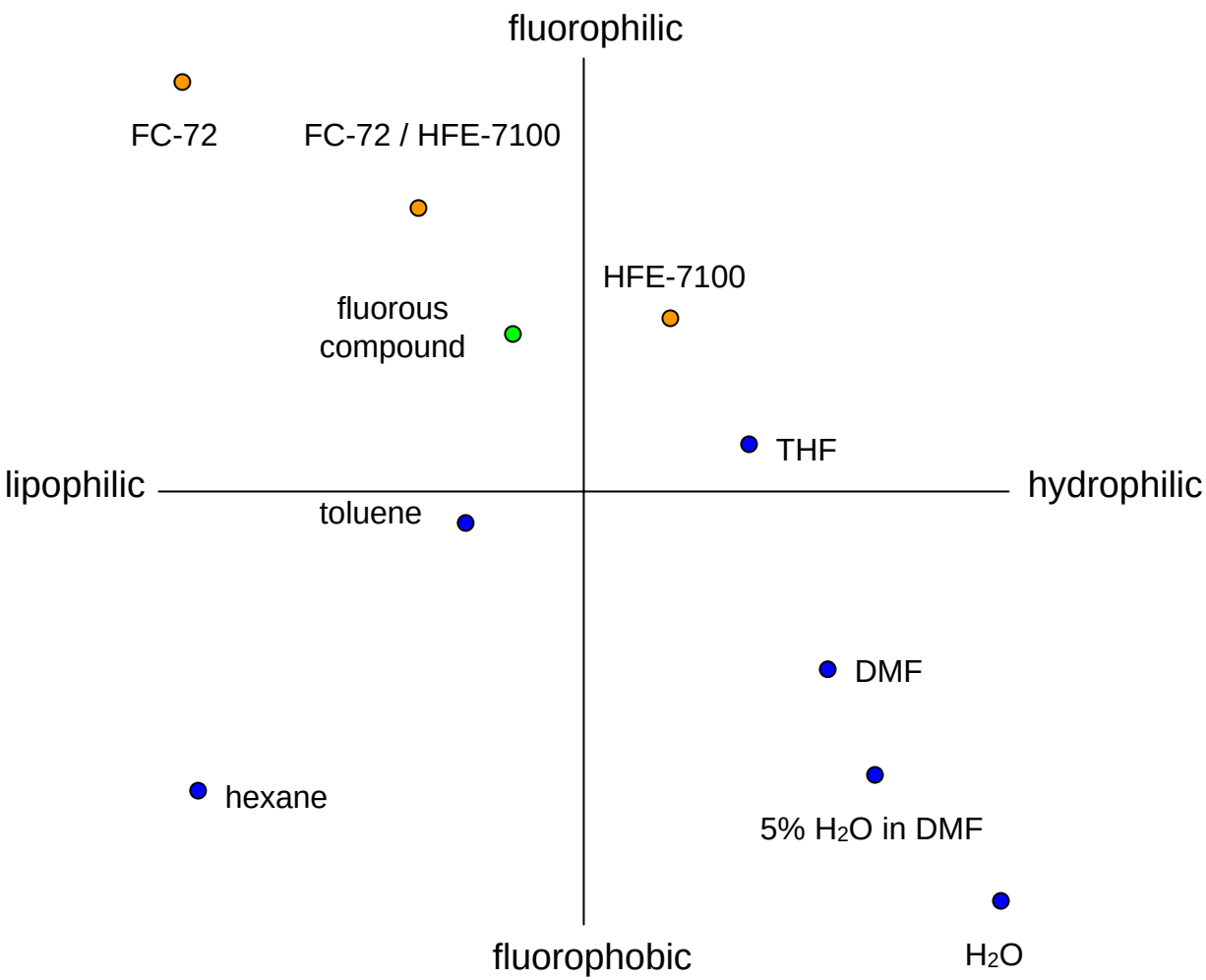
A



B

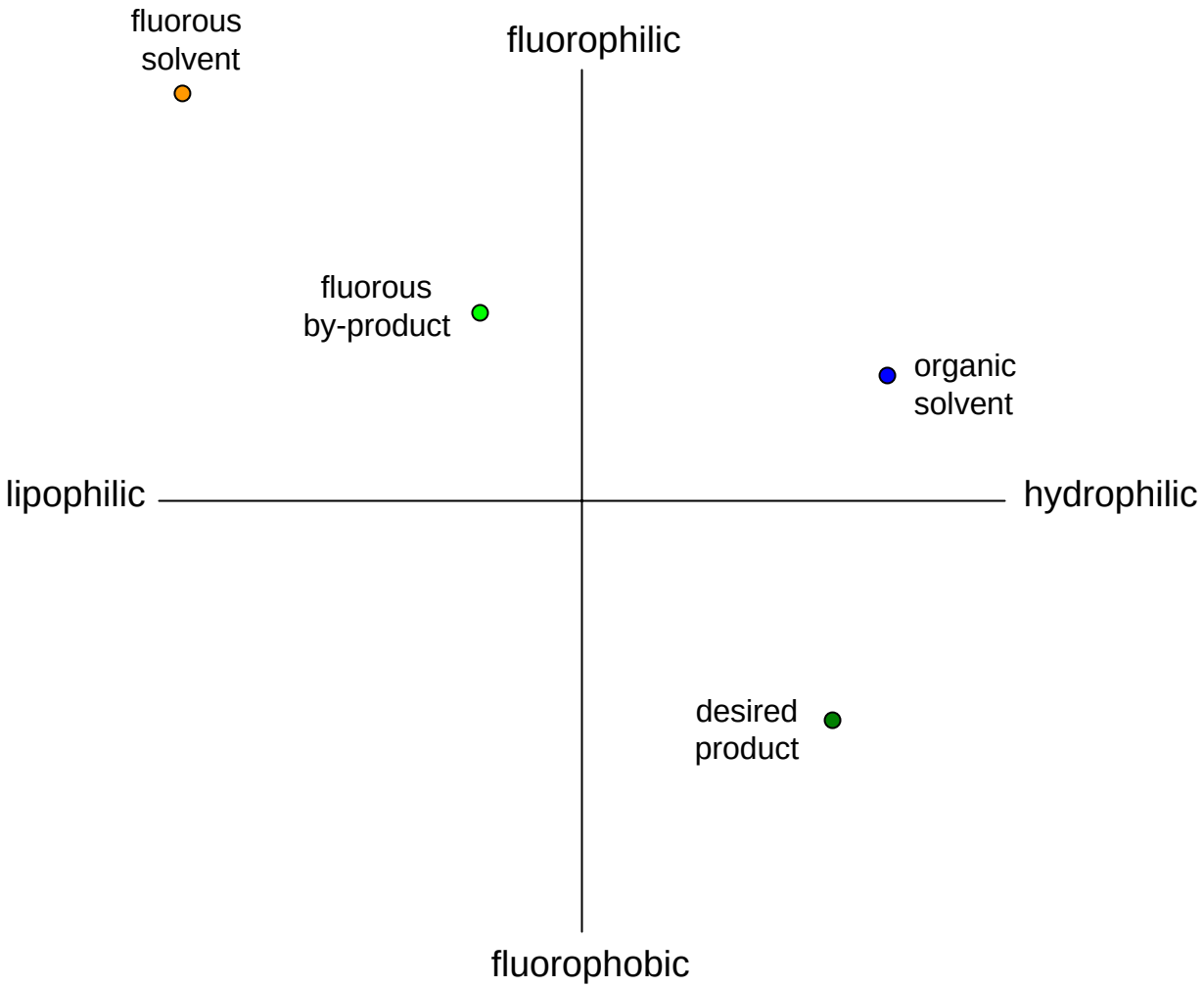
Entry	Compound	Solvent system	% Fluorous	% Organic
1	A	FC-72 / CH_3CN	11	89
2	A	FC-72 / MeOH	NA	NA
3	A	FC-72 / DMF	<0.05	>99.5
4	A	HFE-7100 / 5% H_2O in DMF	>99.5	<0.5
5	A	FC-72:HFE-7100 / 5% H_2O in DMF	98	2
6	B	FC-72 / CH_3CN	<0.5	>99.5
7	B	FC-72 / DMF	<0.5	>99.5
8	B	HFE-7100 / 5% H_2O in DMF	6	94
9	B	FC-72:HFE-7100 / 5% H_2O in DMF	<0.5	>99.5

Qualitative Model for Liquid-Liquid Extractions



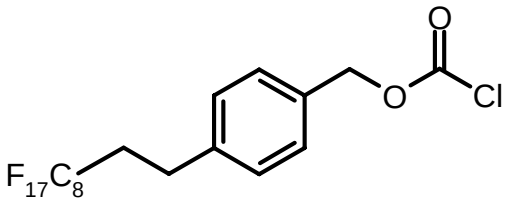
- Solvent miscibility proportional to distance
- P is inversely proportional to substrate distance to two solvents
- Traditional substrate tuning moves fluorous compound up and to the left
- Solvent tuning moves solvents relative to substrate
- Provides an easily tunable system for solution phase peptide synthesis

