Fragment-based Drug Discovery: Comparison of Labeled and Label-free Approaches to beta-Amyloid Secretase (BACE-1)

RapidFire: SPE/MS/MS

Agilent Technologies, Inc.
Life Sciences Group

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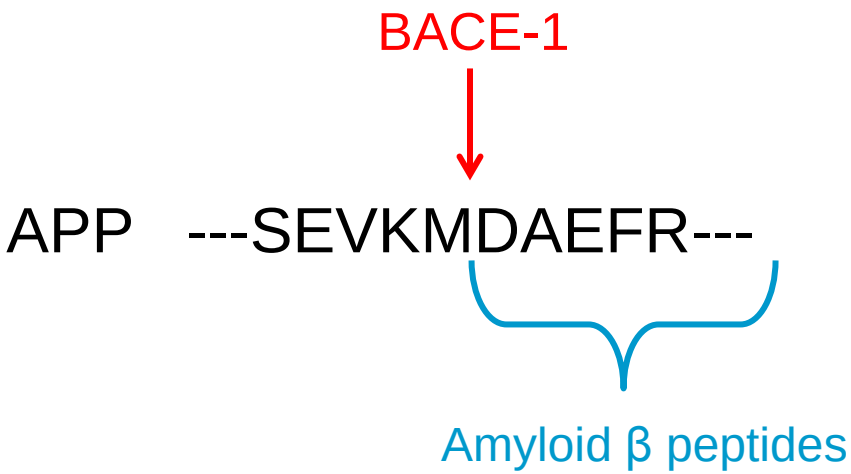
Application Development Scientist, RapidFire

Overview

1. BACE-1 as a model system
2. Fragment Based Drug Discovery
3. MS and RapidFire
4. BACE-1 assay development
5. Screening results
6. Example customer data

BACE-1 as a drug target

- β-site Amyloid precursor protein Cleaving Enzyme 1
  - type 1 transmembrane aspartyl protease
  - cuts Amyloid Precursor Protein (APP) to form amino terminus of Amyloid β peptides



BACE-1 as a drug target

- Imbalance in production and clearance causes Aβ peptides to aggregate into brain plaques
  - key early initiating event in the pathogenesis of Alzheimer's Disease
  - inhibition of β-secretase decreases the production of all of the Aβ peptides
  - Mouse model can be rescued by crossing with BACE1$^{-/-}$ strain

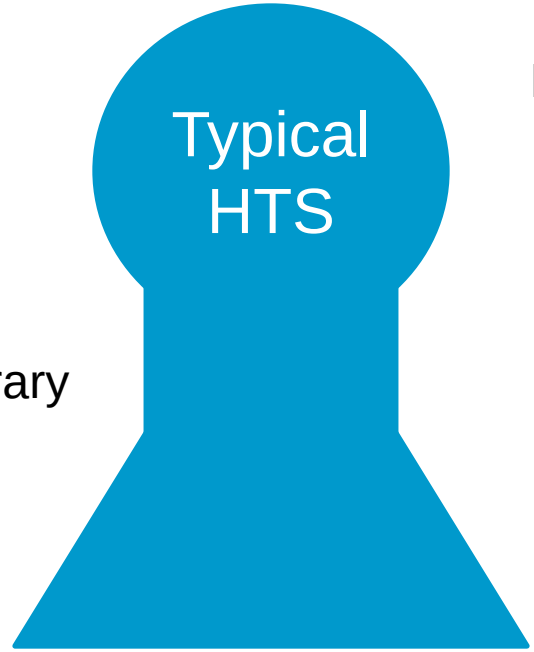
Screening BACE-1

- HTS has met with limited success
  - most inhibitors are peptidomimetic
  - potent in vitro, but large and have many H-bond donors and acceptors
  - poor PK properties and CNS penetration

- Need a small molecule inhibitor with good drug-like properties

What is FBDD?

Large, drug-like molecules



Rule of 5 compliant

~10^6 compounds in a library

High lipophilicity

Vast chemical space – estimated 10^{60} possible compounds

What is FBDD?

