



Self-Templated Synthesis of Enzyme Inhibitors on a Nanoscale

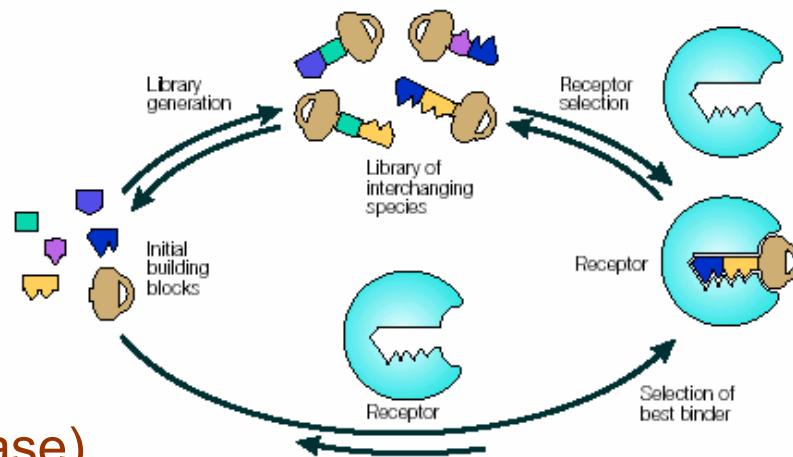
**Paul Schnier, Steve Hitchcock, Klaus
Michelsen, Yuan Cheng, Jim Brown,
Wenyun Qian**

Background

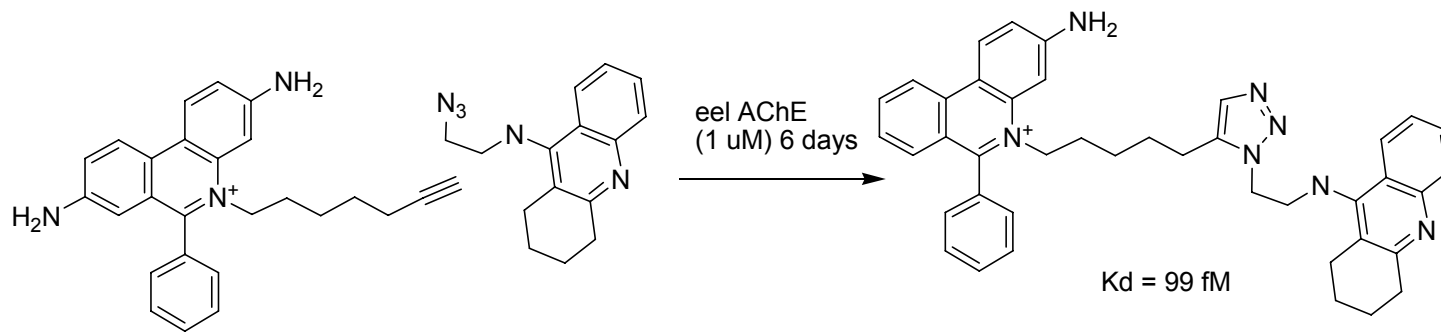
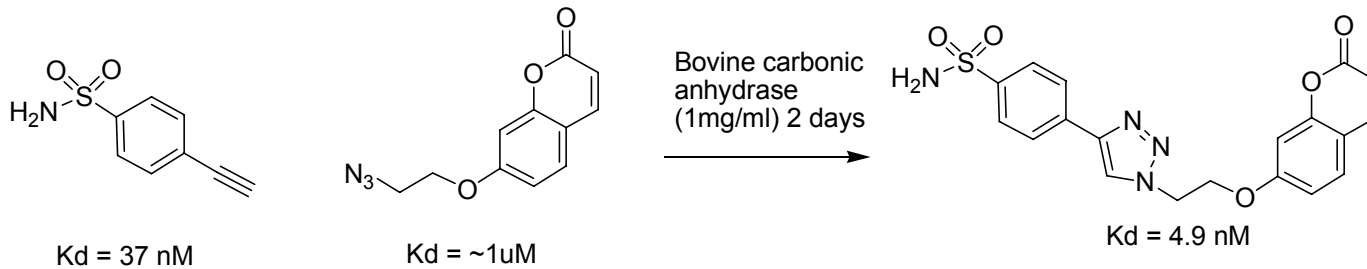
Target-guided synthesis (TGS) 1980's

Dynamic combinatorial libraries 1990's

"Click Chemistry" in situ 2002



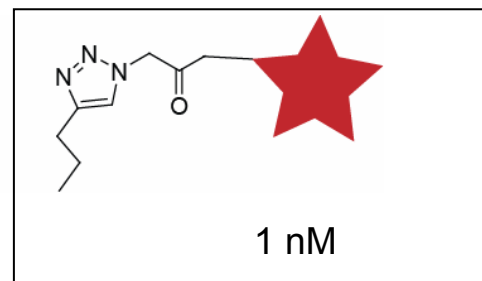
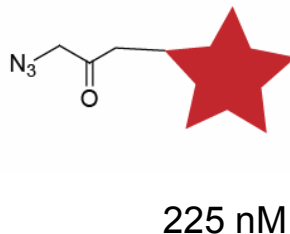
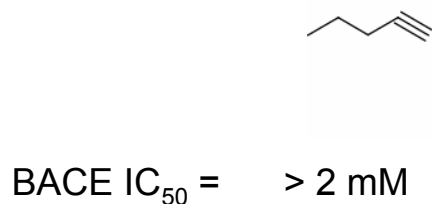
Sharpless - (AChE and Carbonic anhydrase)



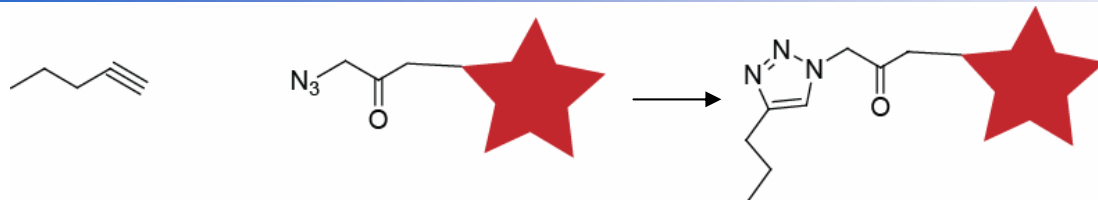
Drawbacks of Existing Methods

- Requires μg of protein per reaction
- High μM reaction conc. required (substrate solubility issues)
- Long reaction times (often days)
- Requires medium-sized fragments each with measurable affinity

Test case substrate “in the flask”

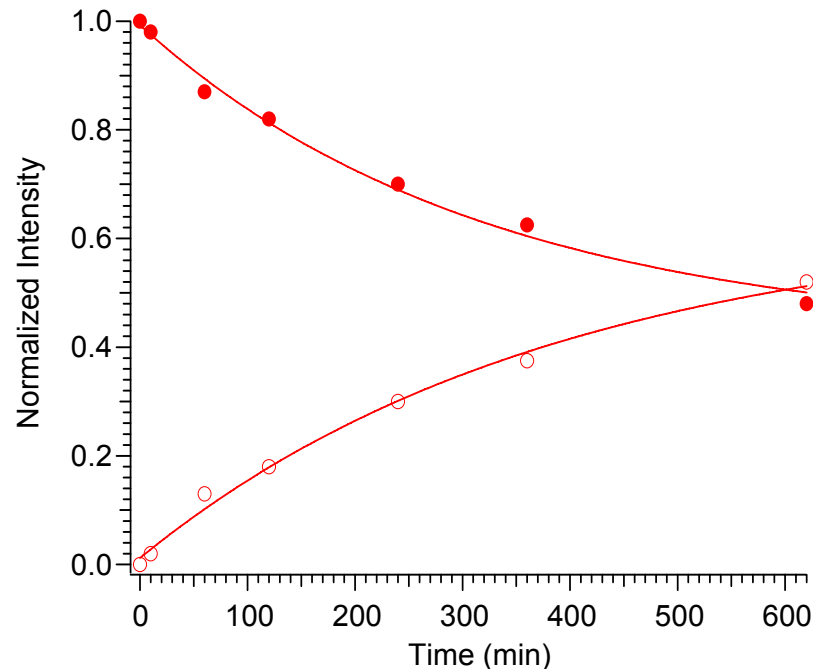
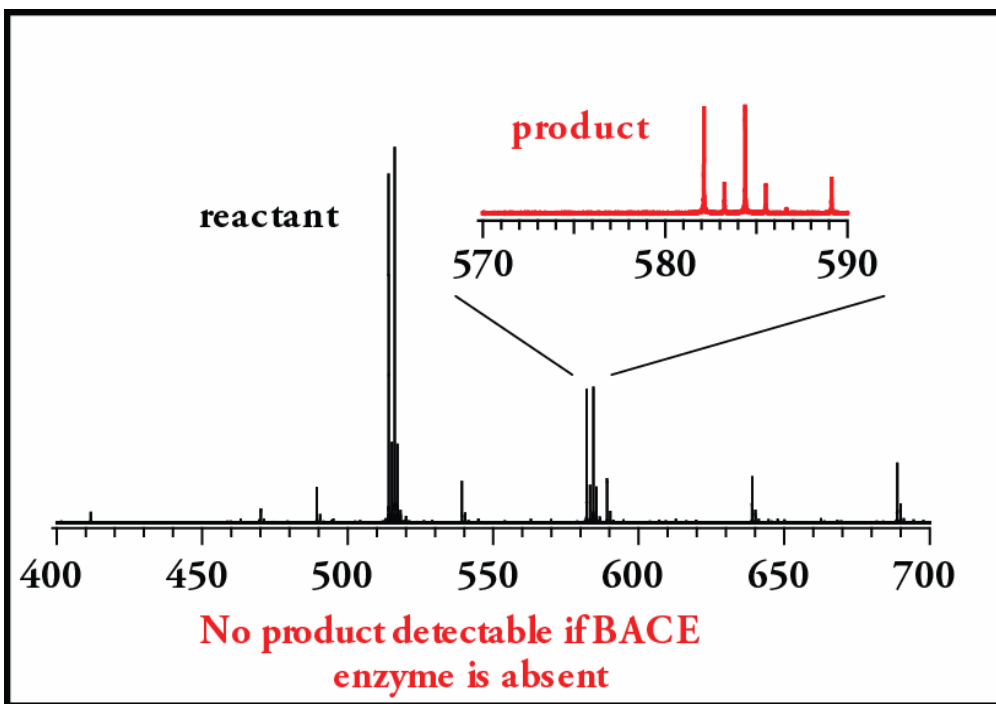