Customizable for Specific Processes

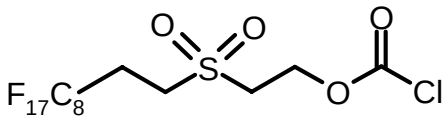


FTI Peptide Synthesis Efforts

- 1st Effort: Repeat van Boom work using f-Z and f-Msc tags



f-Z-Cl

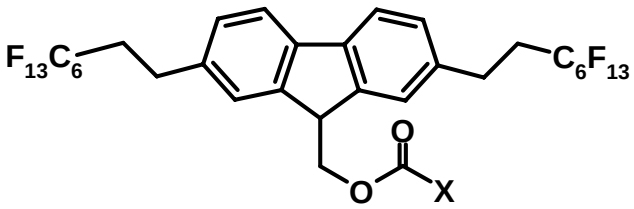


f-MscCl

- f-Z, not surprisingly, was found to detag during acidic cleavage from the resin
- f-Msc was not stable at room temperature, although storage conditions have now been identified.
- Tagging of an amino acid (L or F) with f-Msc was successful, but coupling of f-Msc amino acid was poor.

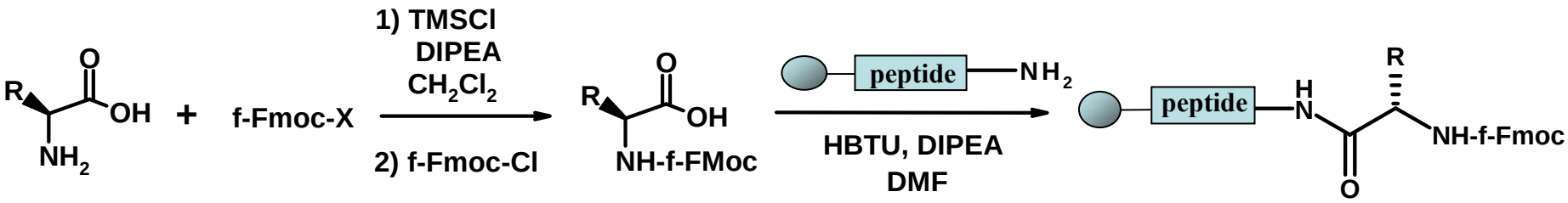
Conclusion: f-Z and f-Msc not particularly good choices for removable fluorous tag