Small compounds

Rule of 3 compliant

~10^{2-3} compounds in a library

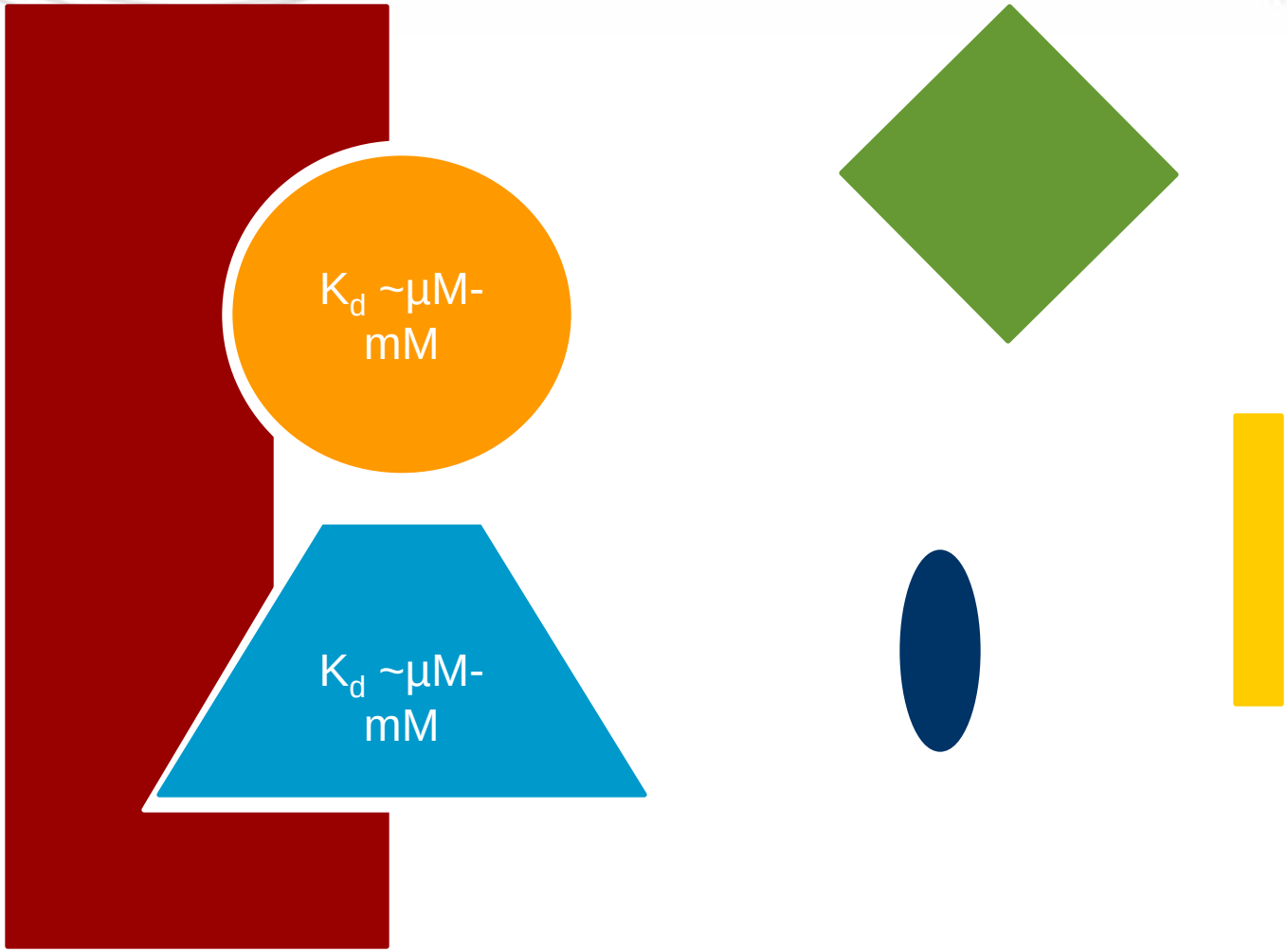
High aqueous solubility

Efficient binders

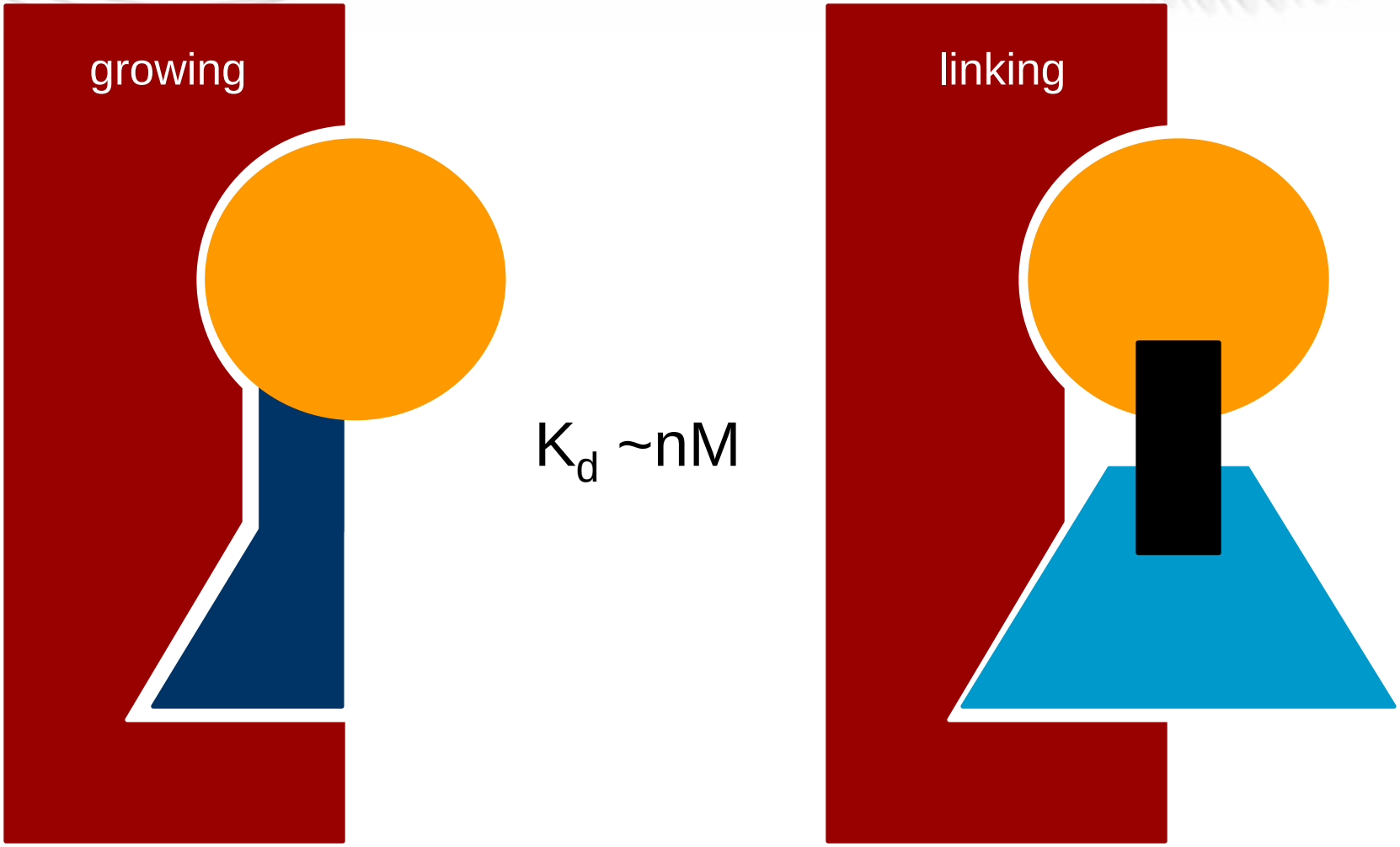
Much smaller chemical space

What is FBDD?

What is FBDD?



What is FBDD?



Biophysical methods are frequently used for FBDD

Method	Pros	Cons
NMR	Sensitive Can give site info	Large quantities of sometimes labeled protein Slow
X-ray Crystallography	Detailed structural info Can handle very large proteins	Must make crystal Slow Not all sites are accessible
SPR	Higher-throughput Gives kinetic and affinity info	No site info
ITC	Gives thermodynamic info	Low throughput Requires high protein concentration
CE	Small samples	No site info
Native MS	Higher-throughput Small samples	Tethering Soft ionization

Biophysical vs Biochemical Screens

Biophysical	Biochemical
Structural information	Functional inhibition
Binding kinetics	Higher-throughput
	Cheaper

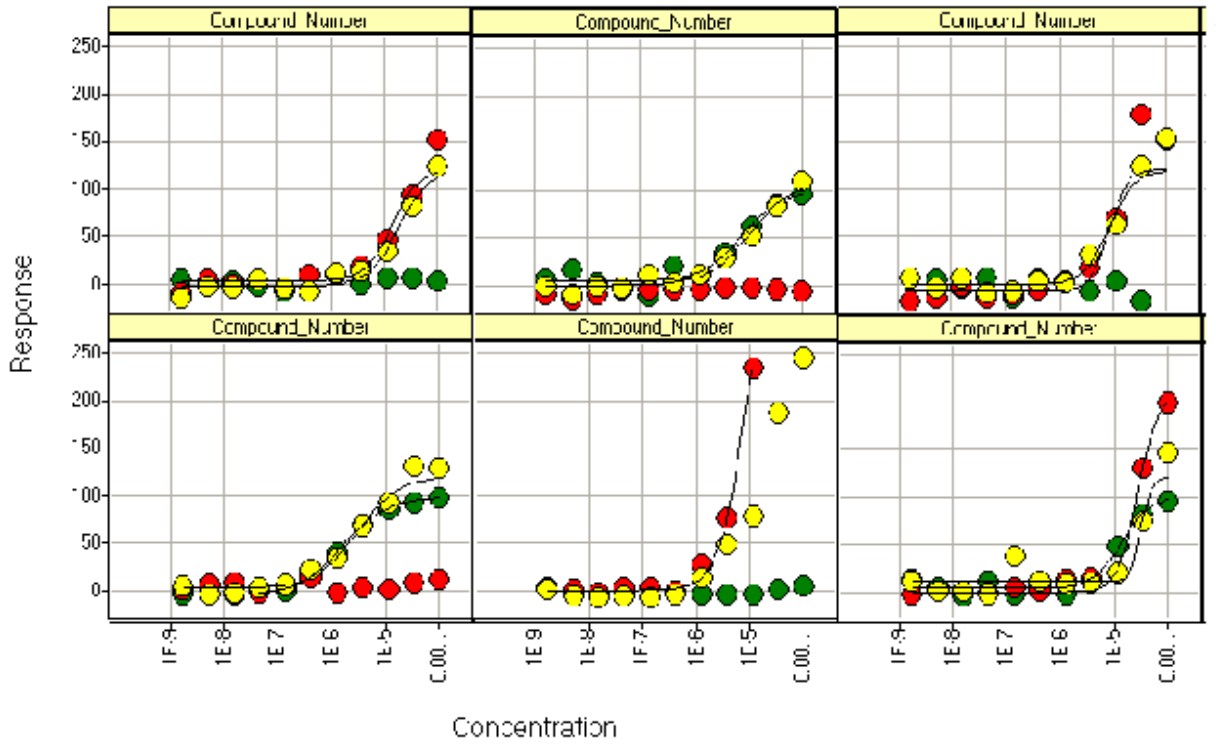
Neither tells the whole story, rather they are complementary

Optical assays common – very fast, relatively cheap, but prone to artifacts

Direct Compound Interference in Optical Assays

Case Study 3: RapidFire for HTS Hit Confirmation

Jmj Demethylase Assay: RapidFire vs. TR-FRET Data



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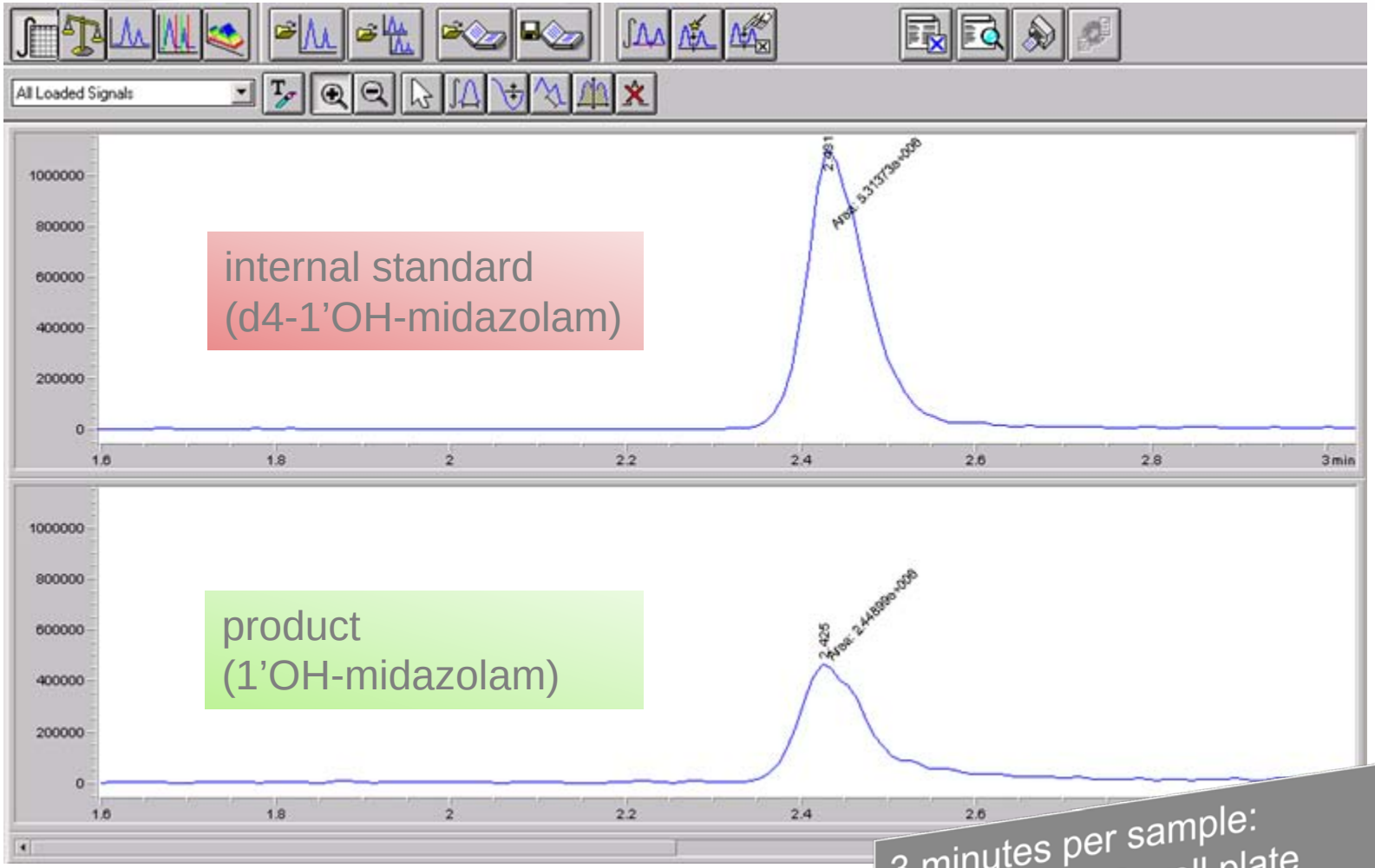
Monitoring Enzymatic Reactions *via* Mass Spectrometry