In situ click reaction catalyzed by BACE



tremendous rate acceleration
in presence of enzyme

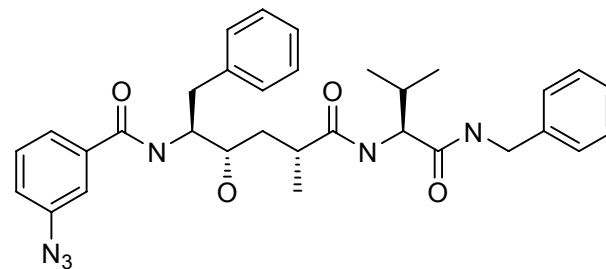
Test case "In the enzyme"



predisposition of reactants in substrate recognition pockets- eliminates entropic component of activation barrier and catalyzes reaction

Library screening in a droplet

- Reaction run in well of chip (nl volume)
- Direct readout by Fourier-Transform Mass Spectrometry
- 400 Reactions per chip
- Sub ng quantities of enzyme per reaction
- Only Azide reactant has affinity for BACE



225 nM

Experiment: 77 Construct: ligand binding domain
Date: 2/7/2003 5:42:58 PM Protein Concentration: 1
Protein: JNK3 Compound Concentration: 1

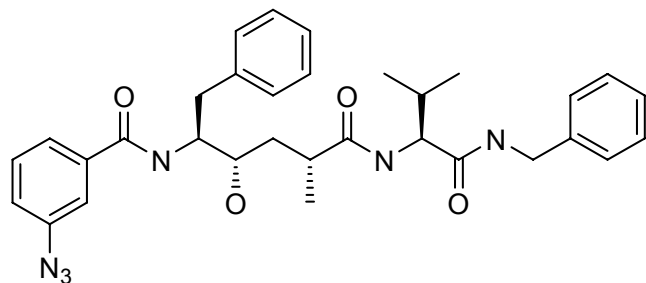
111402-JNK.rpt

Sample	Well	Amgen Name	Exact Mass	Hit	Score	Adducts	Plate Name	RPT File
1	A1	109094#1	467.889	✓	(null)		111402-JNK.r	111402-JNK.rpt
2	B1	109111#1	291.072	✓	(null)		111402-JNK.r	111402-JNK.rpt
3	C1	109075#1	358.997	✓	(null)		111402-JNK.r	111402-JNK.rpt
4	D1	102364#1	211.08	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
5	E1	102369#1	221.156	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
6	F1	68080#2	241.076	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
7	G1	67191#1	236.029	✓	(null)		111402-JNK.r	111402-JNK.rpt
8	H1	68080#1	241.076	✓	(null)	39 23	111402-JNK.r	111402-JNK.rpt
9	A2	69567#1	208.05	✓	(null)		111402-JNK.r	111402-JNK.rpt
10	B2	69548#1	657.964	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
11	C2	658420#1	396.206	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
12	D2	108926#1	236.082	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
13	E2	70809#1	231.048	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
14	F2	86793#1	368.036	✓	(null)		111402-JNK.r	111402-JNK.rpt
15	G2	71451#1	240.114	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
16	H2	90854#2	252.975	✓	(null)		111402-JNK.r	111402-JNK.rpt
17	A3	71616#2	248.086	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
18	B3	108953#1	604.052	✓	(null)		111402-JNK.r	111402-JNK.rpt
19	C3	108954#1	691.864	✓	(null)		111402-JNK.r	111402-JNK.rpt
20	D3	108955#1	374.056	✓	(null)	23	111402-JNK.r	111402-JNK.rpt
21	E3	108956#1	318.14	✓	(null)		111402-JNK.r	111402-JNK.rpt
22	F3	108971#1	311.041	✓	(null)		111402-JNK.r	111402-JNK.rpt
23	G3	108972#1	352.104	✓	(null)	23	111402-JNK.r	111402-JNK.rpt

Structure: CC(=O)Nc1ccc2ccccc12

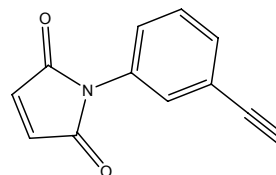
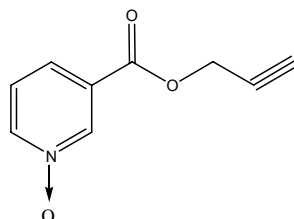
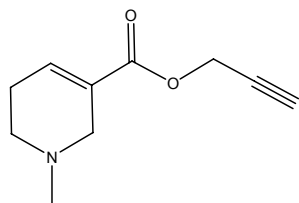
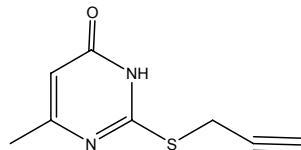
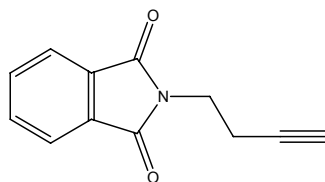
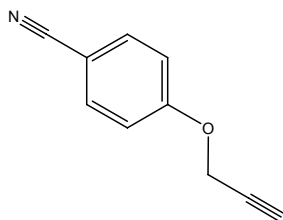
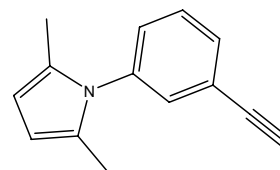
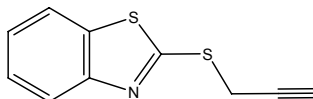
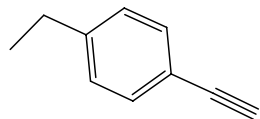
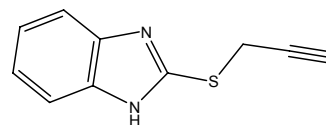
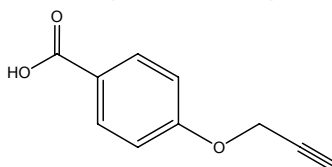
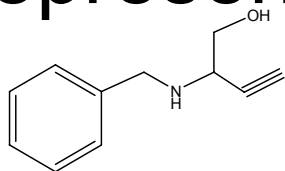
Amgen Name: 68080#1
Exact Mass: 241.073876
Formula: C14H11NO3