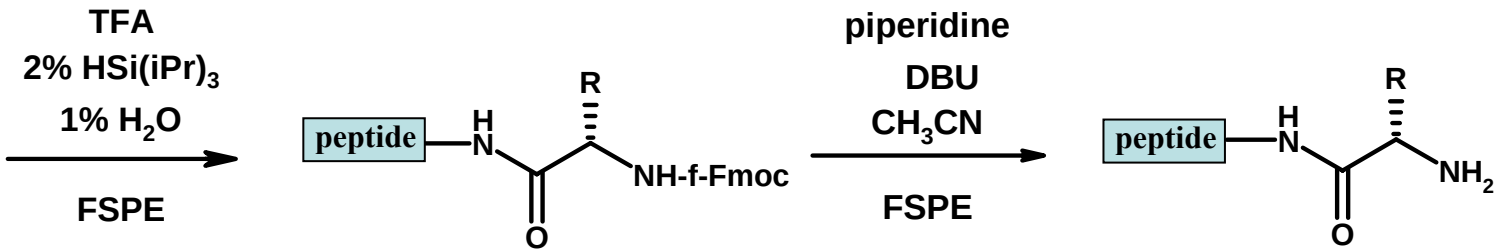
Fluorous Fmoc Peptide Synthesis



X = Cl or OSu

f-Fmoc

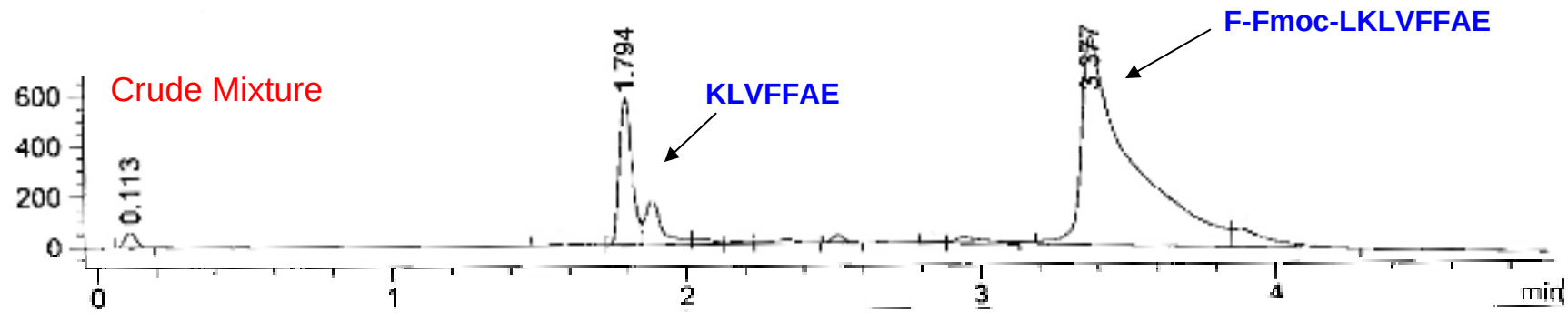


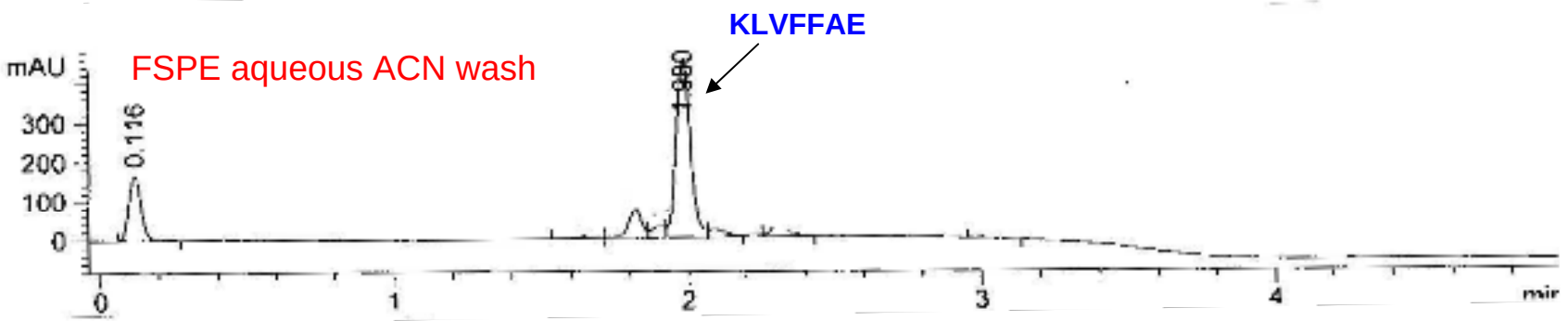


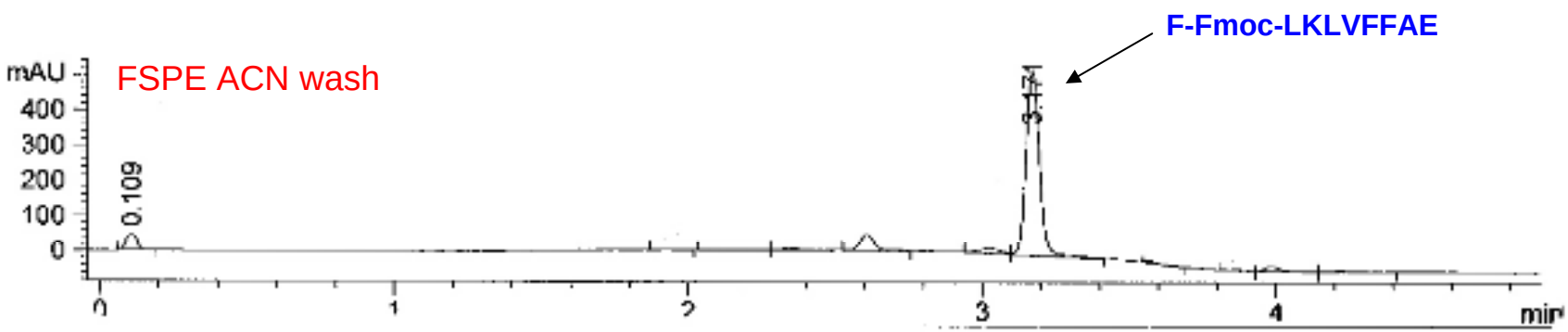
- 10 L-amino acids tagged to date with no difficulties
- “Standard” conditions using Novagel resin employed throughout
- FSPE conducted using 1:1 CH_3CN: water followed by CH_3CN

