Shortening the Time to a Successful Fragment HTL Campaign

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Fragment Based Lead Discovery

Expanding the Drug Discovery Process

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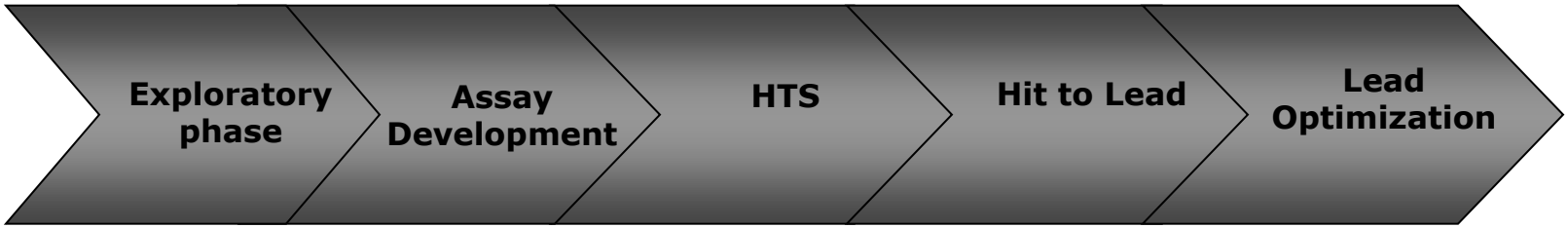
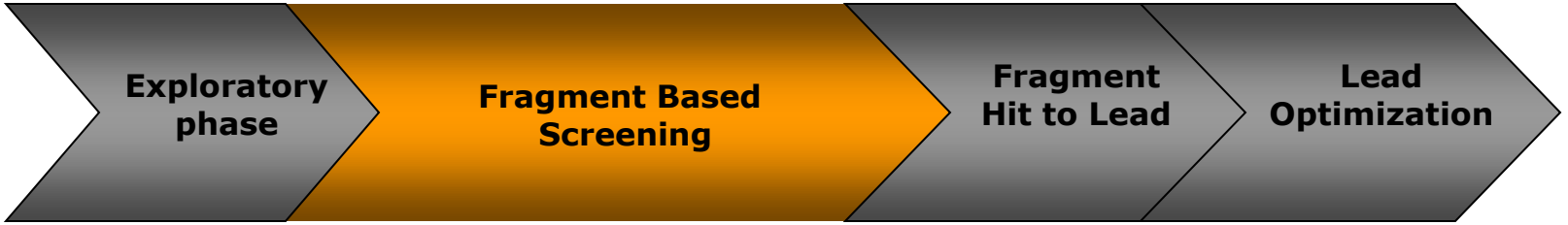


Traditional Lead Identification



Fragment Based Lead Discovery

- Both LI approaches should be run in parallel
- Both can be executed within the same period of time
- Each has its own set of resource requirements and process details

Fragment Based Screening
Comparison to uHTS

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Typical uHTS campaigns screen ~ 10^6 drug-like compounds against a target
Hits are defined as having IC_{50} values < 10μM

Typical fragment-based campaigns screen 10^3-10^4 low-MW compounds against a target
Hits are defined as having IC_{50} values in the 20 – 1000μM range

Composition of the Fragment Screening Libraries

Generic FBS Library

A collection of ~ 2,000 highly characterized, non-proprietary compounds satisfying

$$MW \leq 300;\ 0 \leq ClogP \leq 3;\ HD,HA \leq 3;\ N_{rot} \leq 4;\ N_{fused\_rings} \leq 3;\ N_{chiral} \leq 2$$

Fragment HTS Library

A collection of ~ 26,000 fragment-like compounds from the main compound collection
Limited characterization; many are proprietary; HT assays are required

Fragment Based Screening

How to Detect Initial Fragment Hits?

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Problem

Detection of weakly binding compounds

"Solution"

Highly sensitive biophysical screening methods

- Ligand-detected NMR Spectroscopy *(direct binding or displacement assay)*
- Surface Plasmon Resonance *(direct binding assay)*
- X-ray Crystallography *(direct binding assay)*
- Size-Exclusion Chromatography MS *(displacement assay)*
- Fluorescence Polarization (*displacement assay)*

Characterization of fragment binding by biophysical techniques, e.g., calorimetric methods (binding thermodynamics) and SPR (binding kinetics)

Fragment-target co-structures by High-Throughput X-ray (HTX) analysis (binding mode)

Biological activity of fragment hits by specially designed *in vitro* assays capable of measuring IC_{50} values in the 20 – 1000µM range

Fragment Based Lead Discovery

Typical Workflow

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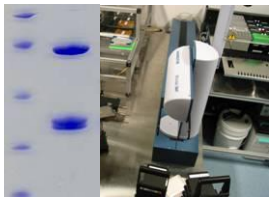
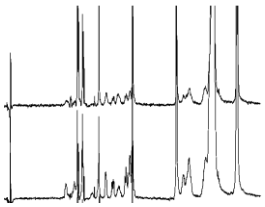
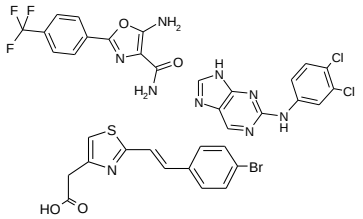
Cloning & Expression