Screen of 1200 Alkynes

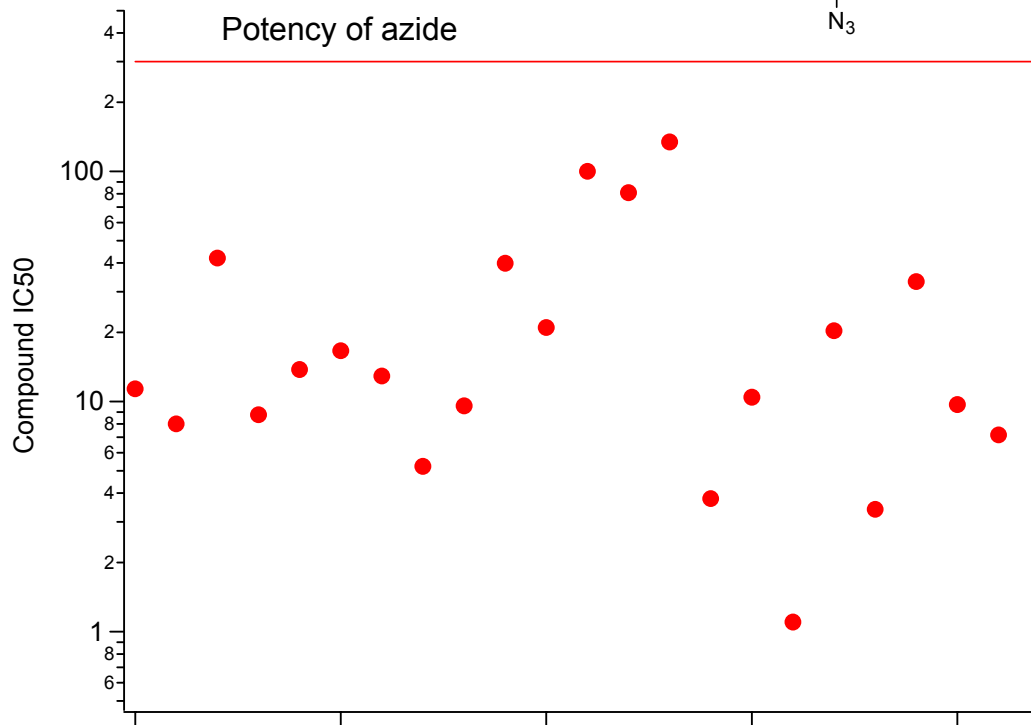
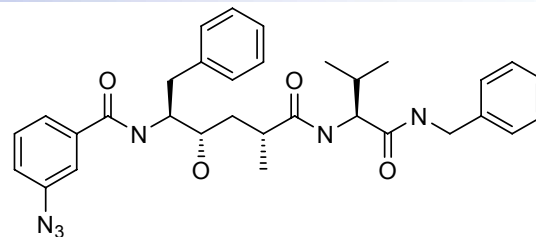


(IC50 ~300 nM)

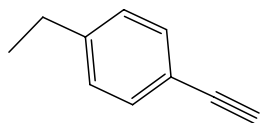
representative hits from alkyne screen:



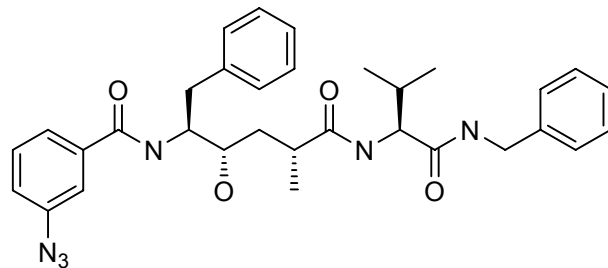
Potency of hits



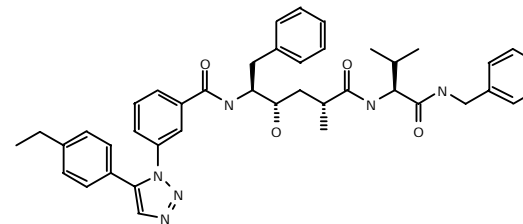
HEA Scaffold



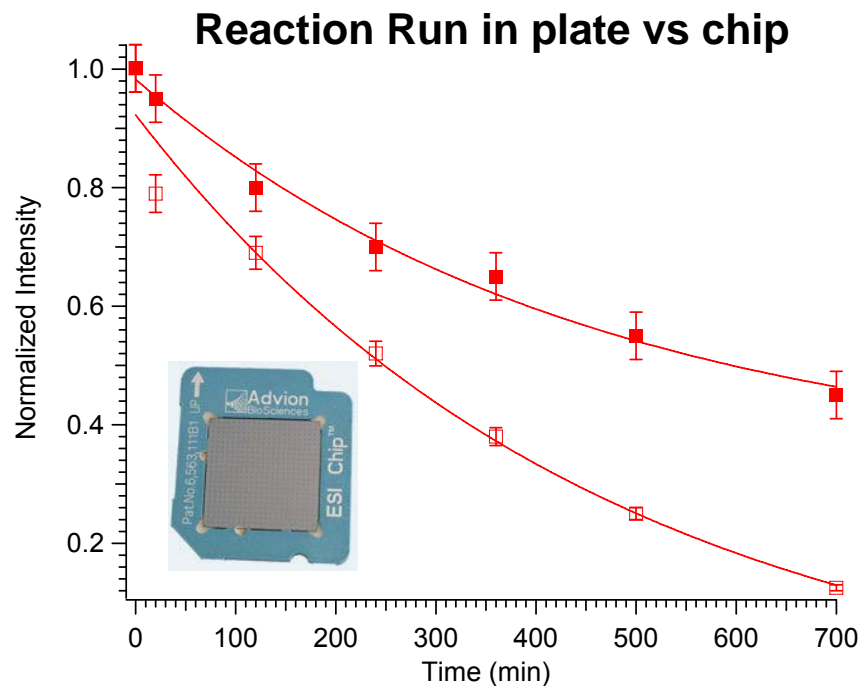
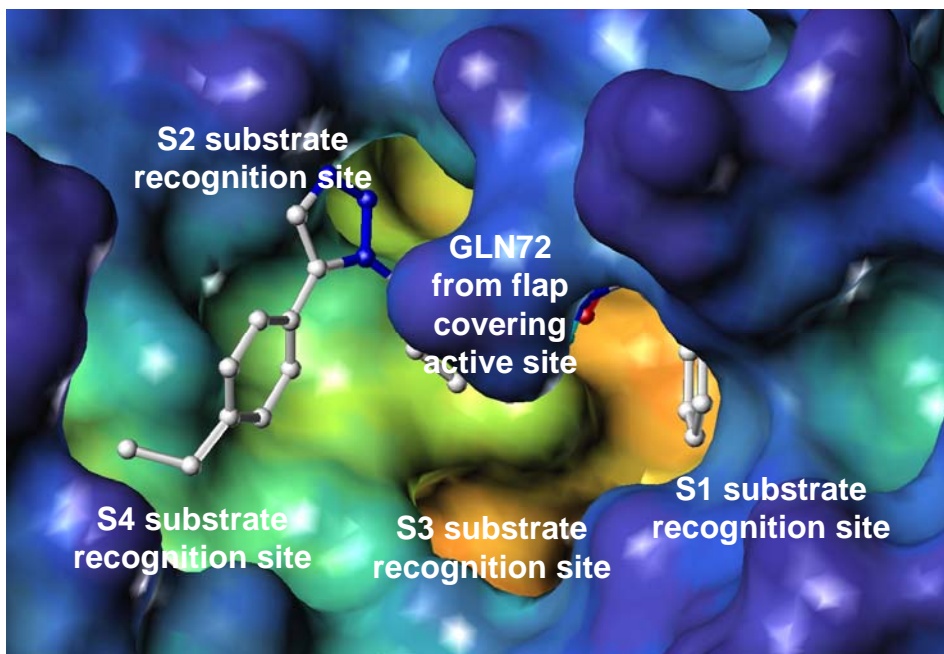
BACE IC₅₀ = > 100 μ M



225 nM

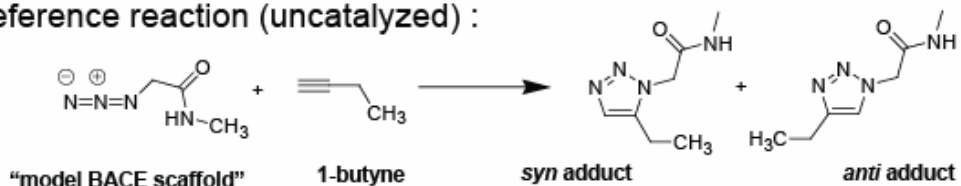


3 nM

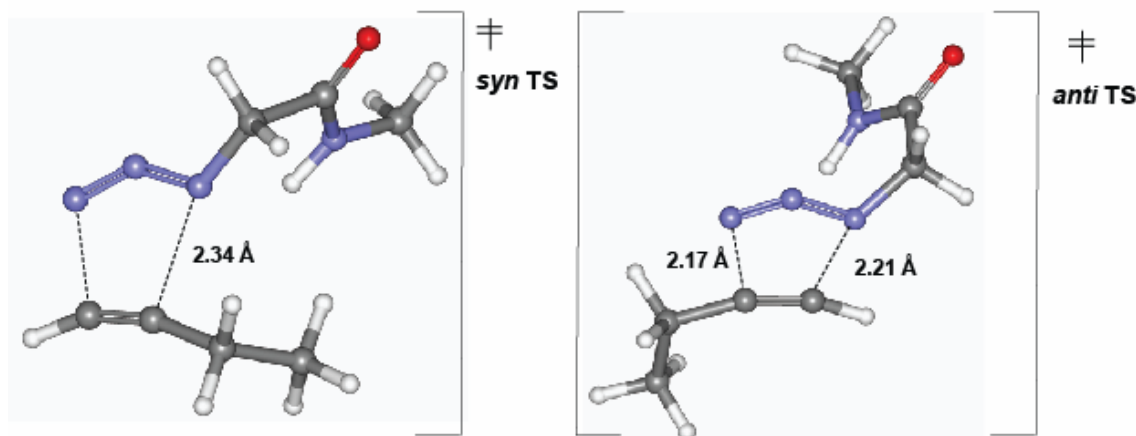


Computational Screening

Reference reaction (uncatalyzed) :



predisposition of reactants in substrate recognition pockets- eliminates entropic component of activation barrier and catalyzes reaction