- Substrate and product are monitored by their unique masses
  - Native reaction substrates & products (no radioactivity, surrogate analytes, or indirect or secondary components)
  - True label-free detection
- MS is highly sensitive and specific
- Direct, quantitative measurements (of 2 (or more) species):
  - Enables data normalization
  - Provides unparalleled information about the biochemical reaction
    - Can track multiple modifications
- Functional biochemical assays (rather than target binding assays)

Traditional Sample Clean-up Using HPLC is Slow

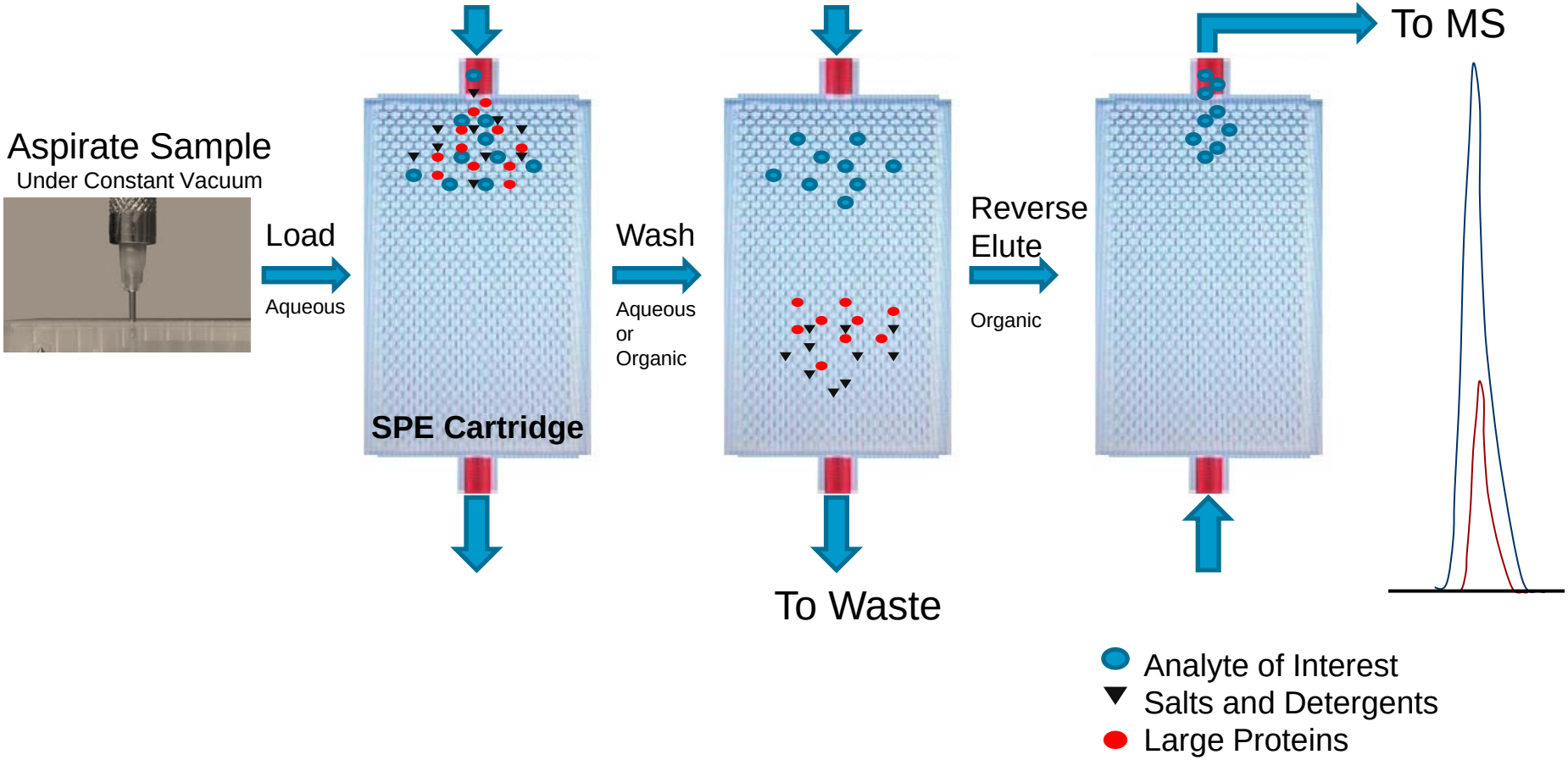


3 minutes per sample:
5 hours per 96-well plate

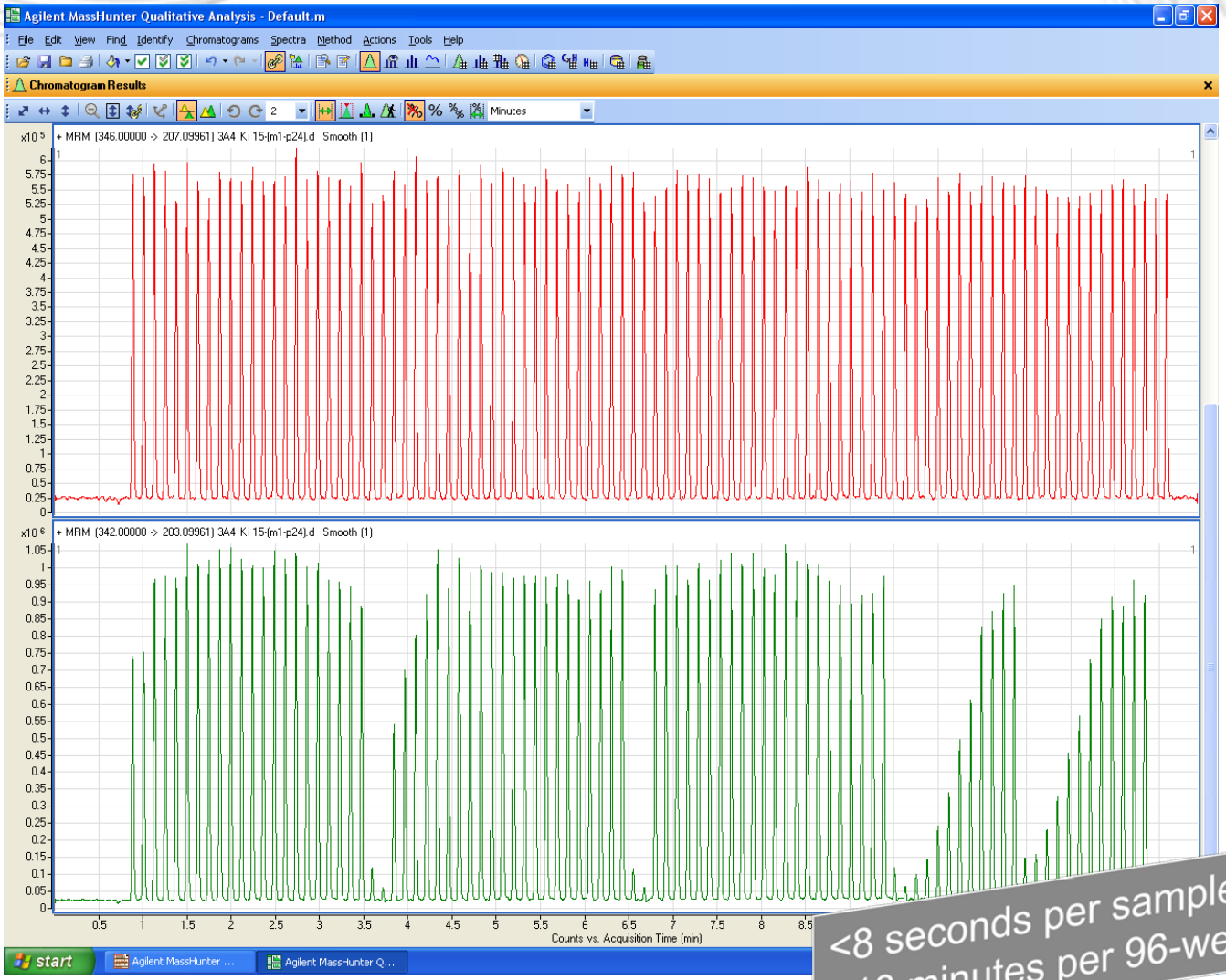
Where RapidFire comes in

- Microscale solid-phase extraction
- Multiple cartridge packing materials
  - can be optimized for small molecules, lipids, peptides, oligonucleotides, and whole proteins
- Compatible with in vitro reaction buffers as well as biological matrices
- Sustained rate of ~8-10 seconds per sample (~13-16 minutes per 96-well plate)
- Compatible with multiple types of MS

RapidFire/MS Sample Cleanup



HTMS Converts a Mass Spec into a Plate Reader



<8 seconds per sample:
<13 minutes per 96-well plate

Can RapidFire be used for FBDD of BACE-1?