Protein Purification, Crystallization and HTX Enablement

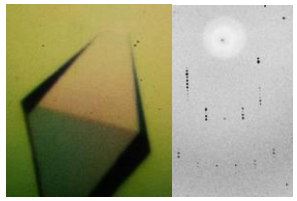


Fragment Screens (enzymatic, FP, NMR, SPR, SEC-MS)

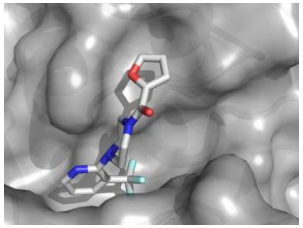


Fragment Hits

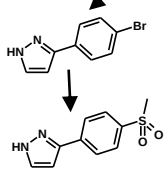
Hit in multiple assays
Chemical novelty
Ligand efficiency
K_d or IC_{50}



HTX Crystallization



HT Co-Structure Determination



Analogs

Synthesis

Fragment Based Lead

Fragment activity / binding assays

FHTL

Fragment Based Screening
Binding Assays

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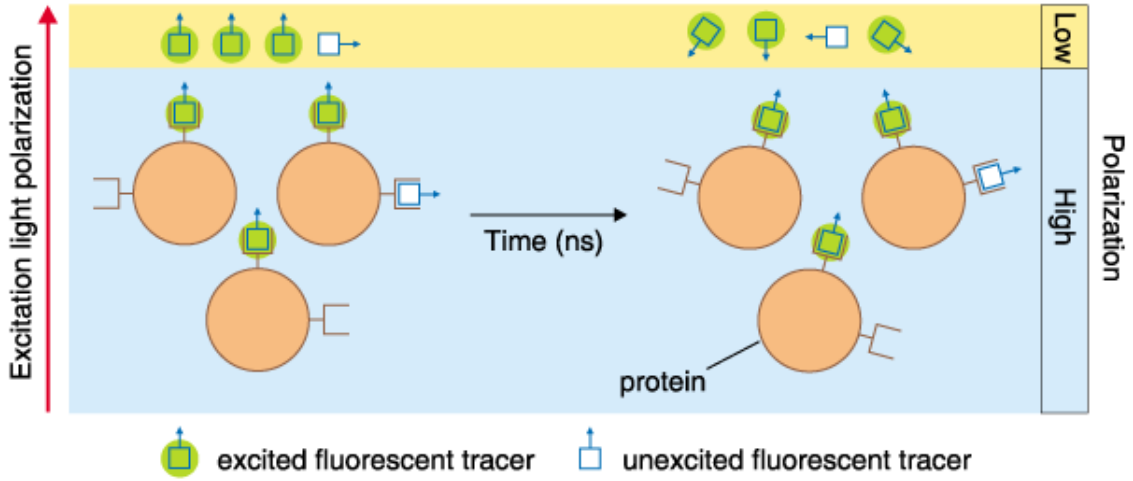
An FBS campaign will always include at least one binding assay.
Three screening modalities are regularly employed at BIPI for binding assays:

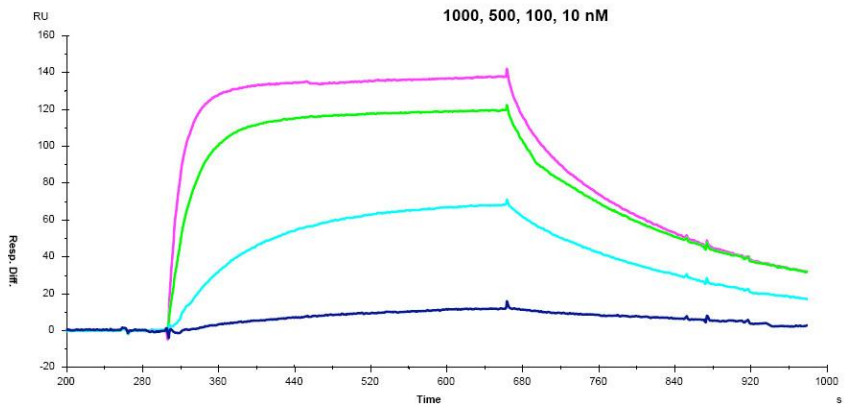
Fluorescent Polarization (FP)

TAMRA or Alexa568 fluoroprobe
Probe K_d = 0.3-3µM
[Probe] ~ 25nM
[Protein] ~ 3-5µM
Assay volume ~ 15µL