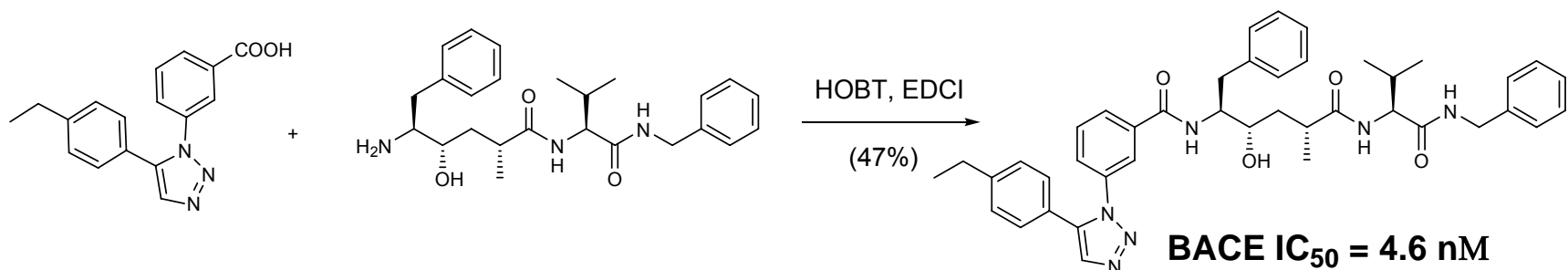
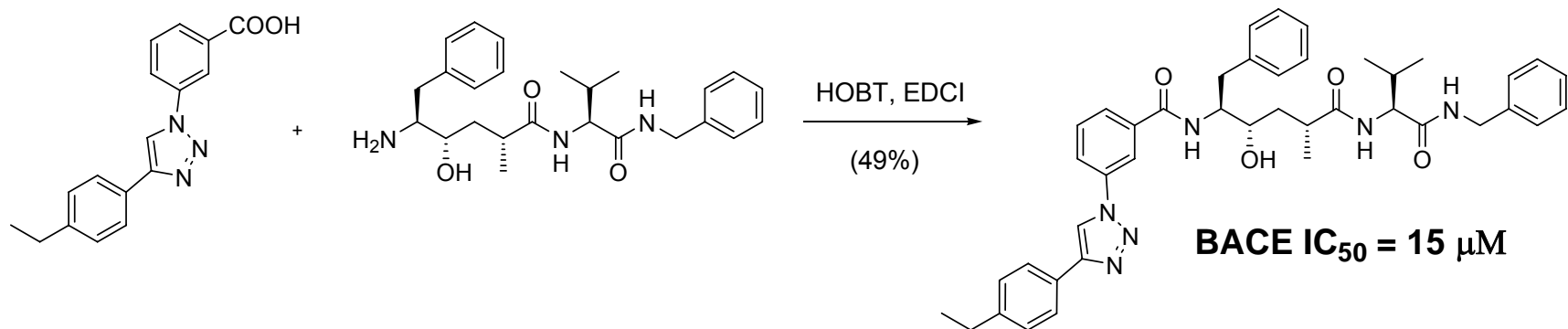
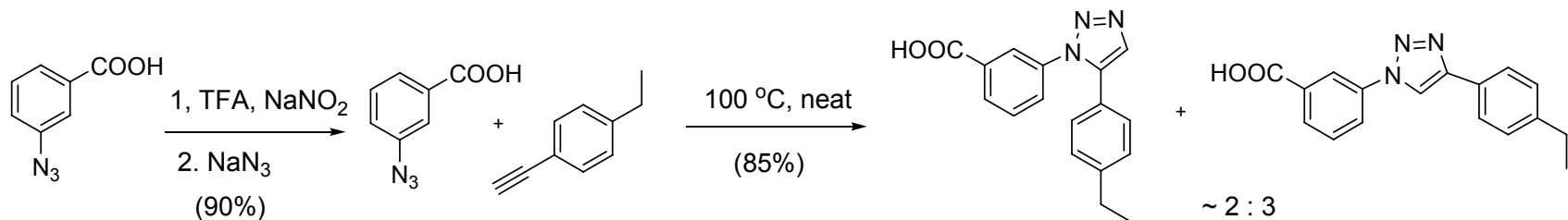
Computed uncatalyzed activation parameters (B3LYP/6-31G*) :

$\Delta E^\ddagger = 15.7$ kcal/mol (water, $\epsilon = 80$)
 $\Delta G^\ddagger = 28.7$ kcal/mol (water, $\epsilon = 80$)

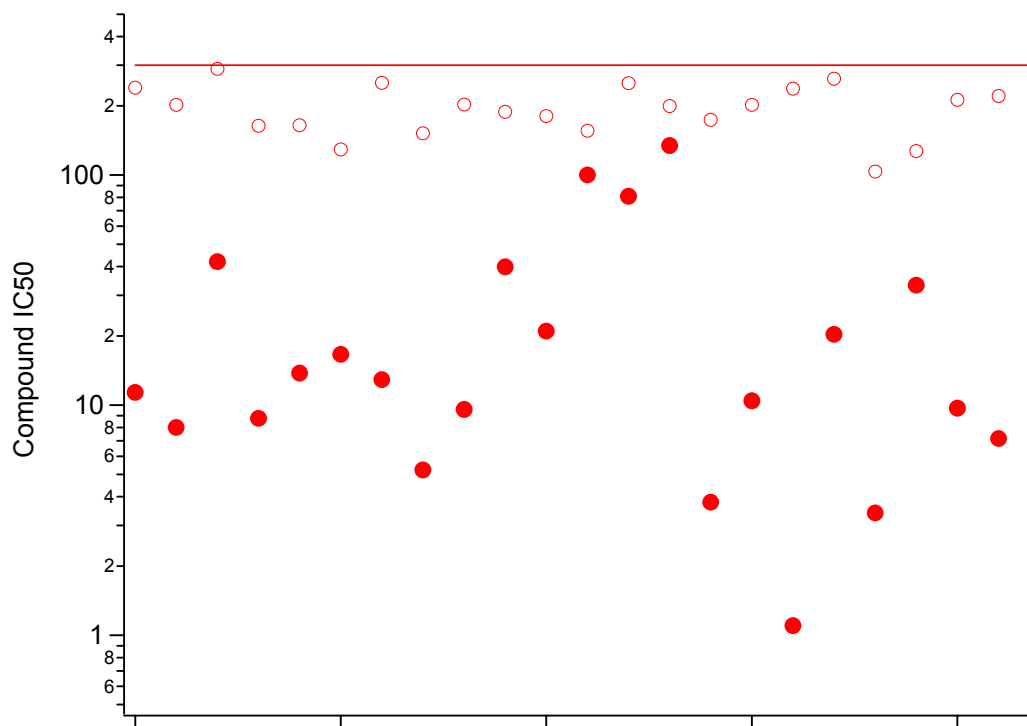
$\Delta E^\ddagger = 16.2$ kcal/mol (water, $\epsilon = 80$)
 $\Delta G^\ddagger = 29.2$ kcal/mol (water, $\epsilon = 80$)

In progress: Currently profiling other, potentially "templatable" reactions (e.g., [4+2], 1,3-dipolar cycloadditions, etc.)- goal is to locate reactions with uncatalyzed activation profile similar to azide click (catalyzable, but negligible background rate)