APP peptide
SEVNLDAEFR
Molecular weight
= 1179.24

BACE-1

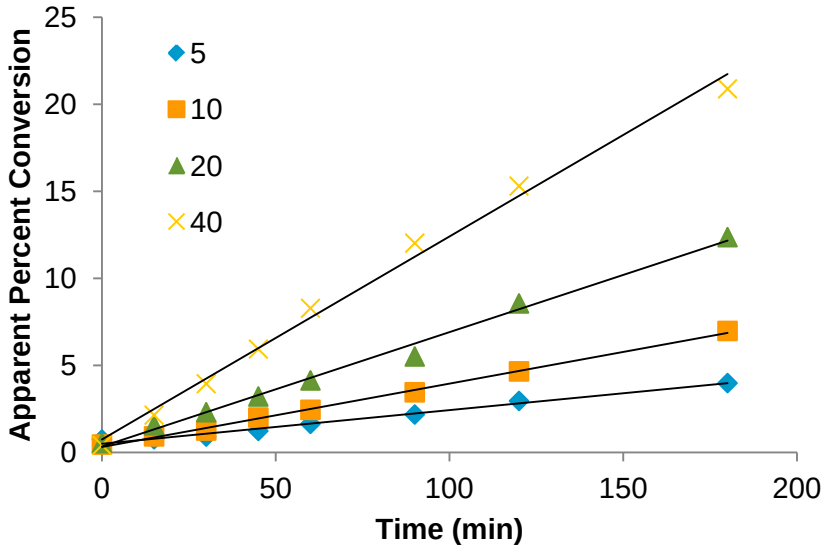
SEVNL
Molecular weight
= 560.60

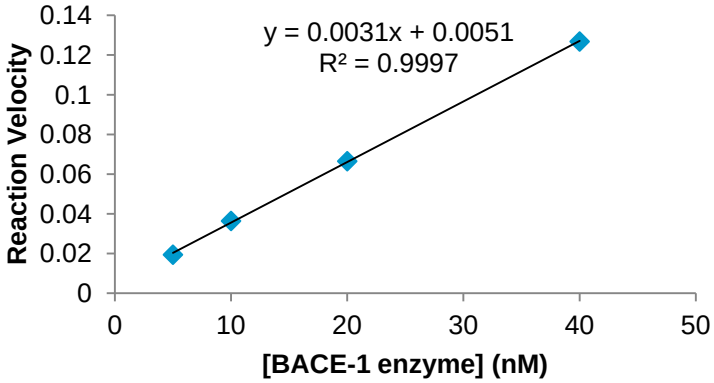
DAEFR
Molecular weight
= 636.65

Assay Development:

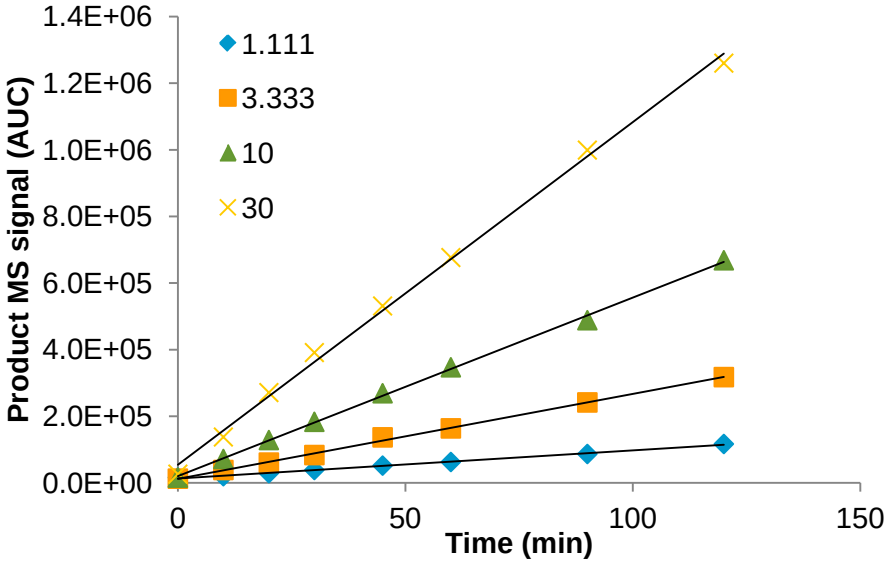
- Linearity
- Kinetics
- DMSO tolerance
- Z' score
- Reference IC_{50}

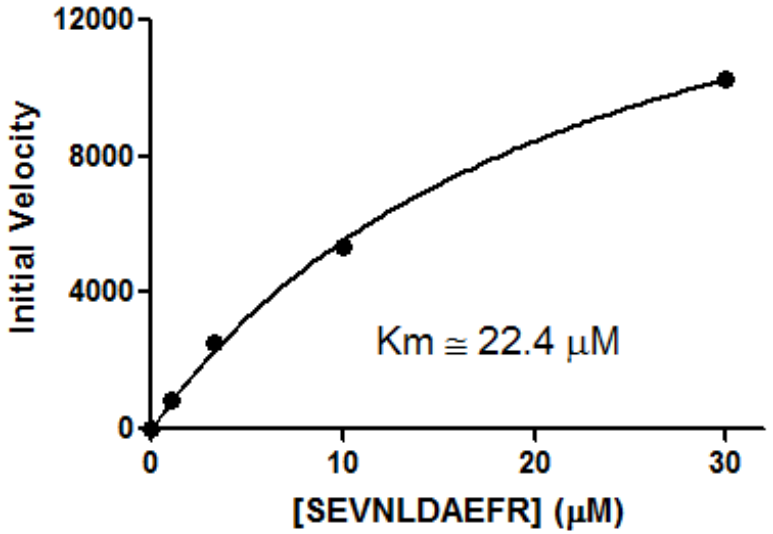
Assay Development – Unlabeled Peptide Enzyme Titration Timecourse





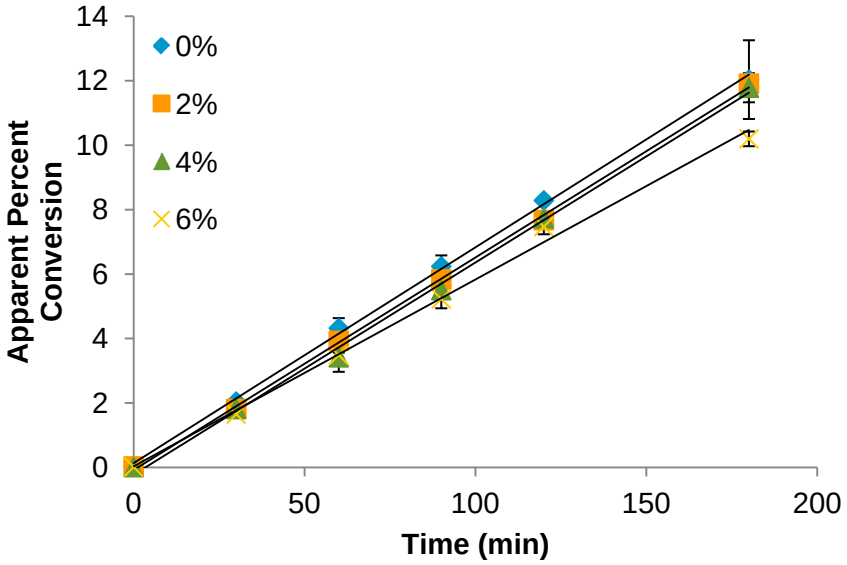
Assay Development – Unlabeled Peptide Substrate Titration Timecourse



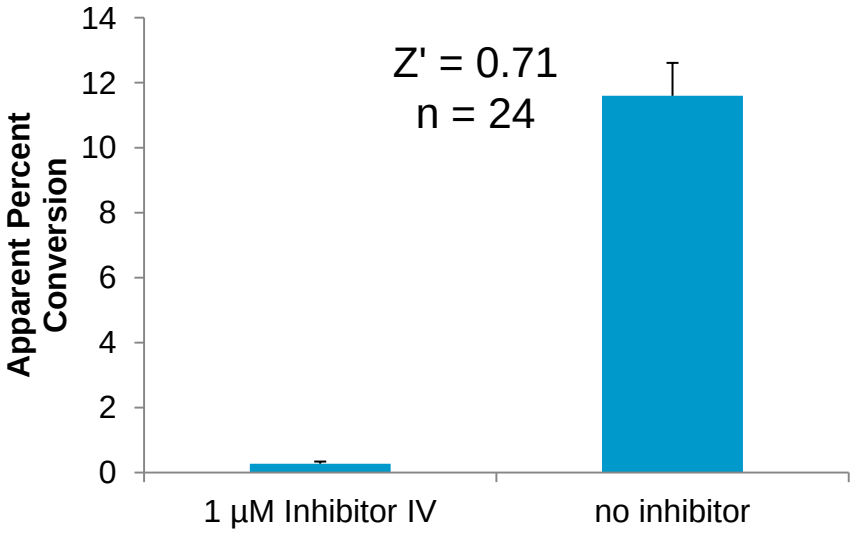


Assay Development – Unlabeled Peptide

DMSO Tolerance



Assay Robustness



How does RapidFire/MS compare to traditional methods?