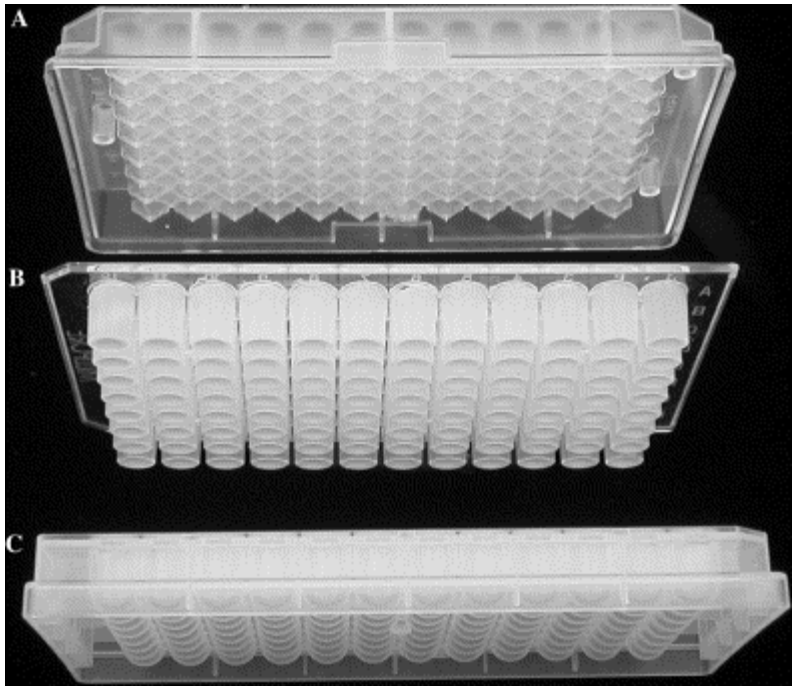
Surface Plasmon Resonance (SPR – Biacore)

Under development
Screen at two fragment concentrations
Initially used 100 and 300µM



Size Exclusion Chromatography MS

- Add target (10µM), fragment (1.7mM) and reference inhibitor (10µM) to each well of pinhole plate (~ 25µL)
- Elute by centrifugation through MultiScreen plate containing SEC media
- Capture eluant in collection plate for LC/MS analysis
- Monitor m/z for reference inhibitor



Fragment Based Lead Discovery
Typical Timelines

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Typical timelines for the steps in the FBLD process are as follows:

Assay Development (protein & FP reagents, validation @ high conc.) 3-6 months

Screening Activities 3 months

- FBS 1° screening (sp-FP, sp-Enzymatic, dd-SPR, mx-NMR, SEC-MS)
- FBS hit confirmation (sx-NMR, re-test hits for other assays)
- FBS hit triage (dr-FP, dr-Enzymatic)

Hit Characterization (HTX, cd-NMR, dr-SPR) 3-6 months

Fragment HTL (Chemistry, HTX, SPR, FP/enzymatic) 9-12 months

- Synthesis (external) or purchase of analogs
- Characterization of analogs
- Synthesis of elaborated fragments
- Characterization of elaborated fragments

Overlapping

Ideally, FBS is run in parallel with conventional uHTS (but this rarely happens!)
Fragment HTL initiated within 2-5 months of completing most Screening Activities

Fragment Based Lead Discovery

Example Goals, Timelines and Final Status for Project #2

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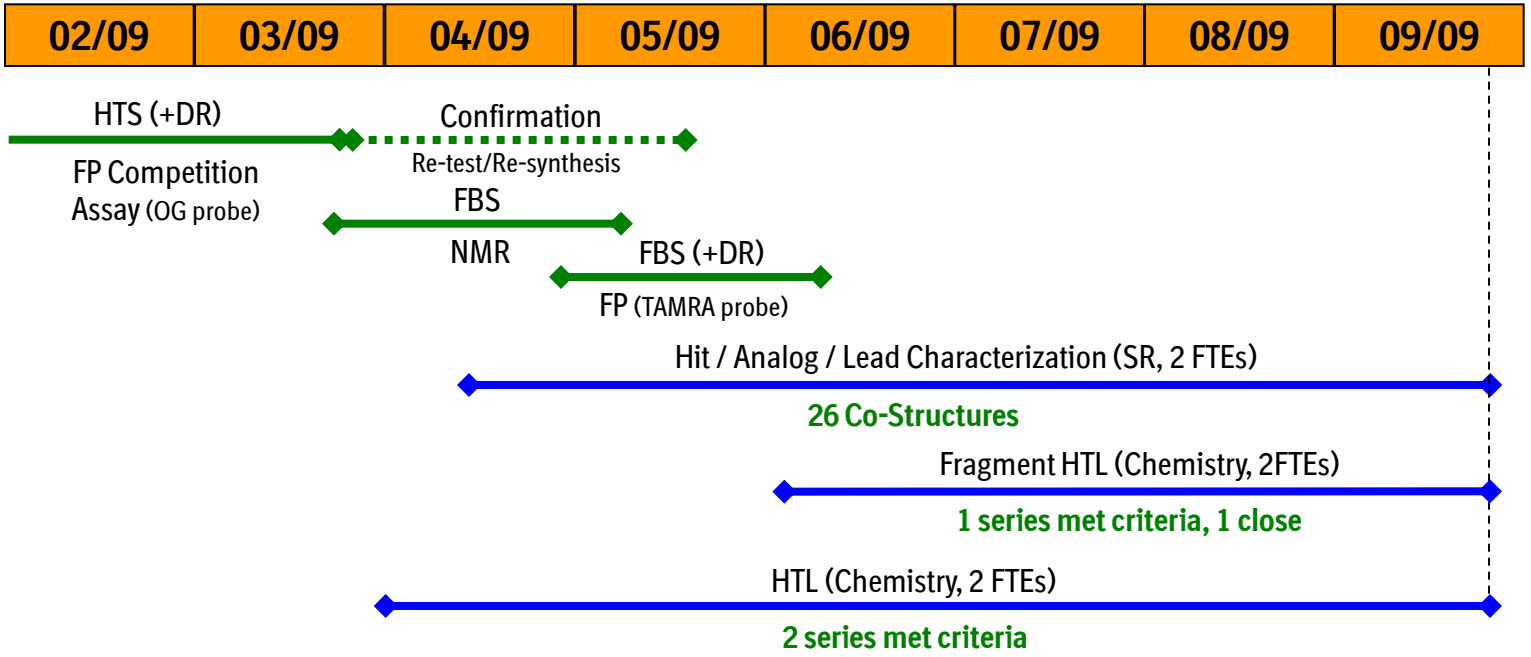
Project Goal: Small-molecule inhibitor for treatment of atherosclerosis