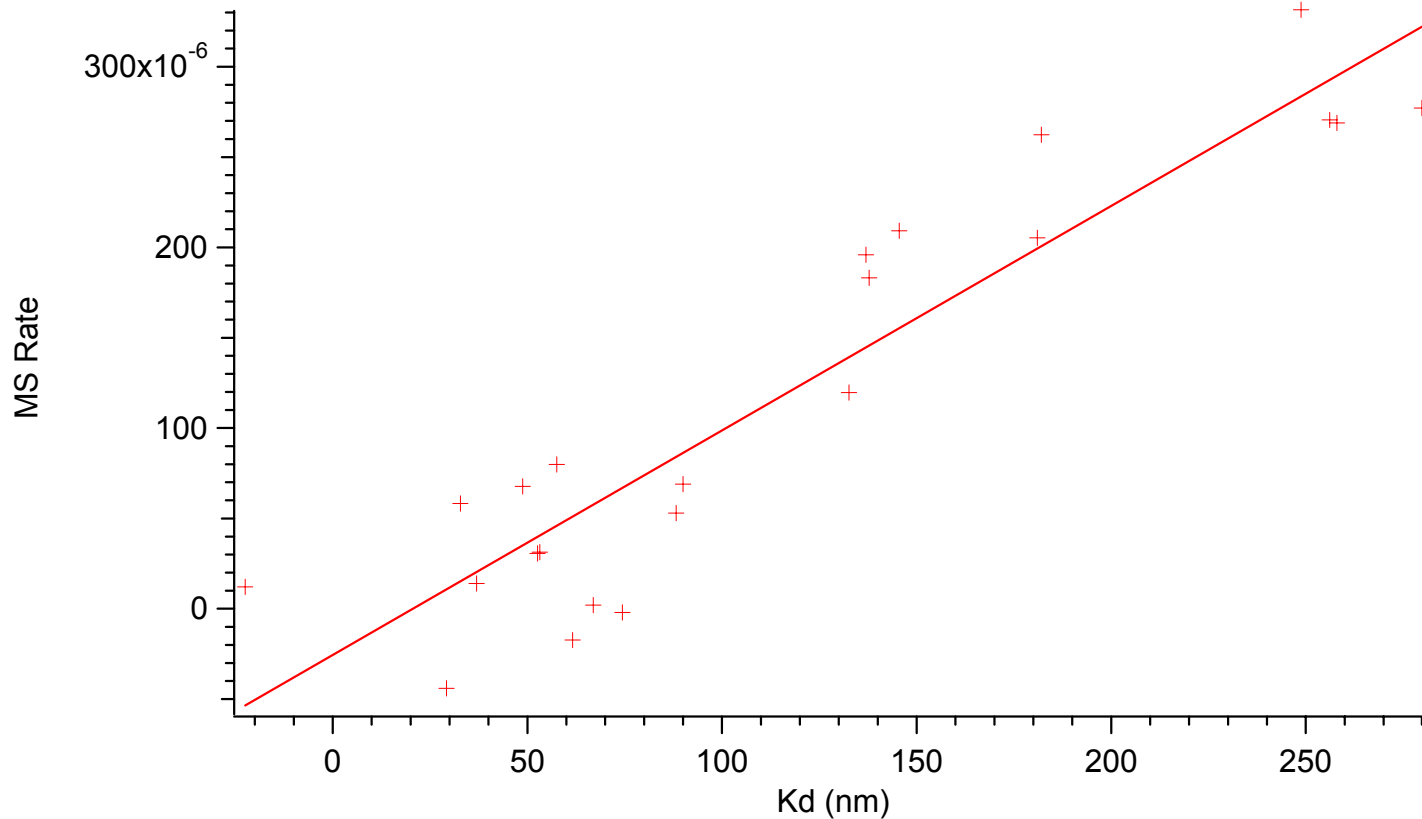
Resynthesis and Confirmation of Hits



Synthesis with random alkynes (control)

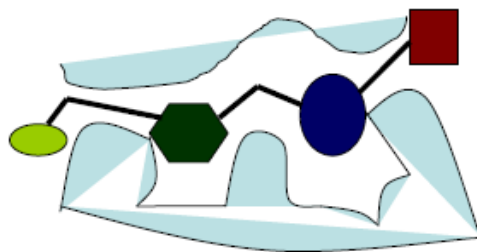


Correlation of Reaction rate and Kd



Exploration of Fragment Approach

Traditional screen hit
MW 350-500
Low LE



Ligand Efficiency (LE):

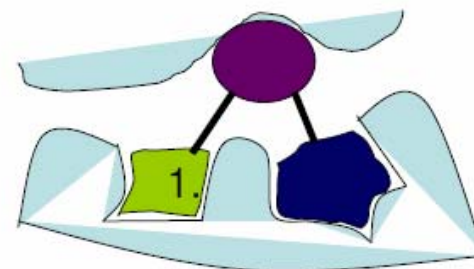
$$LE = \Delta G / \# \text{ non H atoms}$$

$$= -\log(\text{IC}_{50}(\text{M})) / \# \text{ non H atoms}$$

Fragment hit
MW 150-250
High LE



Stepwise
expansion



In situ inhibitor generation (Click chemistry)

Intelligent parallel synthesis + SBDD (beyond Click)

Objective/Challenges :

Find fragments with high LE (>0.3) by SPR

Convert weak affinity leads into sub- μM range

Fragment Libraries

ACRF library

MW < 300
PSA < 30
HBD ≤ 1
acids ≤ 1
bases ≤ 1
rot. bonds ≤ 10
availability > 30 uL of 10 mM stock

(A) 3000 compounds

Maximally-diverse

(B) 600 compounds

Maximally-diverse, highly soluble and not in (A)

322 available (MW 242)

1100 compounds Heterocyclic library

(1) 300 compounds

ADAPT availability

0-1 HBD

no bad/reactive

sorted by PSA

top 300 chosen

244 available (MW 252)

