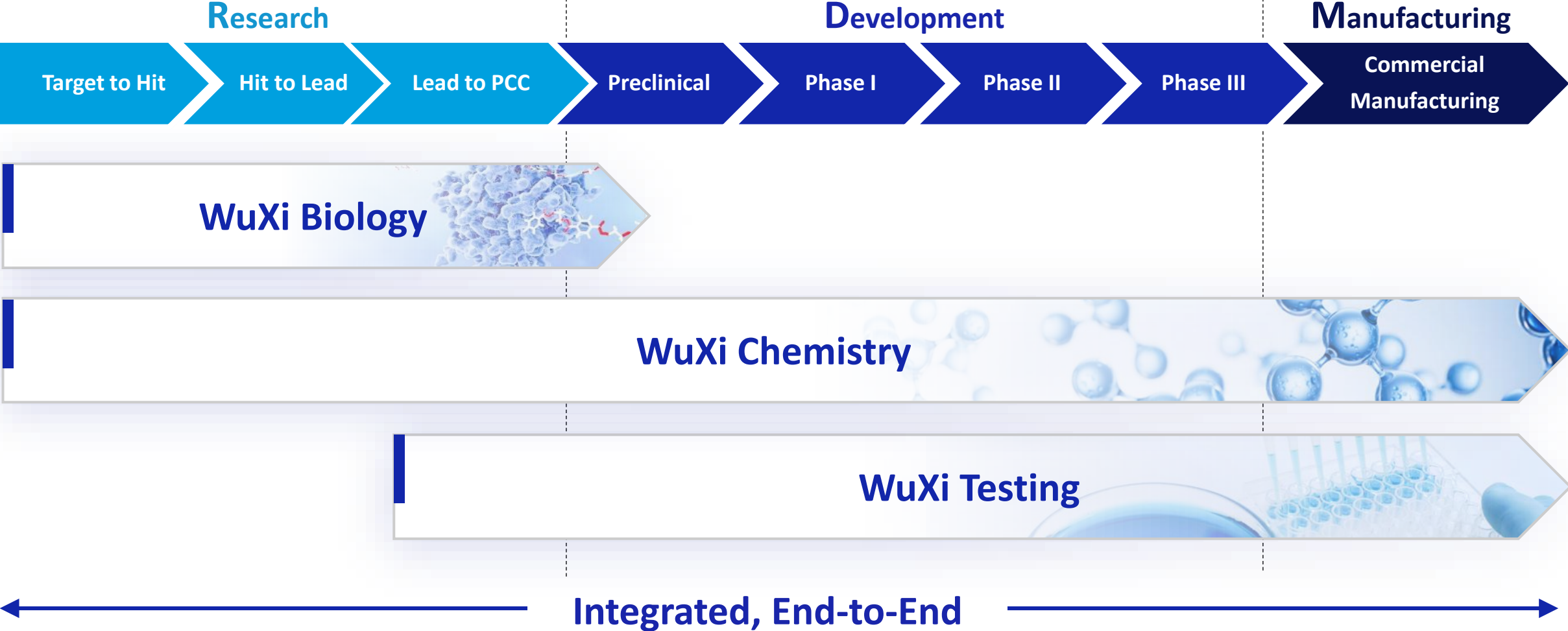


Covalent Fragment-based Drug Discovery

*Early Discovery Platform
In Vitro Biology Unit, WuXi Biology*



Integrated End-to-End CRDMO Enabling Platform



Technology Platforms in Early Discovery

Target

Hit

Lead

PCC

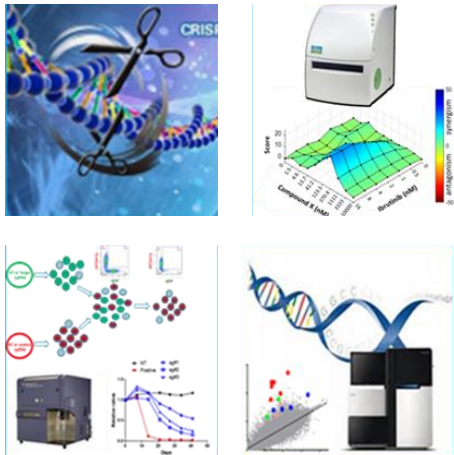
Clinical Trial

Early Discovery

Target Discovery and Validation

Target Discovery & Validation

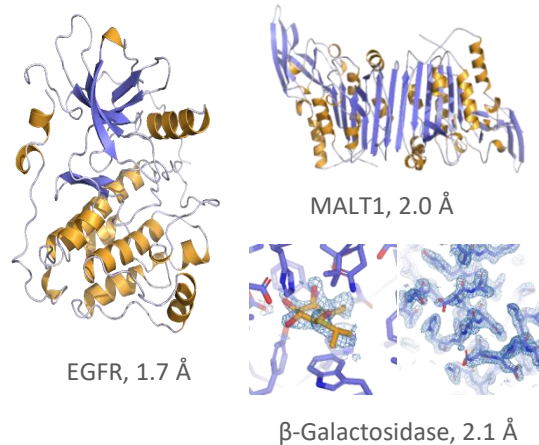
- >120 cell lines screened by gene scissor libraries
- >500 engineered cell lines established
- >100 targets validated