RP- HPLC Progression of F-Fmoc Peptide

FSPE purification following resin cleavage:





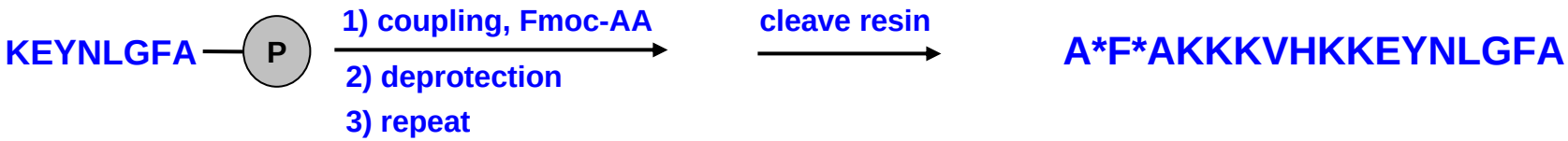


Fluorous Complemented SPPS

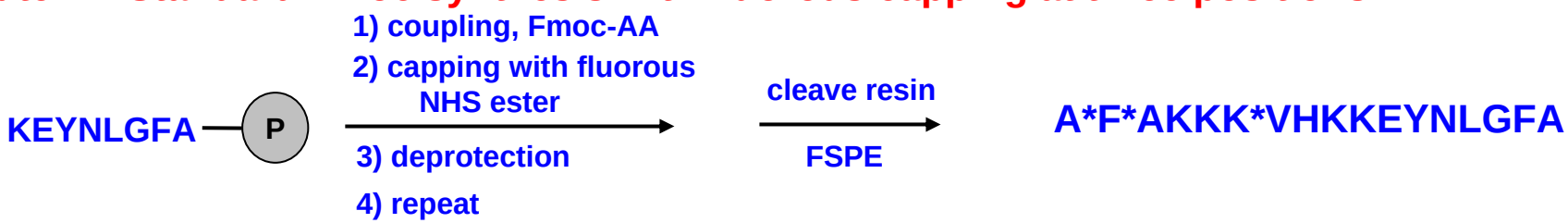
Goal: Compare standard Fmoc based SPPS to fluorous assisted SPPS.

Target 17-mer = AFAKKKVHKKEYNLGFA

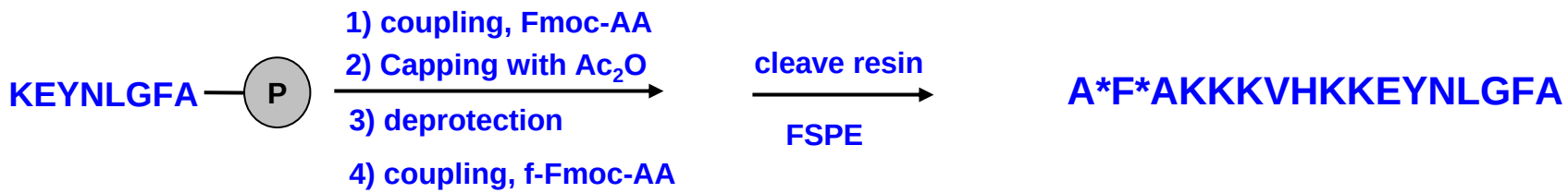
Route A: Standard Fmoc synthesis with no capping



Route B: Standard Fmoc synthesis with fluorous capping at three positions

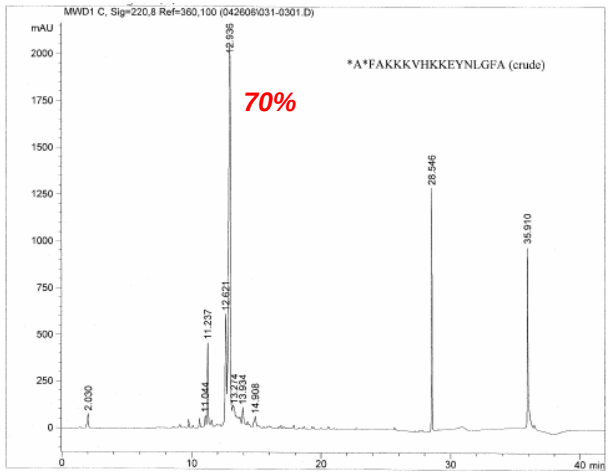


Route C: Standard Fmoc synthesis with acetyl capping and N-terminal fluorous tagging