Labeled APP peptide

Mca-SEVNLDAEFR-K(dnp)-RR_{NH_2}

Molecular weight
= 1999.91

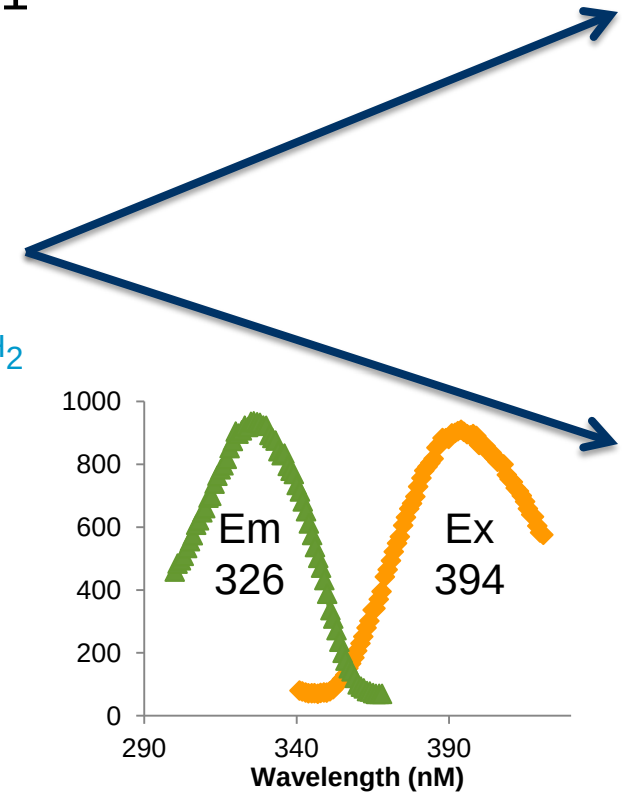
BACE-1

SEVNL

Molecular weight
= 776.79

DAEFR-K(dnp)-RR_{NH_2}

Molecular weight
= 1242.30

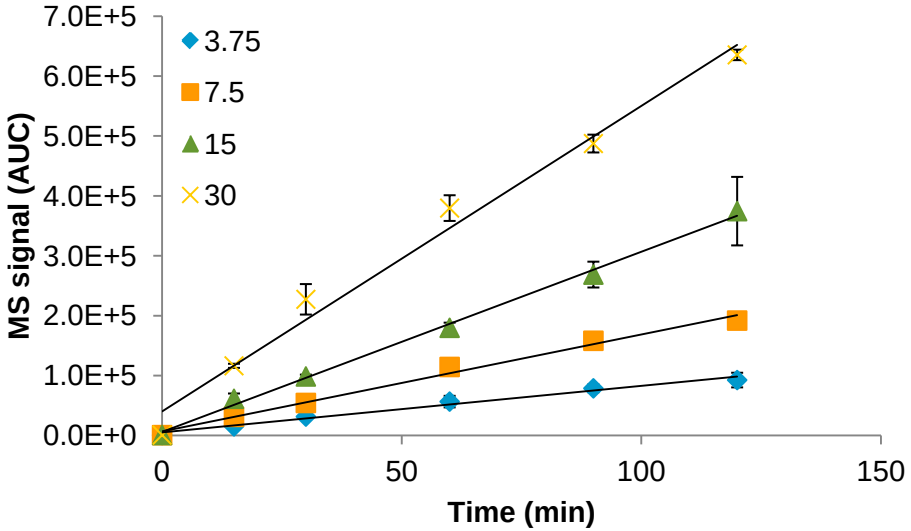


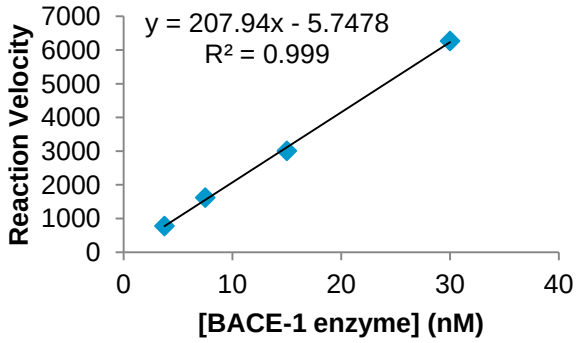
Agilent Technologies

Cary Eclipse Fluorescence Spectrophotometer

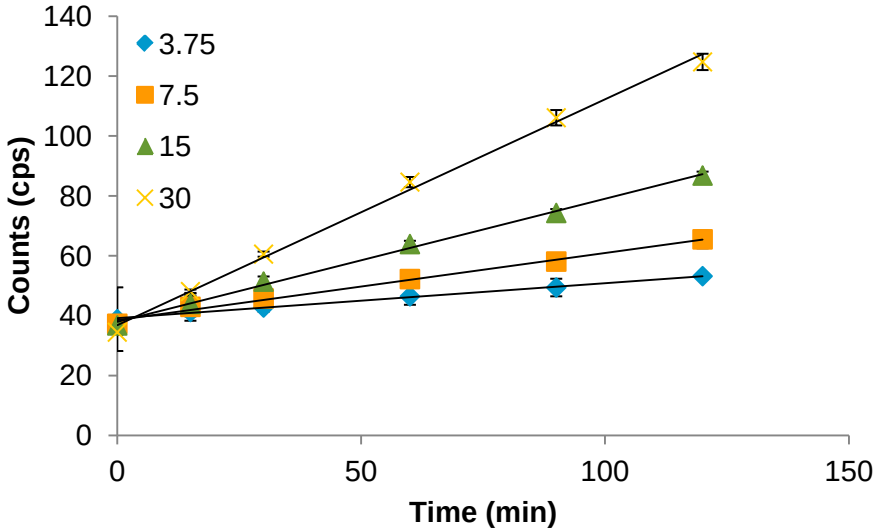
Assay Development – Labeled Peptide Enzyme Titration Timecourse