(2) 50 compounds

ADAPT availability

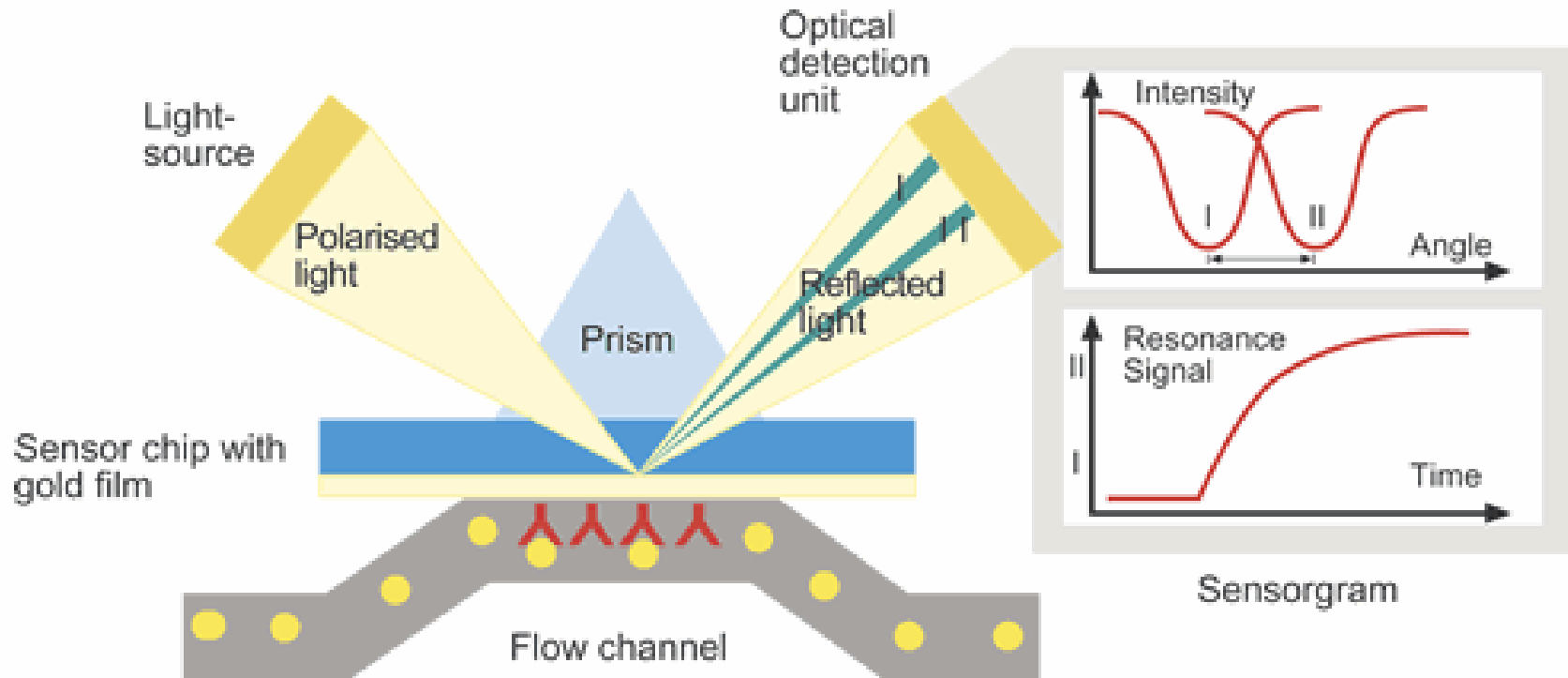
2 HBD

sorted by PSA,

top 50 chosen

Biacore Technology Overview

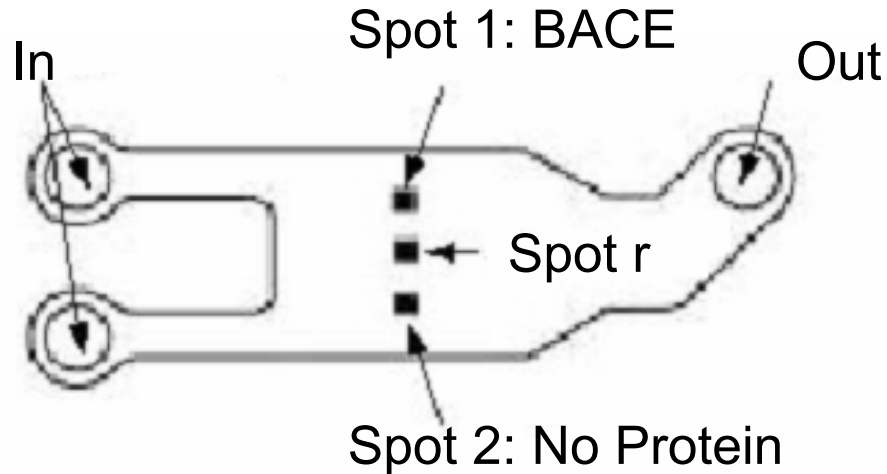
Detection principle: Surface Plasmon Resonance (SPR)



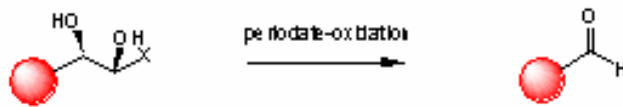
Biacore.com

SPR is sensitive to the refractive index near the gold surface

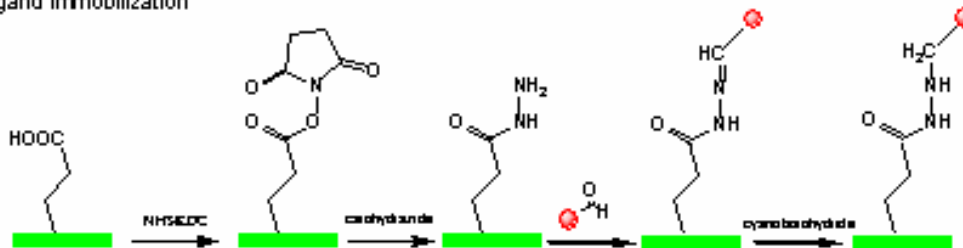
Biacore Assay for BACE-1



Ligand oxidation



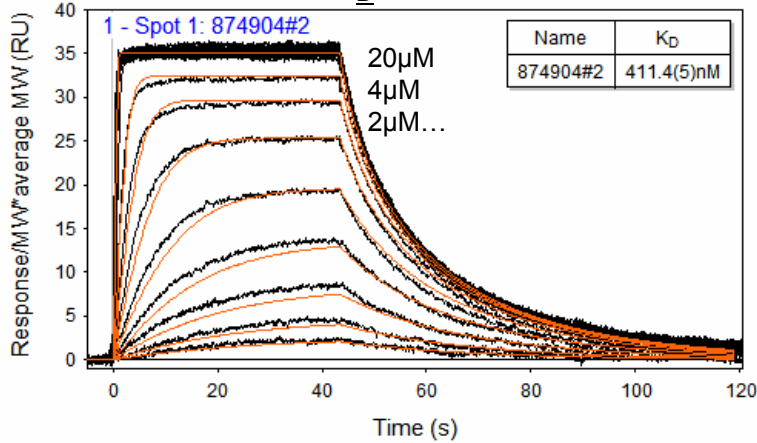
Ligand immobilization



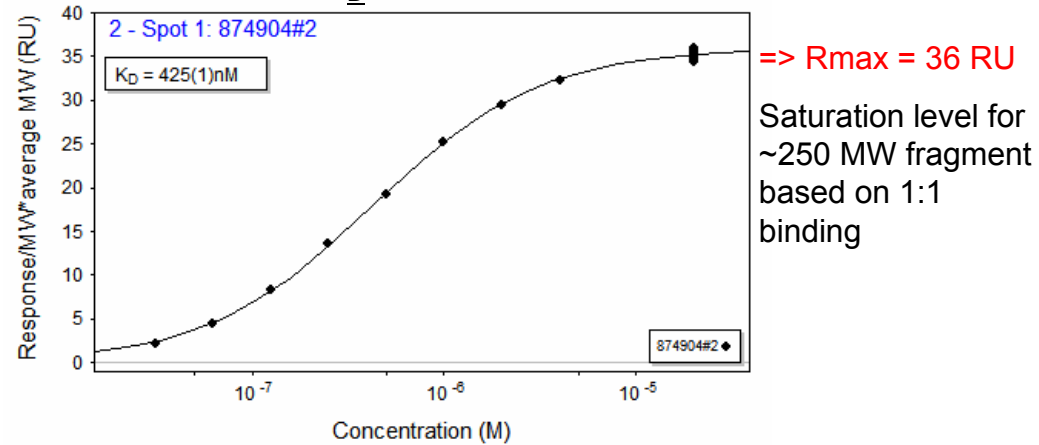
Low protein requirements
(~3.5 μ g / surface)

Assay Performance

Determination of K_D by kinetic analysis

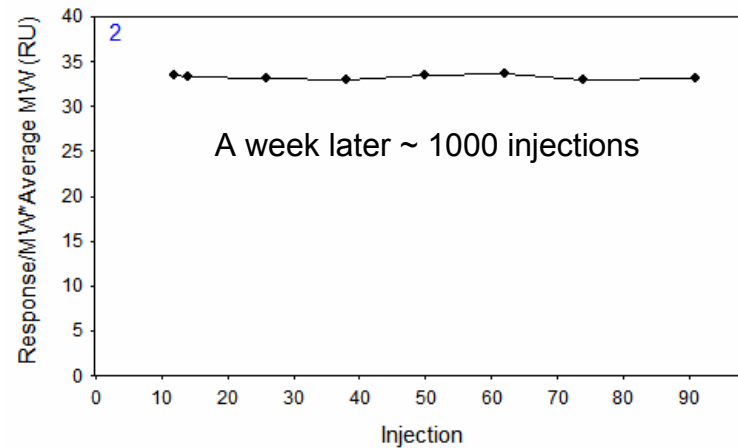
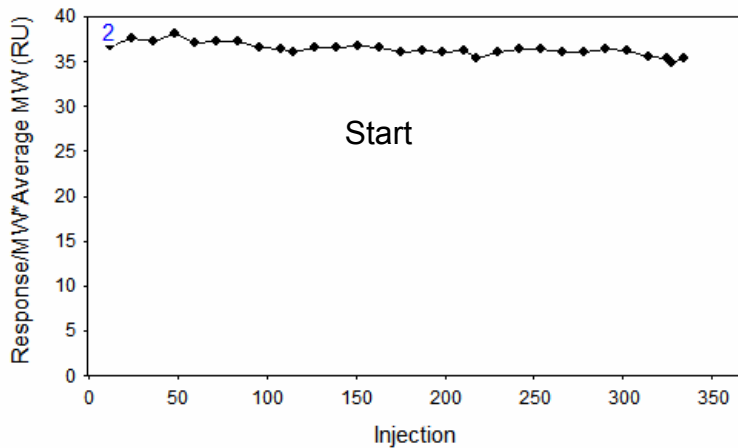