Chemistry Goal: Deliver 2 series that meet the following criteria:

IC_{50} < 500nM, LE > 0.35, $\%Q_h$ < 40, Solubility > 10µg/ml, Cyp IC_{50} > 10µM



- 2 series from HTS met chemistry goals
- 1 series from FBS met chemistry goals, and 1 series was close
- 26 supporting X-ray co-structures (resolution from 2.02 Å to 1.30 Å)

Fragment Based Screening

Fragment Hit Overlap Among Primary Screening Technologies

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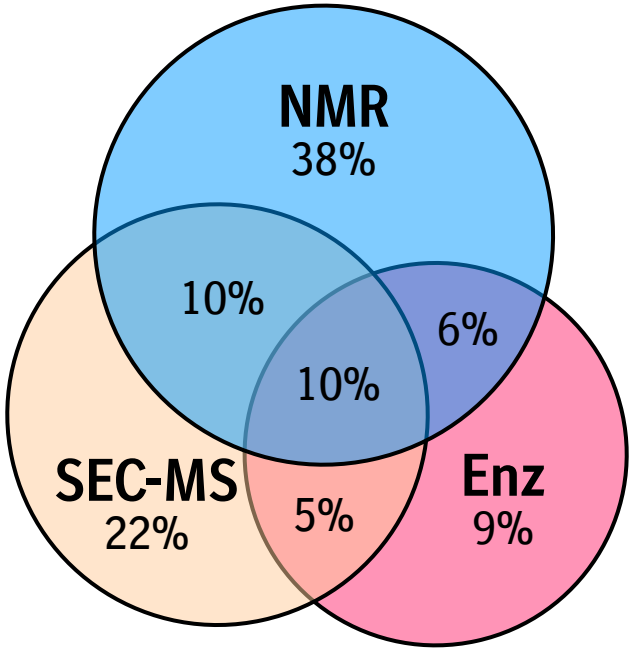
213 Total Unique Fragment Hits



For all fragment hits

Chymase Project

41% of NMR hits test positive by at least one other primary screening technology.

53% of SEC-MS hits test positive by at least one other primary screening technology.

70% of Enz hits test positive by at least one other primary screening technology.

Fragment Based Lead Discovery

Prioritizing Fragment Hits for X-ray Characterization

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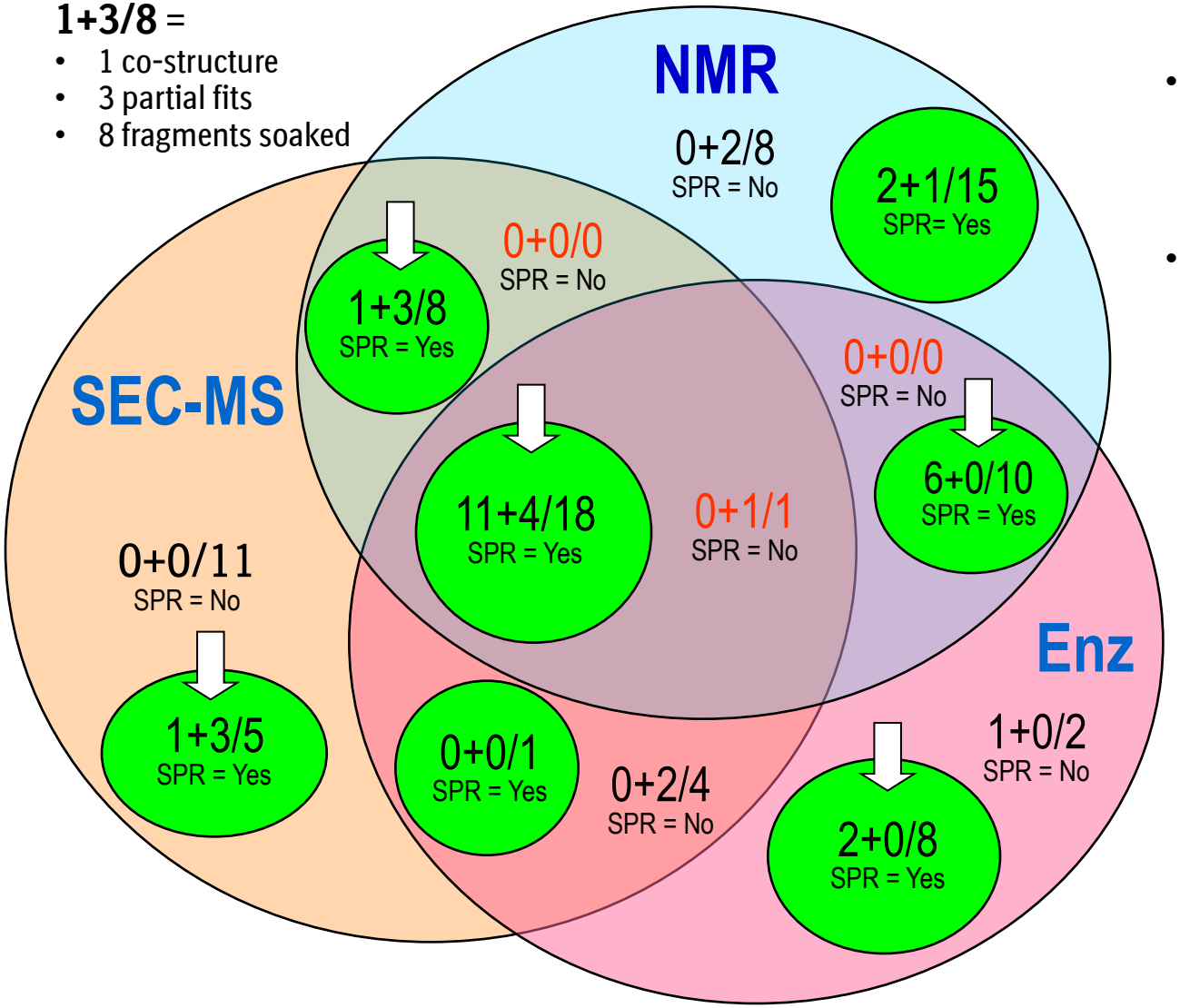
1+3/8 =
- 1 co-structure
- 3 partial fits
- 8 fragments soaked