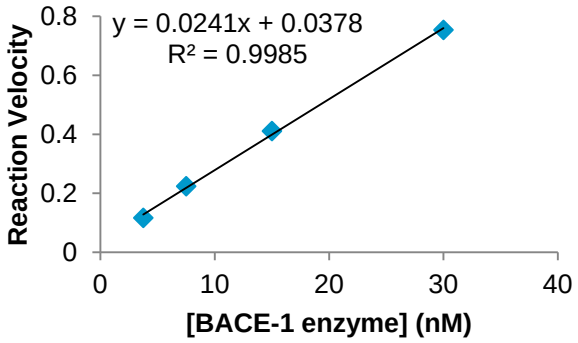
RapidFire-MS





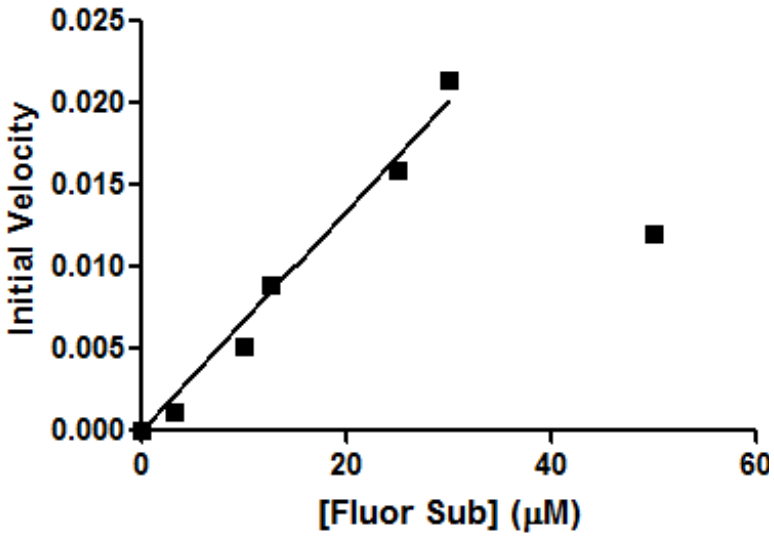
Fluorescence Plate Reader



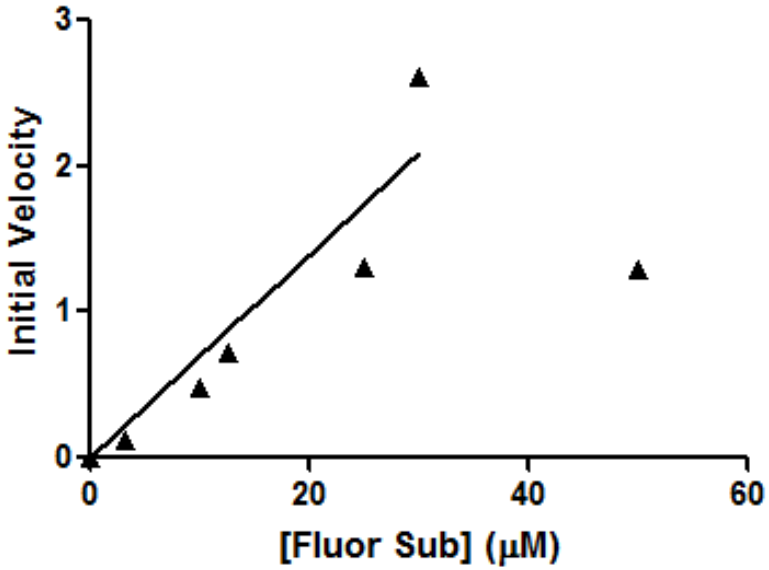


Assay Development – Labeled Peptide Substrate Binding

RapidFire-MS

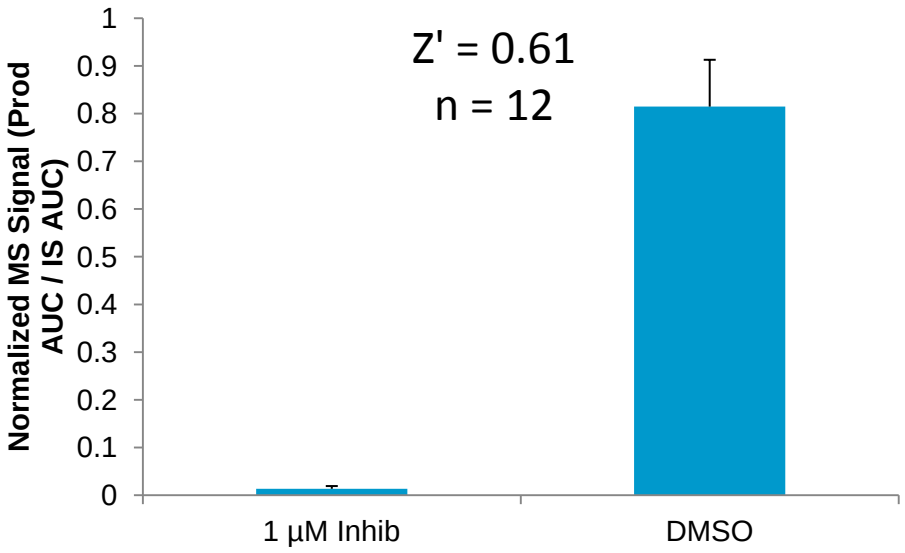


Fluorescence Plate Reader

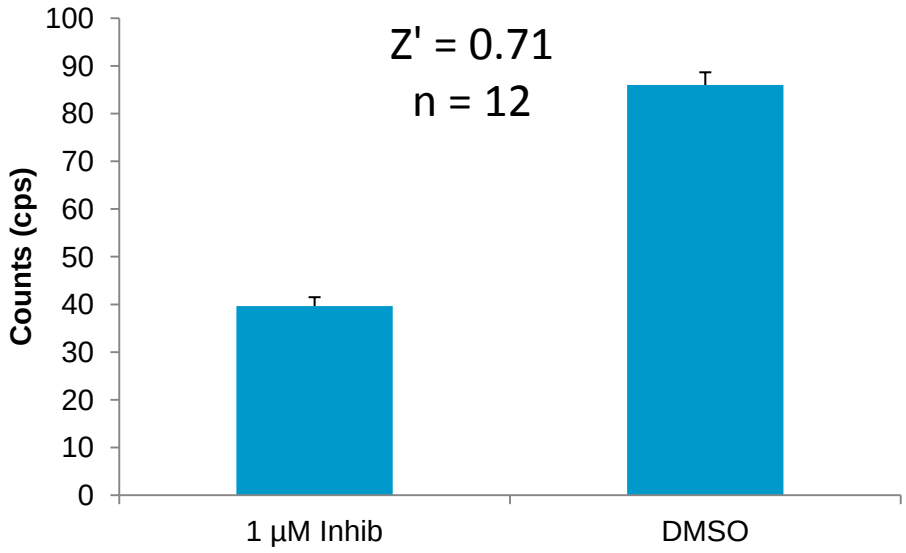


Assay Development – Labeled Peptide Assay Robustness

RapidFire-MS



Fluorescence Plate Reader

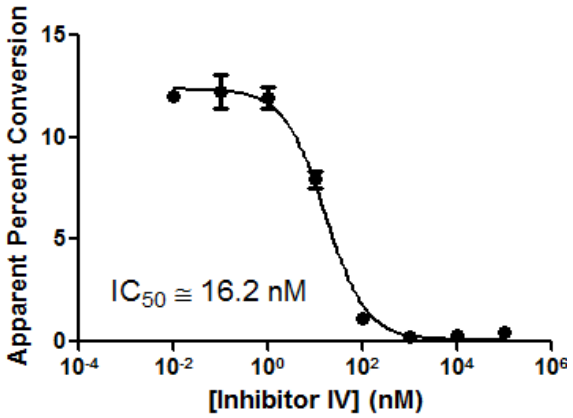


Assay Comparison Matrix

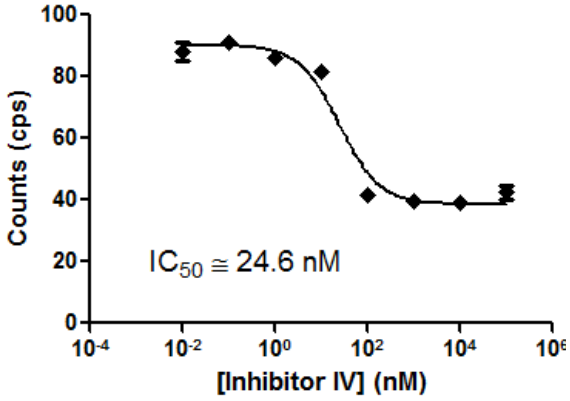
Unlabeled Peptide Mass Spectrometer	Unlabeled Peptide Fluorescence Plate Reader
Fluorescent Peptide Mass Spectrometer	Fluorescent Peptide Fluorescence Plate Reader

Assay Characterization

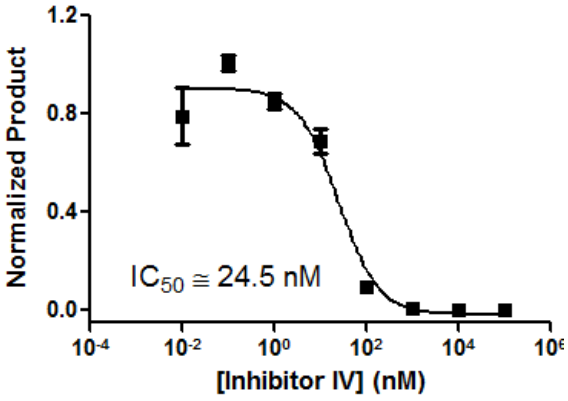
Unlabeled Substrate by MS (UMS)



Fluorescently Labeled Substrate by FS (FS)



Fluorescently Labeled Substrate by MS (LMS)



New: 565794 InSolution™ β-Secretase Inhibitor IV Calbiochem®

N-(1*S*, 2*R*)-1-Benzyl-3-(cyclopropylamino)-2-hydroxypropyl)-5-(methyl(methylsulfonyl)amino-N′-((1*R*)-1-phenylethyl)isophthalamide

Published IC_{50} = 15 nM

Can RapidFire be used for FBDD of BACE-1?

APP peptide
SEVNLDAEFR
Molecular weight
= 1179.24

BACE-1

SEVNL
Molecular weight
= 560.60

DAEFR
Molecular weight
= 636.65

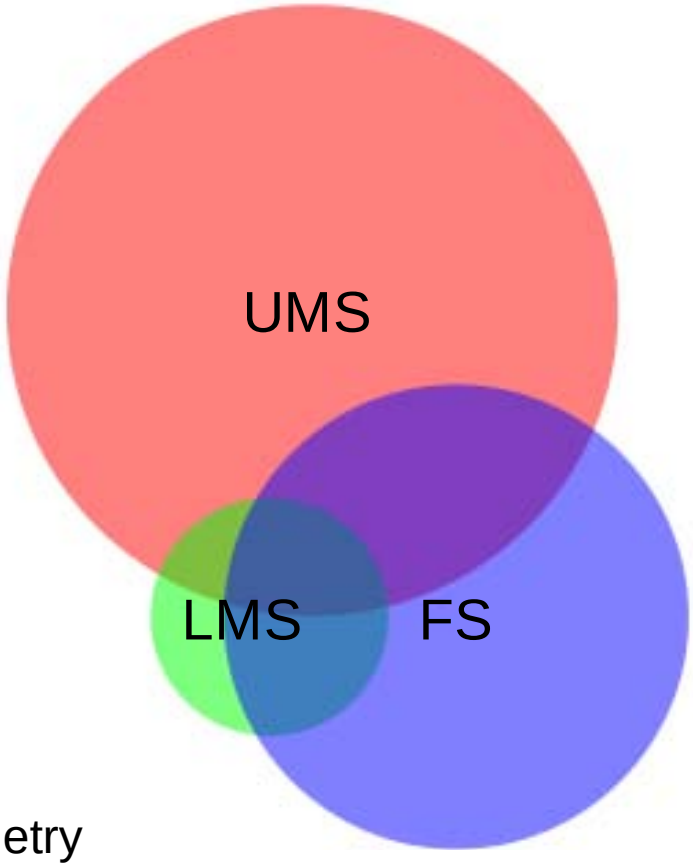
Fragment Based Screen:

- Hits appear as inhibition of product formation
- Cutoff set at 3σ below uninhibited control wells