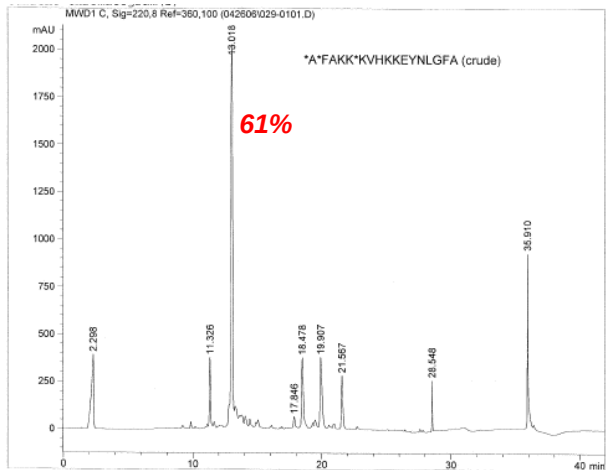


Fluorous Complemented SPPS

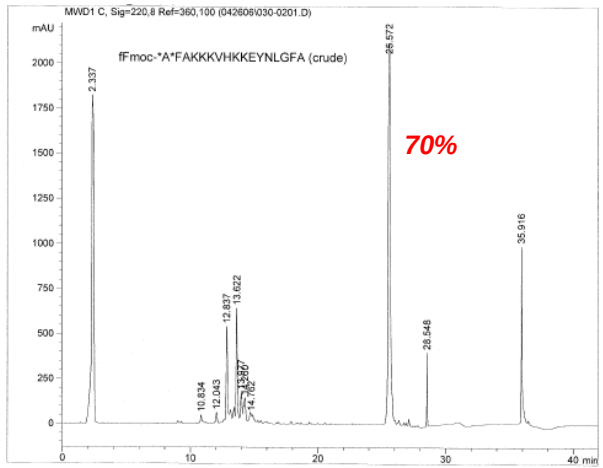
Route A (standard Fmoc SPPS)



Route B (fluorous capping before FSPE)

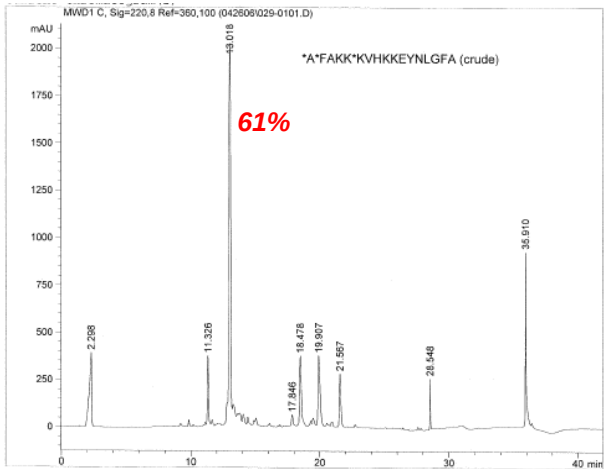


Route C (F-Fmoc N-terminal tagging before FSPE)

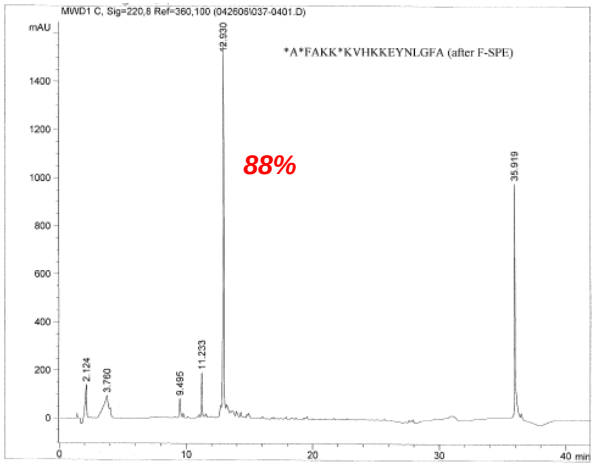


Fluorous Complemented SPPS

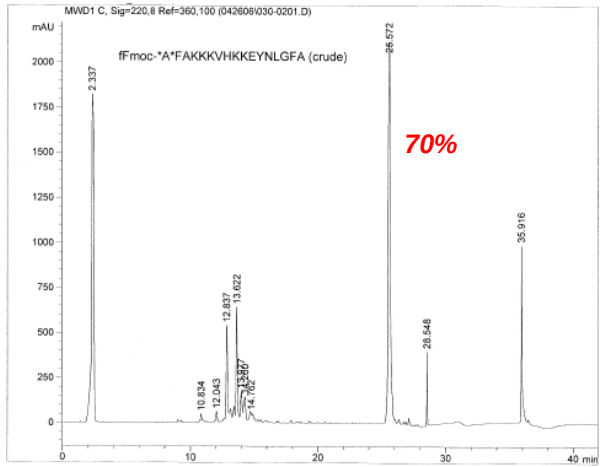
Route B (fluorous capping before FSPE)