Determination of K_D by steady state analysis

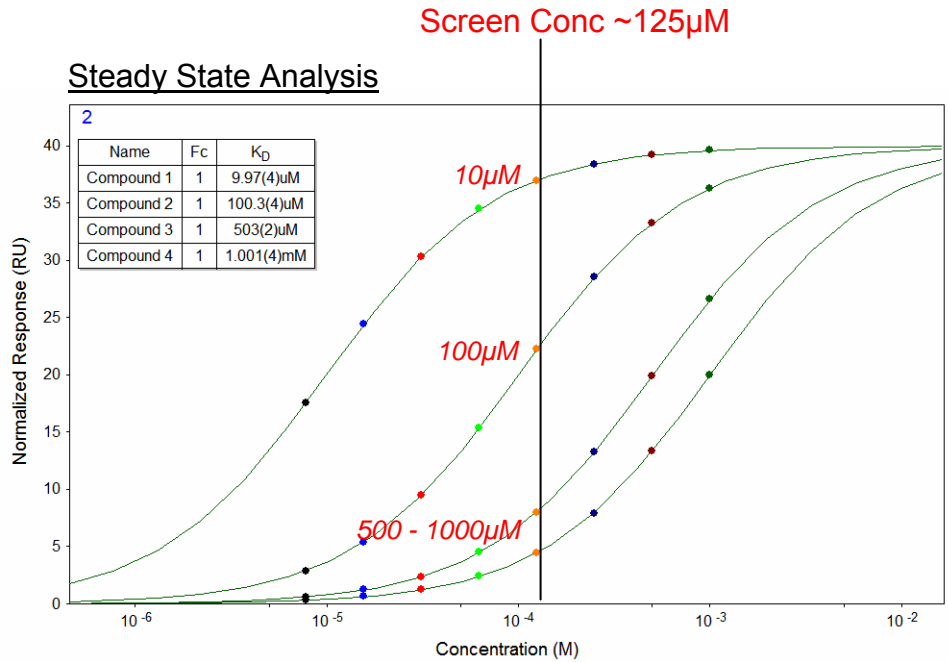
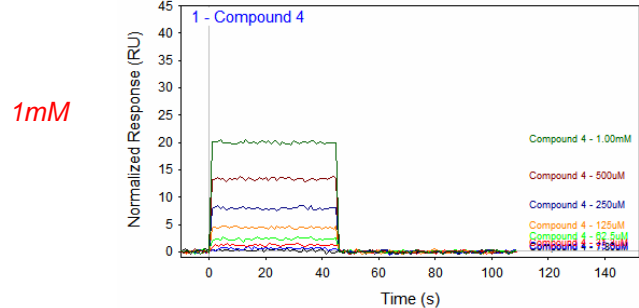
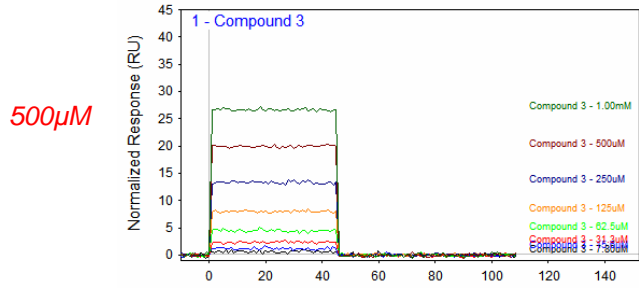
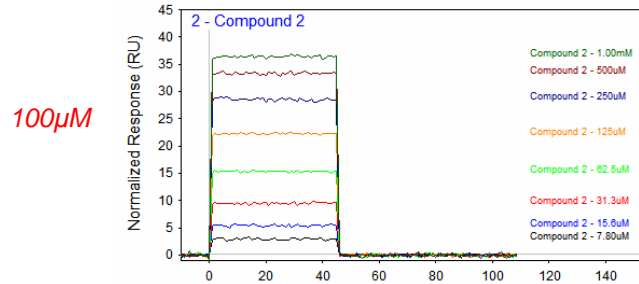
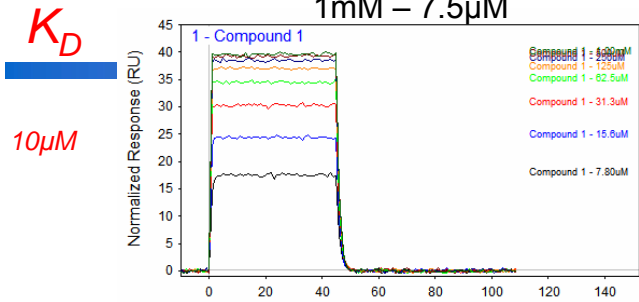


$$R_{max} = \text{MW (compound)} / \text{MW (BACE-1)} * \text{Immob Level}$$

Surface performance (20µM 874904#2)

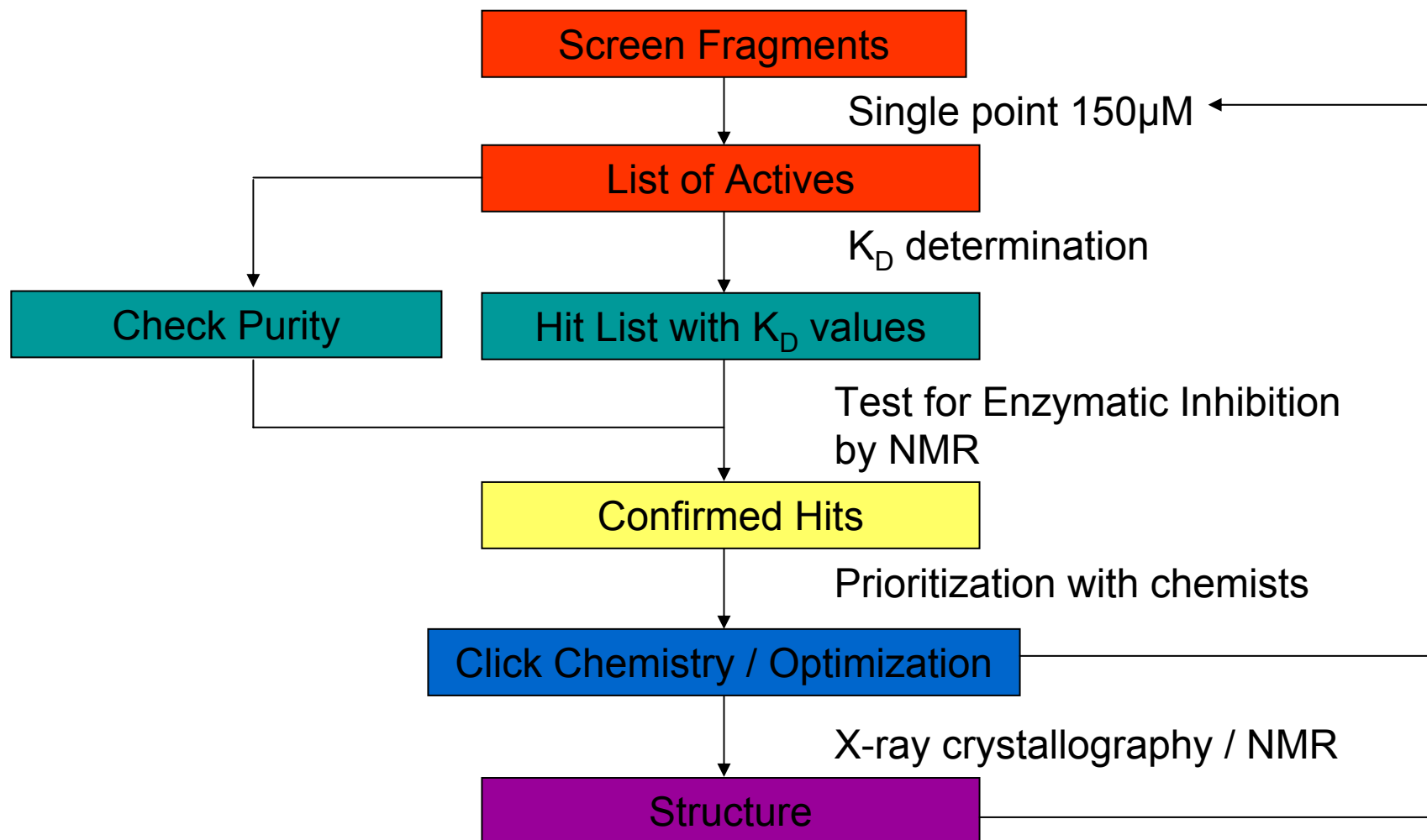


What concentration to screen at ?