- Unique SEC-MS hits do yield a significant number of X-ray co-structures and partial fits
- SPR significantly improves the X-ray success rate for unique SEC-MS hits

91 fragments analyzed
Complete data on all
24 co-structures
16 partial fits

Fragment Hit to Lead

Three Possible Approaches

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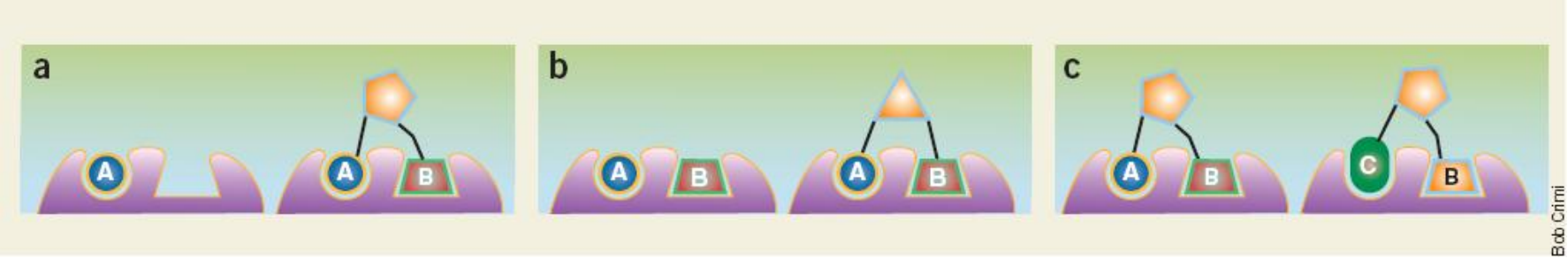
A fragment hit will generally not be sufficiently potent to be considered a “lead”

A fragment hit having high “ligand efficiency” can be evolved chemically using several strategies:



Jhoti Nat. Biotech. 2005 23 184

Grow	Link	Replace
Extend the fragment hit into adjacent pockets to gain potency (*de novo* design, chemical libraries)	Join adjacent fragment hits to gain potency (“SAR by NMR”)	Exchange part of a lead associated with a liability (e.g., PK) with an over-lapping fragment hit

Fragment Based Lead Discovery

The "Replace" Approach to Fragment HTL

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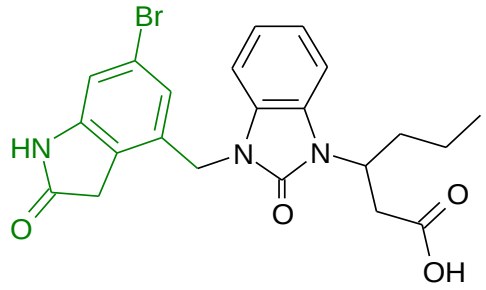
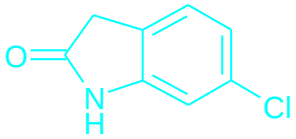
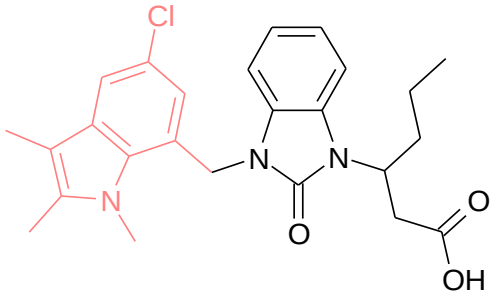