Protein Science

Protein Production & Structural Biology

- Over 1500 proteins produced
- 1200 Assay grade protein delivered
- >250 Crystallization grade protein delivered
- Xpress portfolio of >150 proteins to be delivered within 8 weeks



Hit Discovery and Validation

Proprietary Library & Screening Platform

- DEL: >1500 client, >500 target screened, validated molecule has entered the Phase III clinical trials.
- HTS/ASMS/FBDD: >160 projects delivered successfully
- New platforms including covalent, TPD, D2B etc.
- Integrated biophysics platform to support screening and validation



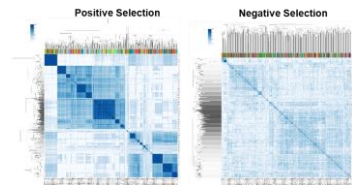
Non-covalent inhibitors of 3CLpro

Phase III clinical trials

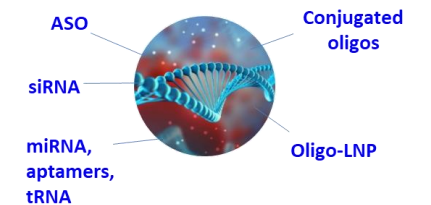
Peptide and Oligonucleotide

Peptide and Oligonucleotide Discovery

- >1000 projects delivered
- Integrated service from library design and construction to screening to characterization
- Extended platforms including LNP delivery, SELEX etc.



mRNA display



Global Facilities

USA



Natick, MA

DEL, Biophysics
Logistic Center

10+



China

400+



Shanghai
(Waigaoqiao)

Screening (DEL, FBDD, ASMS),
Library, Biophysics, Oligo Discovery



Suzhou

Protein Production,
Crystallography

Germany



Gräfelfing, Munich

Protein production, FBDD, Crystallography,
Biophysics, DEL

80+



Shanghai
(Zhangjiang)

Screening (HTS/HCS), OBOC
DEL, Peptide Discovery



Chengdu

Oligo and mRNA Discovery

Drug Discovery as An Exploration of Chemical Spaces

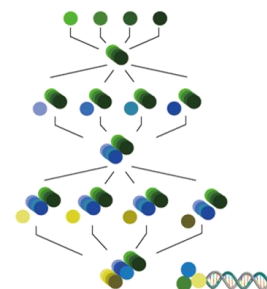
Hit Generation Technologies

High Throughput Screening



10^5 - 10^7 compounds

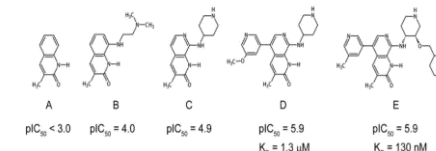
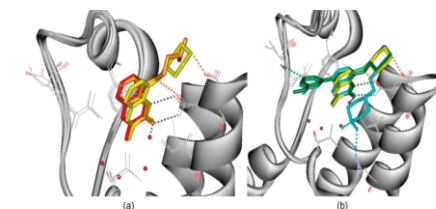
DEL-Screening



Split-and-mix synthesis
(mixtures with DNA-barcode)