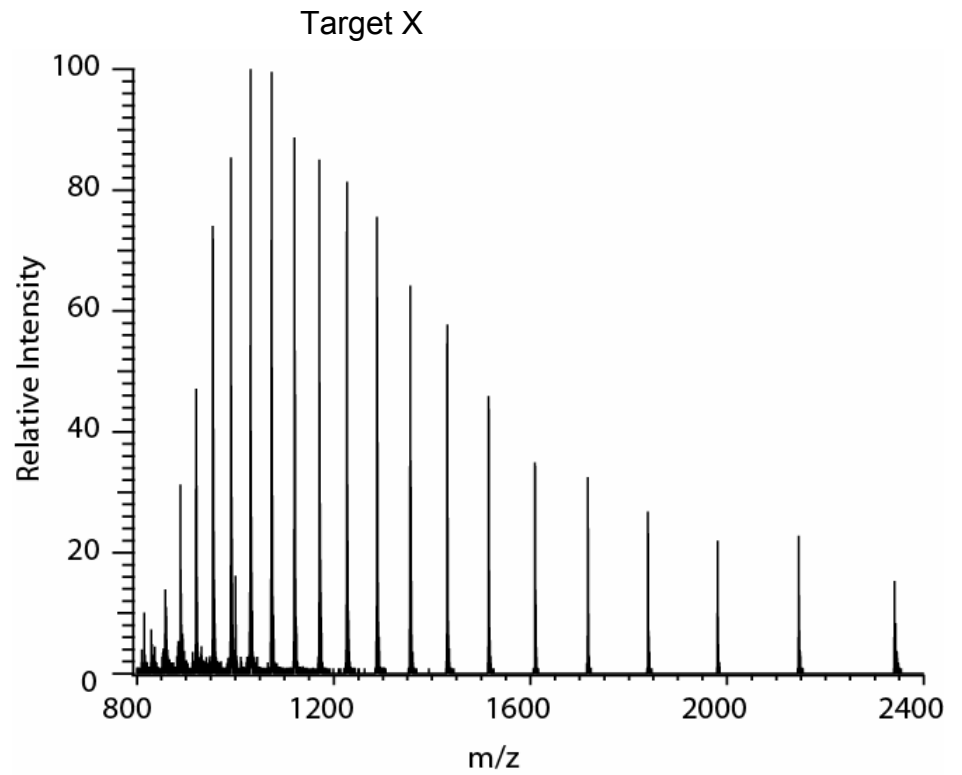
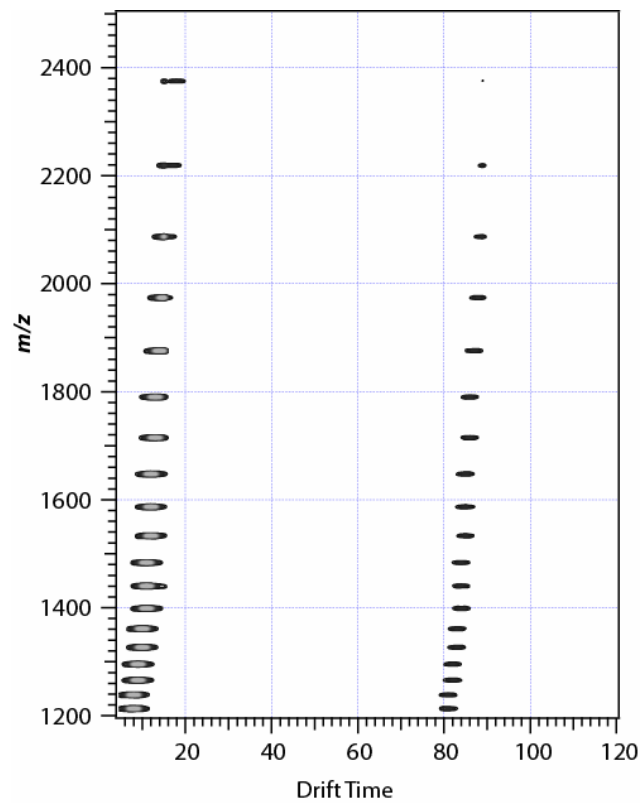


**20 compounds with binding > 10 RU
(Kd's 80 μ M – 500 μ M)**

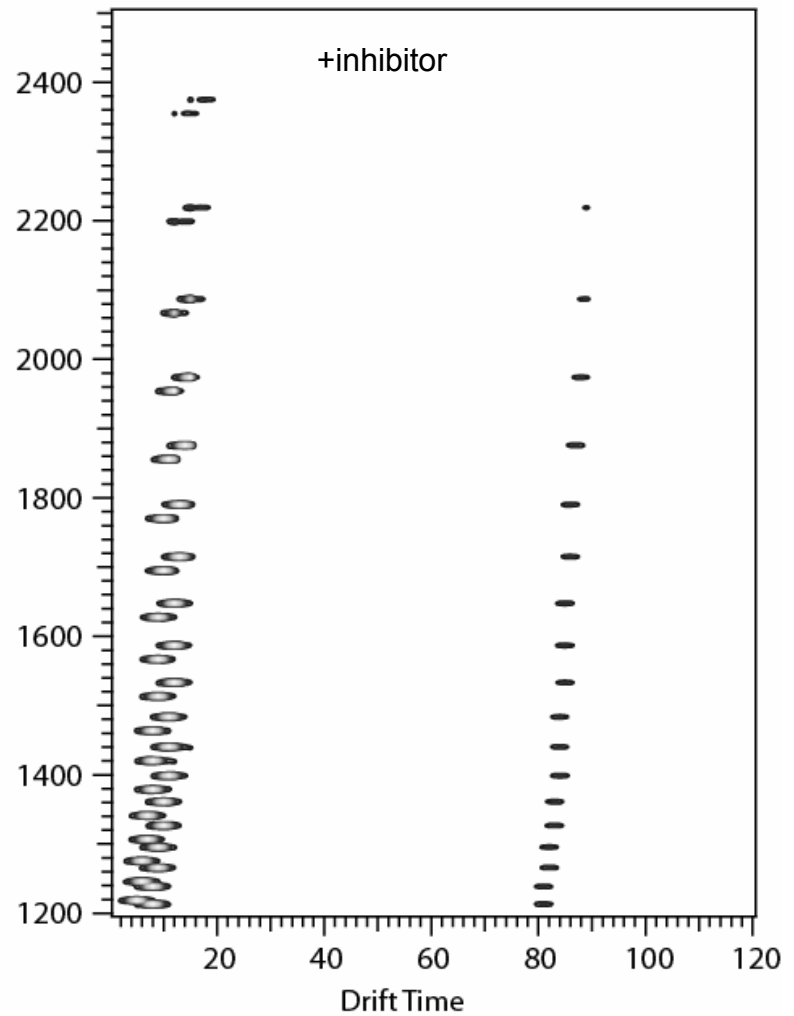
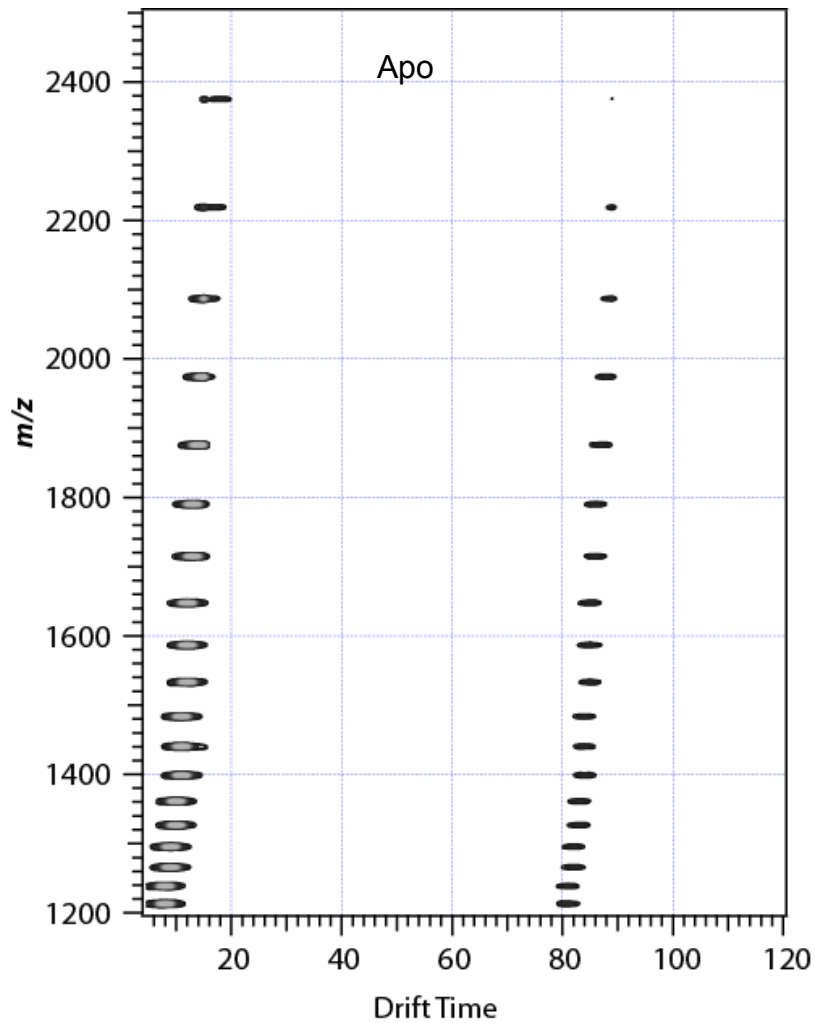
Fragment Screen Flow scheme



Protein Conformation



Protein Conformation



Conclusions & Next Steps

Potential to accelerate early lead optimization via rapid screening of large collections of readily accessible coupling partners on sub ng scale

- Synthesis and affinity from the same experiment
- No purification step necessary
- Accounts for dynamic nature of target protein

Future Scope

Assembly of acetylene and azide fragment libraries in progress

6 BACE substrates (deep P2 and P3 binders) completed

Test case screen against 2 highly homologous proteins - look for unique (selective) hits

Investigate other chemical reactions beyond Click

Investigate membrane bound proteins

Acknowledgements

- Dhanashri Bagal
- Steve Hitchcock
- Wenyuan Qian
- Jim Brown
- Yuan Cheng
- Wenge Zhong
- Michael Bartberger