IC_{50} (Chymase) = 50 nM
IC_{50} (Cat G) = 5,500 nM



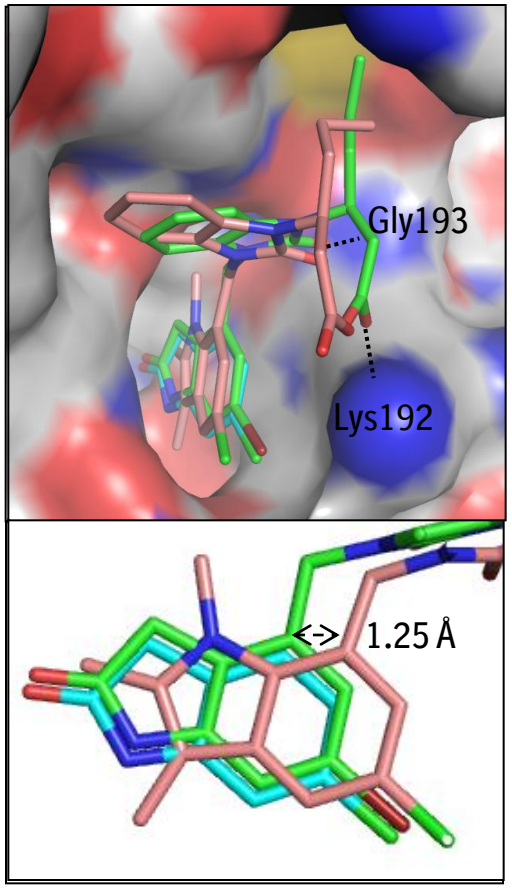
IC_{50} = 510 μM
Fragment Hit



IC_{50} = 31 nM
LO Series

Issues:

- GSH adduct formation
- Cat-G selectivity



- P1 moiety of compound at top binds as deeply into S1 as the oxindole fragment
- Benzimidazolone core of compound at top repositions itself to enhance its network of interactions

Fragment Based Lead Discovery

Profiling the Fragment Replacement in an LO Series

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		SoLO		"Fragment"		Current Top LO Compounds		
				Compound at top				
Structure								
Human Chymase IC_{50} (nM)		85		41		12		5
Chymase with HSA IC_{50} (nM) (fold)		NT		230 (5.6), n = 2		67 (5.6), n = 8		28(7), n = 2
CatG IC_{50} (nM) (fold)		220 (2.5)		5400 (130)		230 (19)		176 (35)
GSH adduct		detected		not detected		detected		detected
HLM ($\%Q_h$)	RLM ($\%Q_h$)	38	25	22	11	45	15	22	6
CYP 2C9 / 2C19 / 3A4 IC_{50} (μM)		>30/>30/>30		14/>30/>30		2.1/8.8/>30		29/>30/>30
	HT Sol (μg/mL) | Caco2 (AB/BA)	10/64	6/16	41/>100	2.4/28	3.3/103	20/27	
	AUC (ng*hr/mL) | Vss (L/kg)	38636	0.13	239	1.84	12653	0.34	Rat RACE: Conc @ 6h (10mg/kg) 1.85 μM
	$T_{1/2}$ (hr) | MRT (hr)	1.12	1.6	0.90	0.41	3.6	4.3	
Rat PK	Clearance ($\%Q_h$)	6.0		108		2		
	C_{max} (μM) | T_{max} (hr)	24	0.42	0.33	0.33	2.3	4.7	
	Bioavailability (%)	87		21.4		120		
		PO 10 mg/kg PEG water 70/30 IV 1 mg/kg PEG water (70/30)		PO 10 mg/kg PEG water 70/30 IV 1 mg/kg PEG water (70/30)		PO 10 mg/kg PEG water 70/30 IV 1 mg/kg PEG water (70/30)		