Initial Screening Results

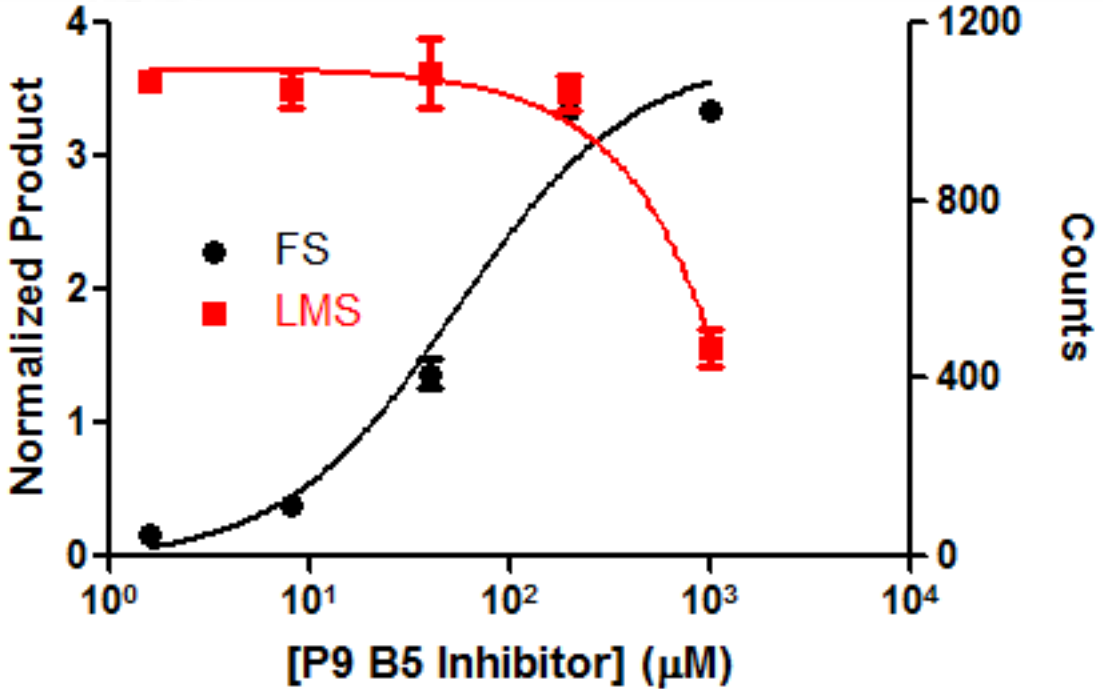
Hits by Assay Format

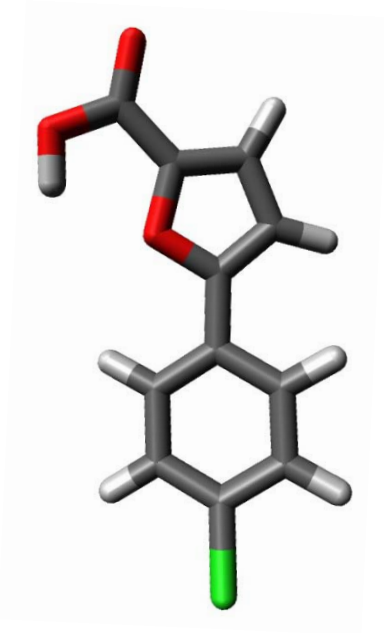
	UMS	LMS	FS	all 3
UMS	211	14	41	-
LMS	14	32	22	-
FS	41	22	122	-
all 3	-	-	-	8



UMS = Unlabeled peptide by Mass Spectrometry
LMS = Labeled peptide by Mass Spectrometry
FS = Labeled peptide by Fluorescence Spectroscopy

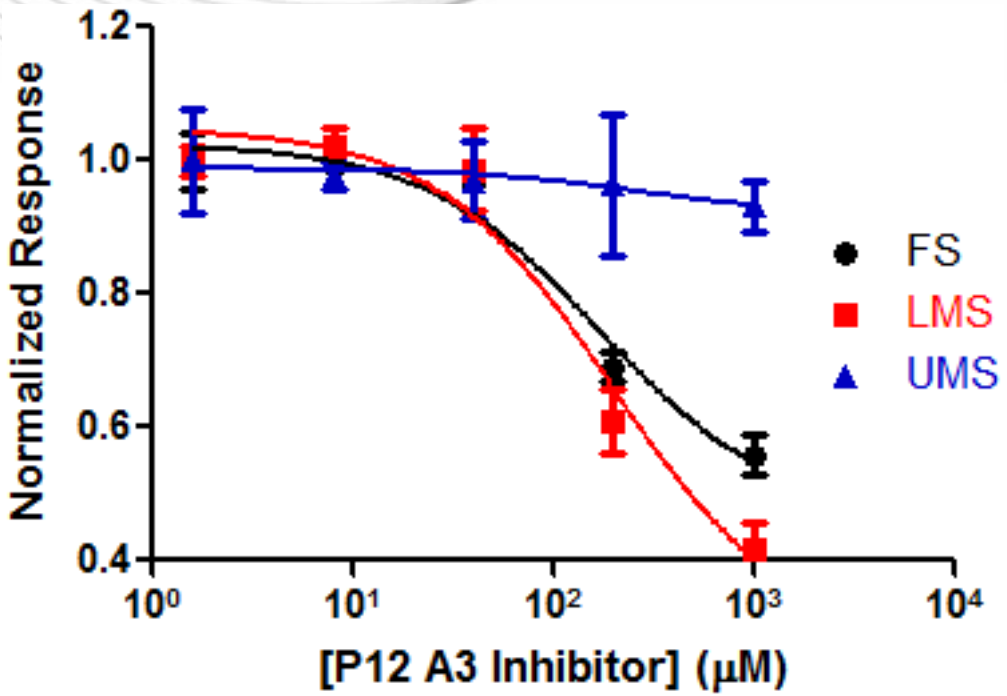
Hits Observed by MS Only

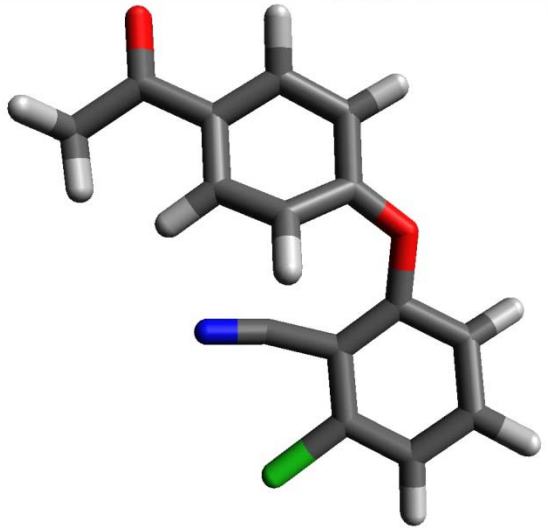




- FS showed concentration-dependent increase in signal, suggesting AF
- MS data were consistent with a traditional inhibition curve

Hits Observed with the Labeled Peptide Only



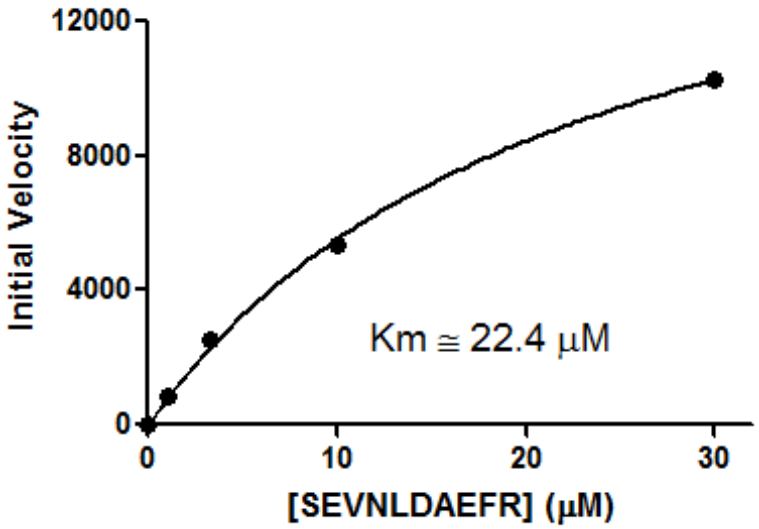


- Both assays employing the labeled substrate (LMS and FS) showed inhibition
- MS data for the unlabeled substrate showed no significant decrease in product formation
- Non-native substrates can generate misleading data, causing follow-up of compounds that don't inhibit the biologically-relevant reaction

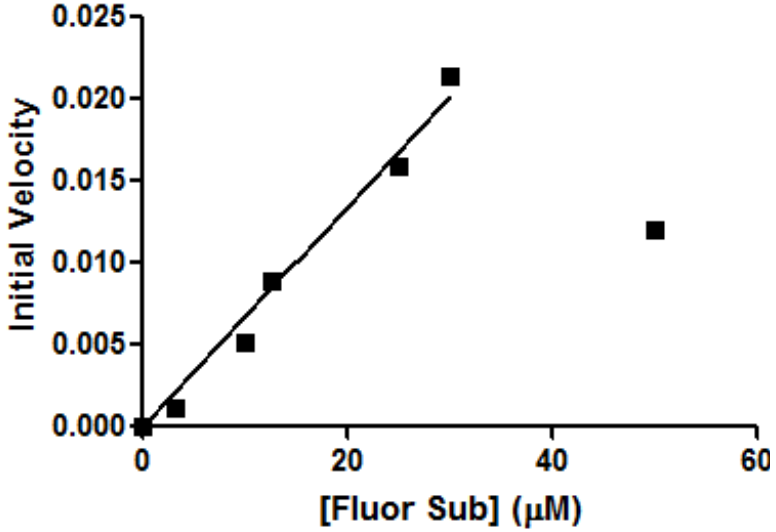
Hits Observed with the Unlabeled Peptide Only

- Some compounds appeared as hits in the unlabeled assay but not in either of the labeled assays
- Likely due to the altered binding of the enzyme and the two substrates
- Further underscores the need for careful substrate selection – biologically-relevant hits could be missed

Unlabeled Substrate by MS (UMS)