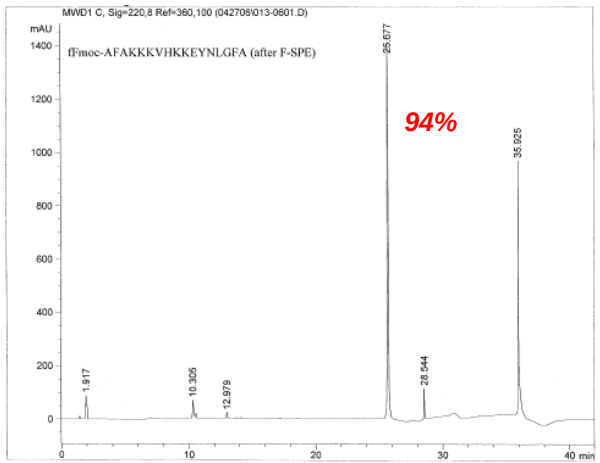
FSPE



Route C (F-Fmoc N-terminal tagging before FSPE)



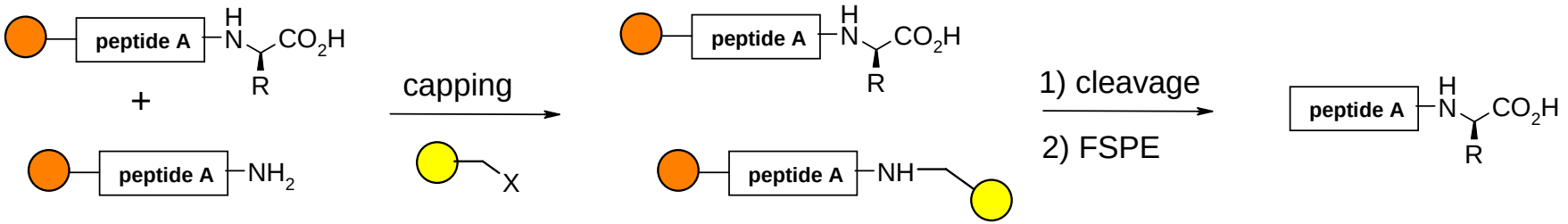
FSPE



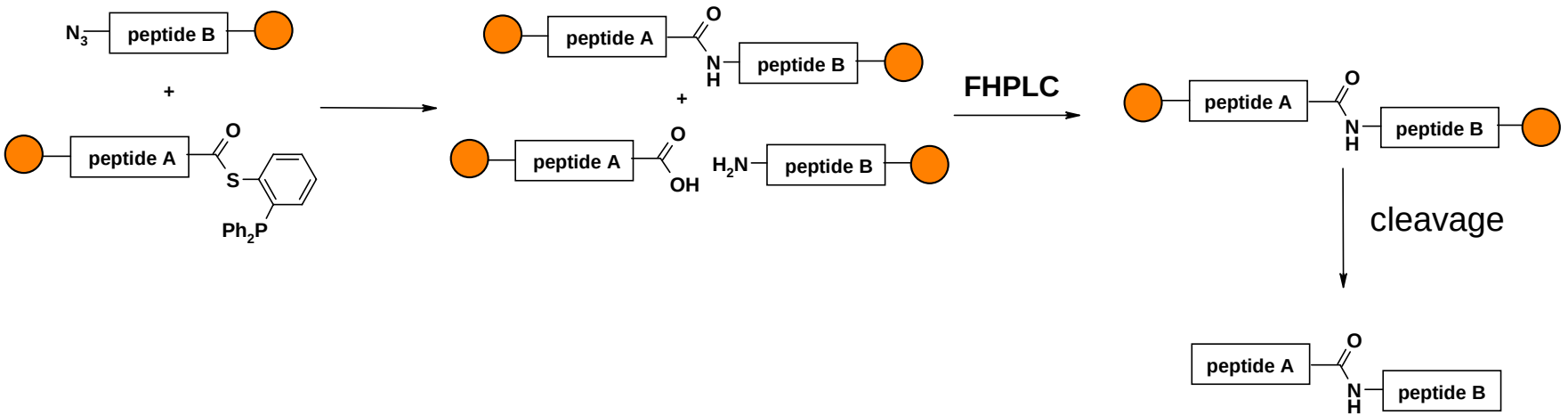
Other Potential Strategies

= removable fluorous tag = permanent fluorous tag

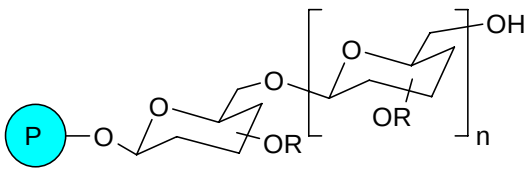
Solution phase peptide synthesis with fluorous capping:



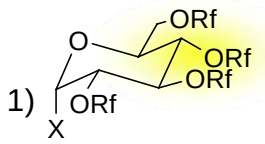
Fluorous tags in segment condensation/chemical ligation



Potential Glycopeptide Synthesis

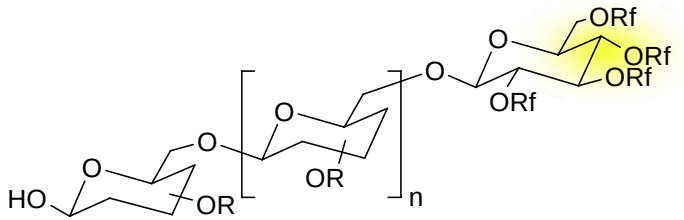


automated or manual oligosaccharide synthesis with capping



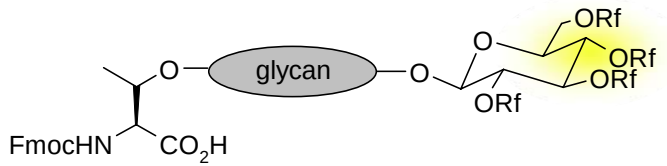
2) cleavage from resin

3) FSPE



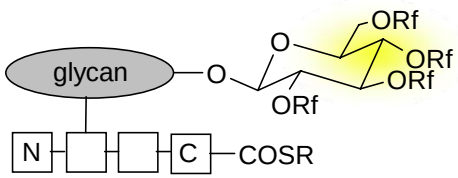
Glycan synthesis with terminal fluorous tagging

amino acid glycosylation

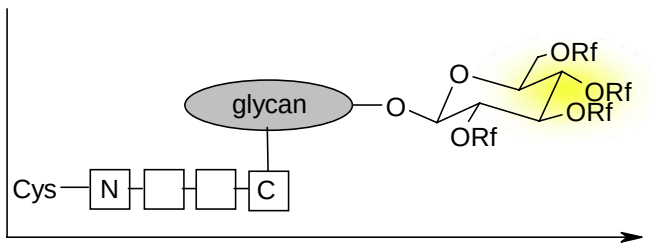