The "Grow" Approach to Fragment HTL

Comparative Progression of HTS, VSL and FBS Hits

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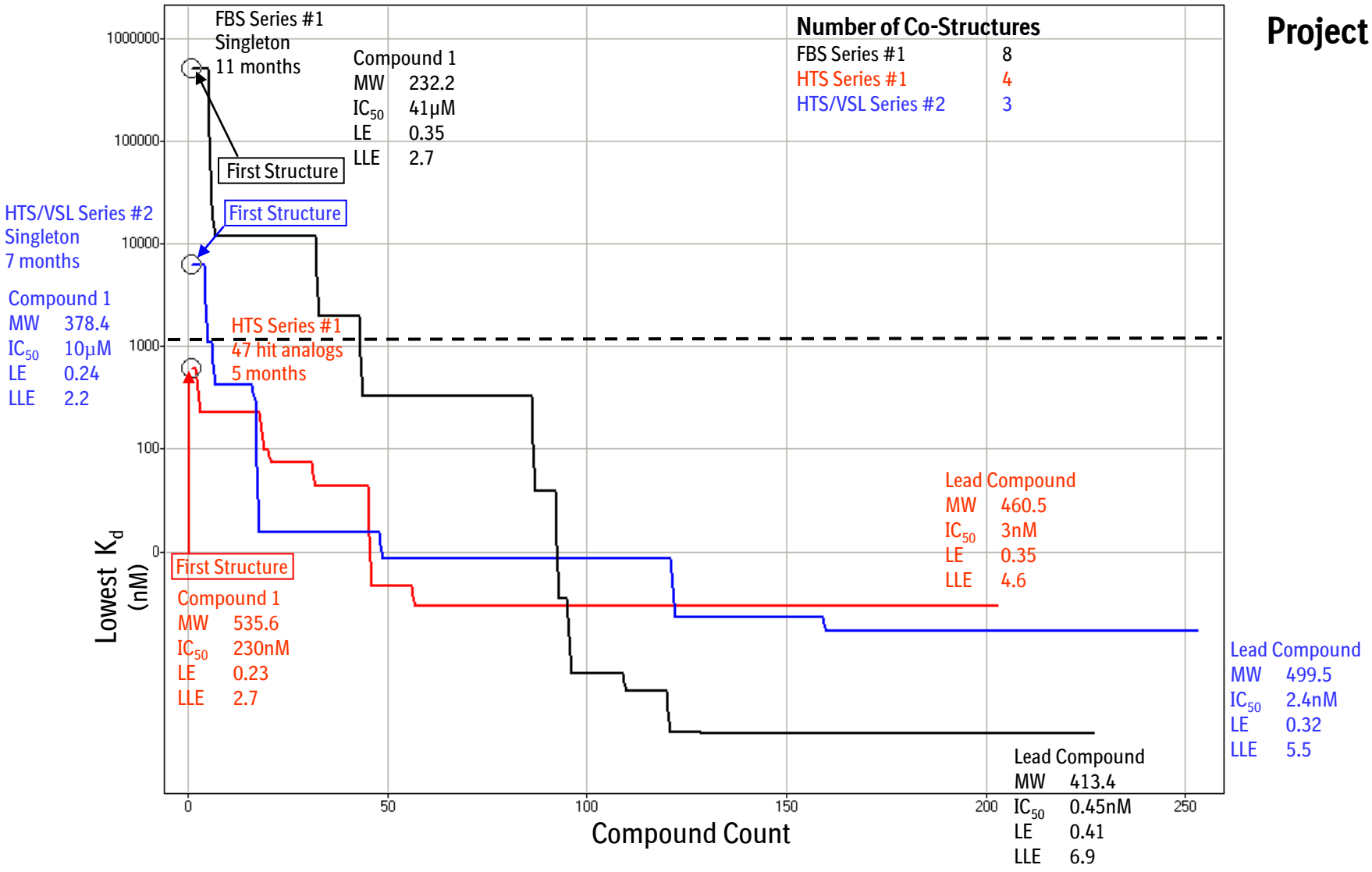

Project #1 Lead Series

Profile Data for Representative Lead Compounds

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	HTS Series #1	HTS/VSL Series #2	FBS Series #1
IC_{50} (nM)	3	2	0.5
LE	0.35	0.32	0.41
HT-Sol 7.4 ; 4.5 (µg/ml)	1.7 ; 0.4	22.9 ; 0.8	0.46 ; 6.8
HLM (Q_h)	< 11.4%	47%	27%
IC_{50} (µM) CYP3A4BFC	> 30	> 30	-
IC_{50} (µM) CYP2D6	> 30	> 30	3
Other IC_{50} (nM)	-	325	1.1
Ex vivo IC_{50} (nM) *best value from series	52.8nM*	> 4µM*	24.5nM