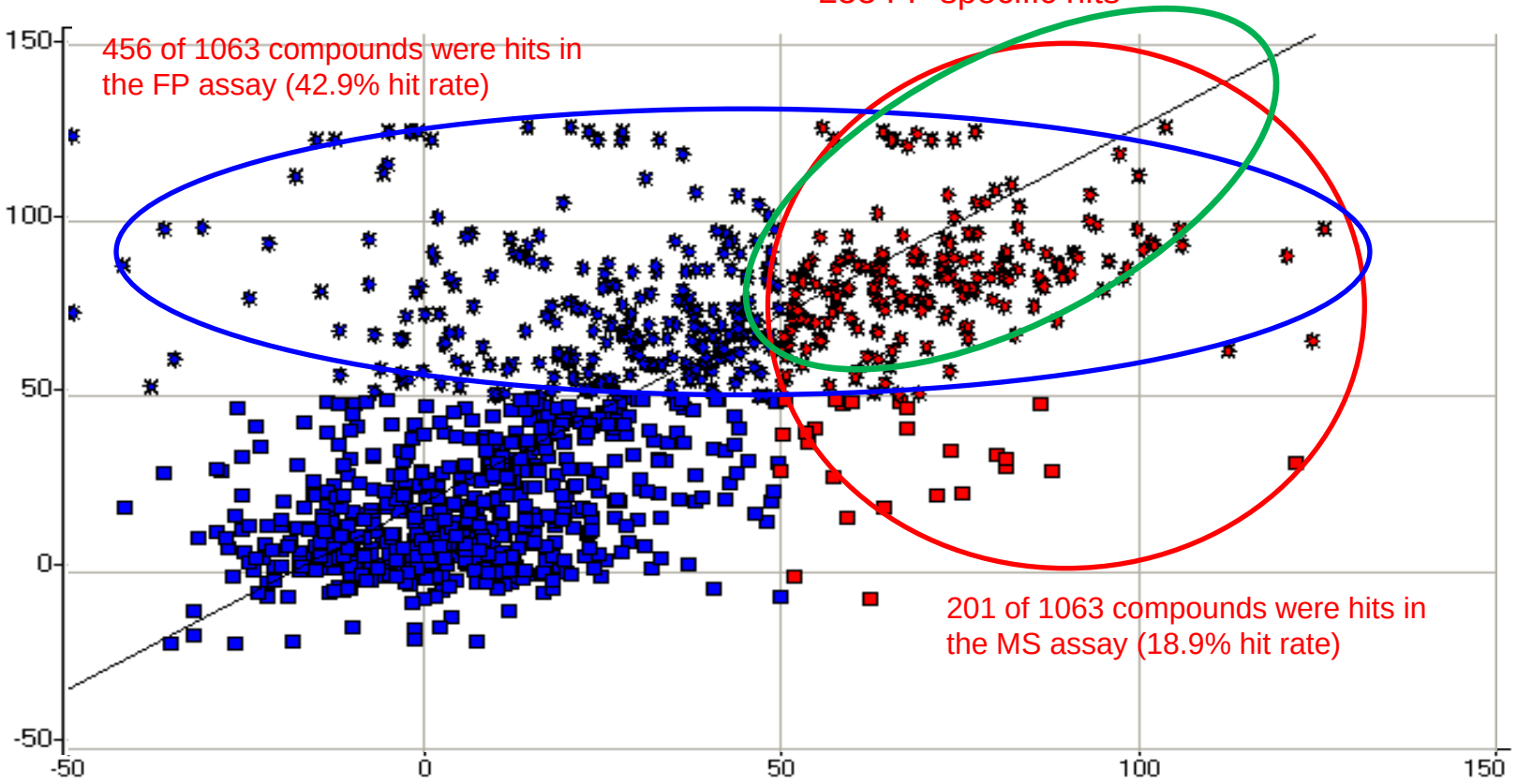
Fluorescently Labeled Substrate by MS (LMS)



Fragment Based Drug Discovery at Customer Sites

GlaxoSmithKline

- 175 of the hits overlapped both assays.
- 26 MS specific hits
- 255 FP specific hits



Presented at MipTec 2011

Fragment Based Drug Discovery at Customer Sites

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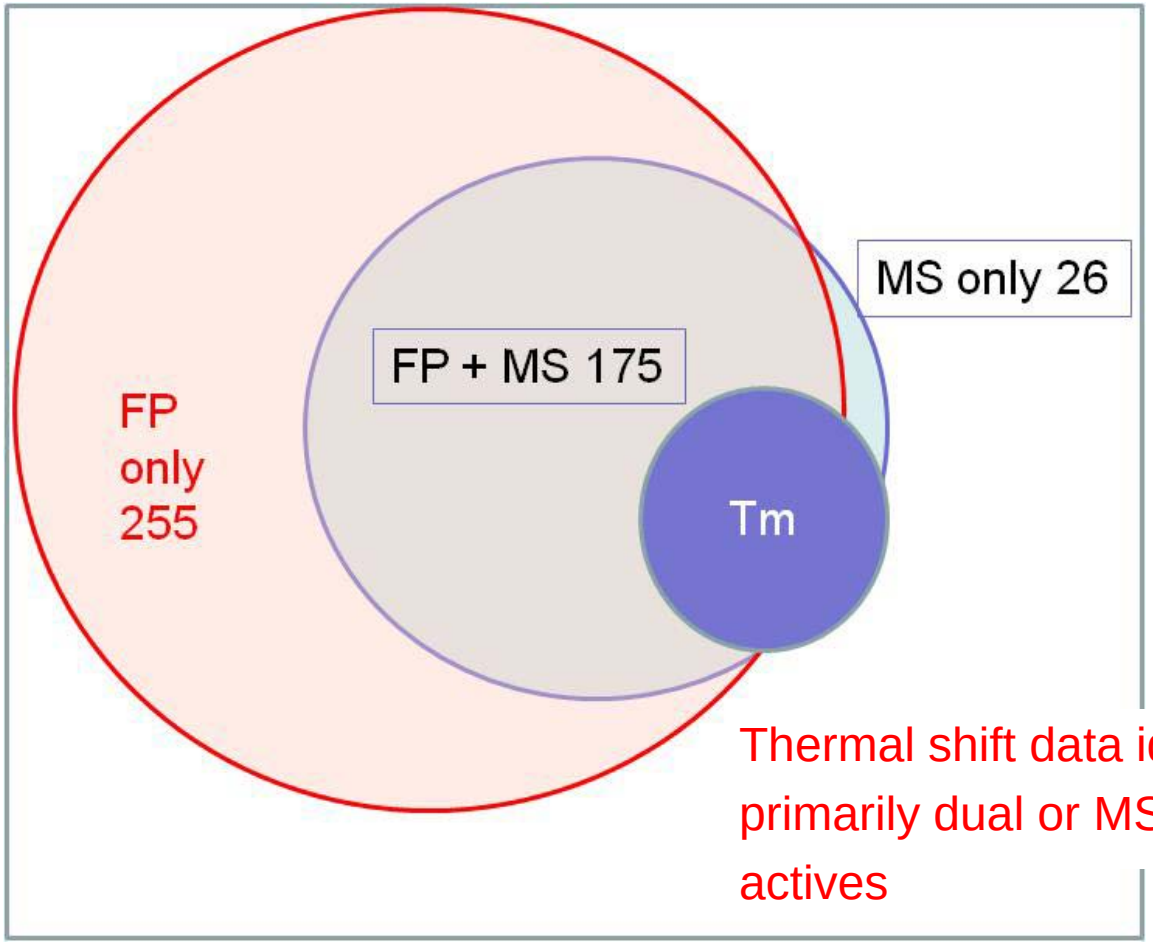


Thermal shift data identified primarily dual or MS-specific actives

Presented at MipTec 2011

Conclusions

- Robust BACE-1 assays were developed
  - for both a labeled and an unlabeled substrate
  - with detection by MS and FS

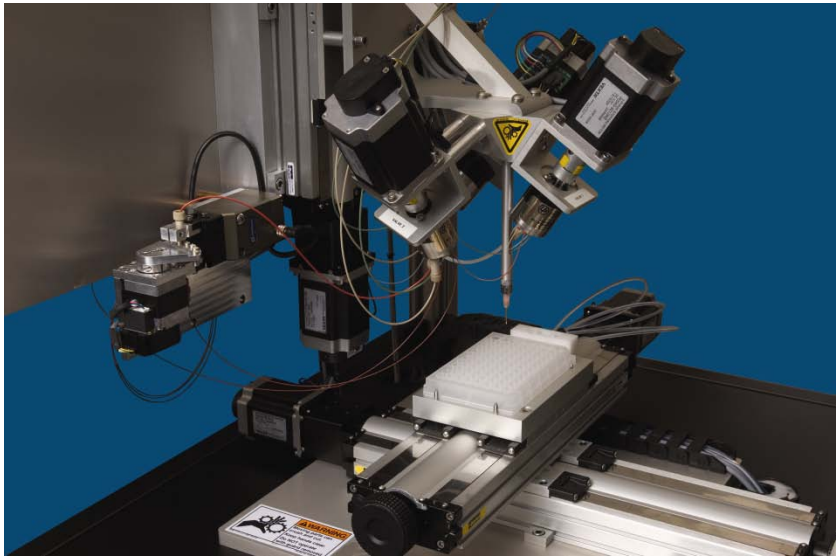
- A fragment library was screened by all 3 assays
  - hit rates varied widely
  - three disparate hit sets were found

- FS and MS produced different hit sets when analyzing the same samples
  - some MS hits (including the most potent) were obscured by autofluorescence
  - AF did not account for all of the differences between the methods

Conclusions

- MS generated different hit sets for the labeled and the unlabeled peptide
  - demonstrates the importance of substrate selection
  - RapidFire/MS allows study of the most native substrate

- RapidFire High-throughput MS is a valid tool for FBDD
  - shows functional inhibition
  - label-free - allows study of more native substrates
  - less susceptible to confounding factors such as AF



Acknowledgements

Thank you to Melanie Leveridge at

Questions?

More about **RapidFire** technology:

agilent.com/lifesciences/rapidfire

Learn about **Agilent Discovery Services**, contract research using RapidFire technology.

agilent.com/lifesciences/discoveryservices

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