1) Fmoc based peptide synthesis

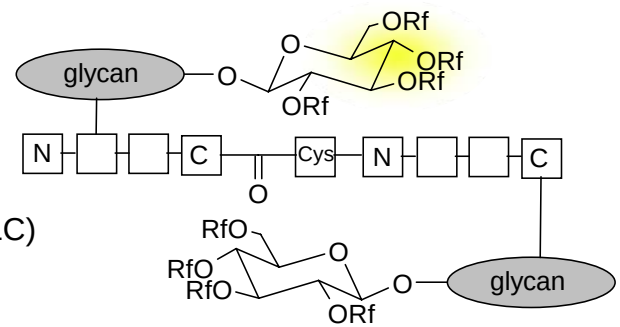
2) FLLE or FSPE purifications between couplings



solution phase glycopeptide synthesis using fluorous glycan as support



"Native Chemical Ligation"



Fluorous based separation (FSPE, FLLE, or FLHPLC)

Conclusions

- Fluorous techniques provide an orthogonal purification method distinguished by its simplicity and high selectivity

- Fluorous chemistry has been effectively applied to the synthesis and purification of peptides
  - Solid phase synthesis with capping
  - Solid phase synthesis with N-terminal tagging
  - Solution phase synthesis using fluorous supports or fluorous reagents

- Fluorous techniques can be envisioned in many applications including glycopeptide synthesis, chemical ligation strategies, etc.

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