The "Grow" Approach to Fragment HTL

Comparative Progression of HTS and FBS Hits

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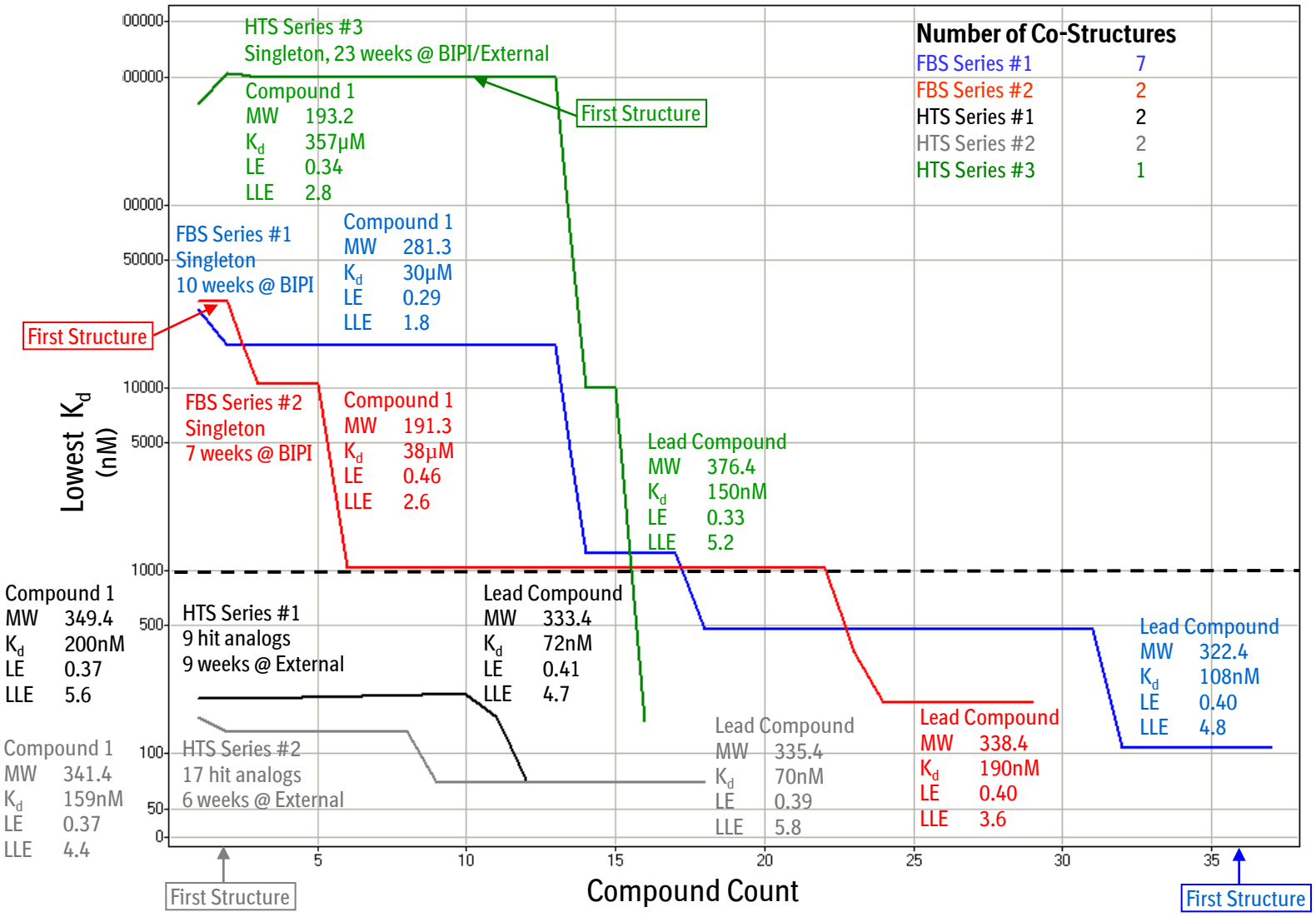
Project #2



Project #2 Lead Series

Profile Data for Representative Lead Compounds

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	HTS Series #1	HTS Series #3	FBS Series #1	FBS Series #2
K_d (nM)	130	70	108	190
LE	0.37	0.39	0.40	0.40
HT-Sol 7.4 ; 4.5 (µg/ml)	> 35	> 33	6.8 ; 6	< 1
HLM (Q_h)	< 30%	< 30%	< 30%	54%
IC_{50} (µM) CYP3A4	12.4	> 30	> 30	30
IC_{50} (µM) CYP2D6	12.8	22	> 30	30
IC_{50} (µM) CYP2C9	0.8	> 30	> 30	15
MDL HitProfiler	No significant responses	Not Tested	$5HT_{2B}$ 62% inhibition @ 10µM	Not Tested
Cytotoxicity	No findings to 100µM	No findings to 100µM	No findings to 100µM	No findings to 30µM Major toxic effect at 100µM
LPS/TNF	86% inhibition @ 30mg/kg	83% inhibition @ 30mg/kg	Analog showed 84% inhibition @ 30 mg/kg	Not Tested

Comparative Progression of Hits

Observations from > 2 Completed Fragment HTL Campaigns

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- Fragment hits and HTS hits can be progressed to the same point in a comparable period of time, but such progression of fragment hits requires more "Faith-Based Synthesis" given the often weak potency during the initial 20-50 compounds
- Progression of a high µM fragment hit to below 1µM may require that 20-50 single-point analogs be made to maintain a high LE (> 0.35) and to drive LLE into its desired range (5-7) for drug-likeness
- Parallel synthesis may be contraindicated in the initial stages of fragment evolution because there is a more intimate relationship between "core" and substituent, and because ADME properties should be minimally retained but preferably improved while potency is being driven below at least 1µM
- Fragment SAR, guided by structure, can be used to more rapidly optimize parts of a larger, more potent compound *(applicable even to HTS hits ➔ deconstruct HTS hits to most efficient core)*
- Project #1 shows the benefit of having an initial fragment hit with a high LE (0.35)
- Project #2 shows with FBS Series #1 that a high-quality docking model can drive fragment evolution
- HLM, CYP and HT solubility data should be profiled early on even in Fragment HTL

Fragment Based Lead Discovery

Organizational Requirements

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- Dispensary support for unique FBS processes
- HTS support for enzymatic and/or other non-biophysical HT fragment assays
- Integrated Structural Biology (X-ray, NMR) and Biophysics (SPR, NMR, ITC, FP)
- Dedicated group of chemists (≥ 3)
- Use of external chemistry resources for simple analoging
  - Confirm stability of binding mode
  - Generate fragment SAR
  - Synthesize I- or Br-containing fragment analog(s) for X-ray

Acknowledgements

Fragment Based Lead Discovery

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