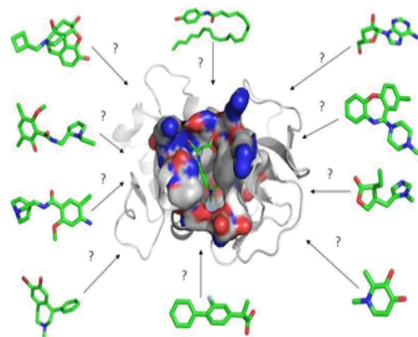
10^9 - 10^{12} compounds

Fragment Based Drug Discovery



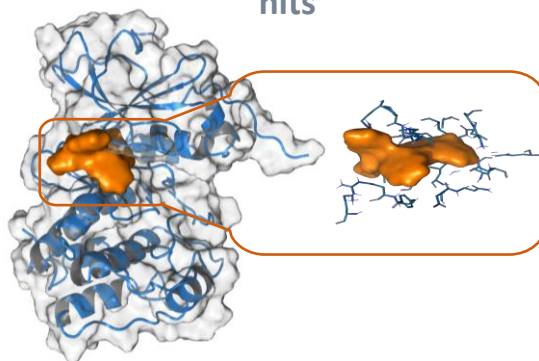
10^3 - 10^4 compounds

Virtual Screening

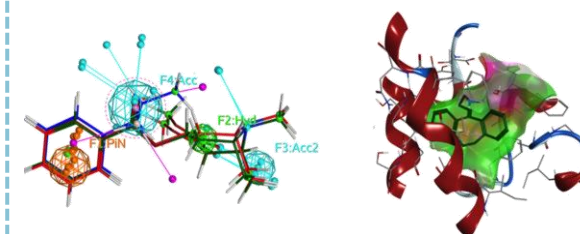


10^6 - 10^9 compounds

hits



Structure-Based Drug Design



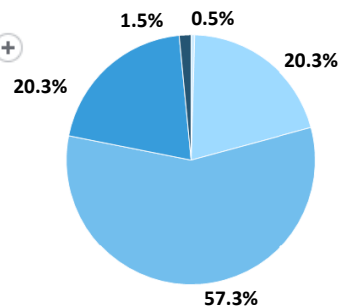
10^2 - 10^3 compounds

Integrated Fragment Based Drug Discovery

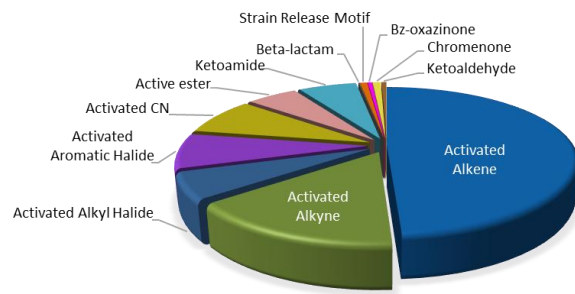
Various Proprietary of in-house Libraries

Color by:

RCount ▾ +

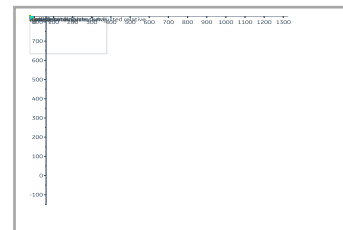


Conventional

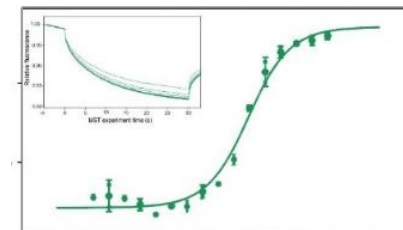


Covalent

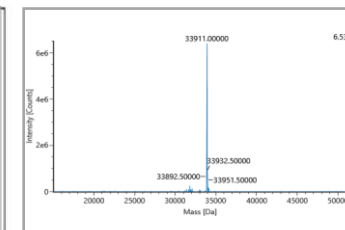
Biophysics & Screening Platform



Single-dose screening

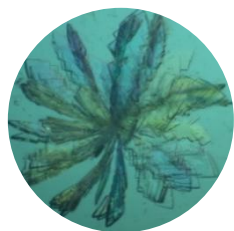


Affinity ranking

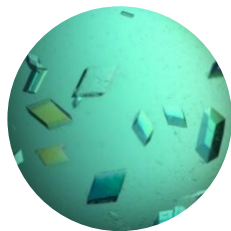


MS-based screening/validation

Crystallography



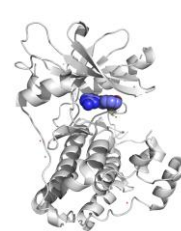
Screening



Optimization

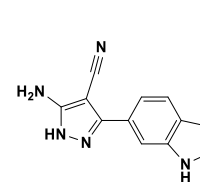


Apo reference structure

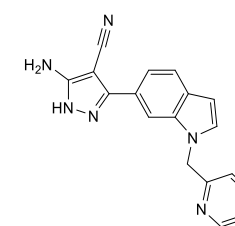


Co-crystal structure

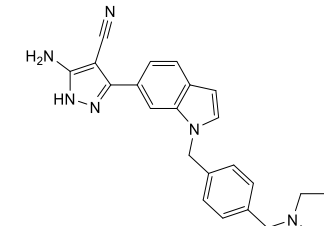
Medicinal Chemistry & CADD & Synthesis



1
Kd = 5.0 μ M
LE = 0.42



26f
Kd = 0.5 μ M
LE = 0.36



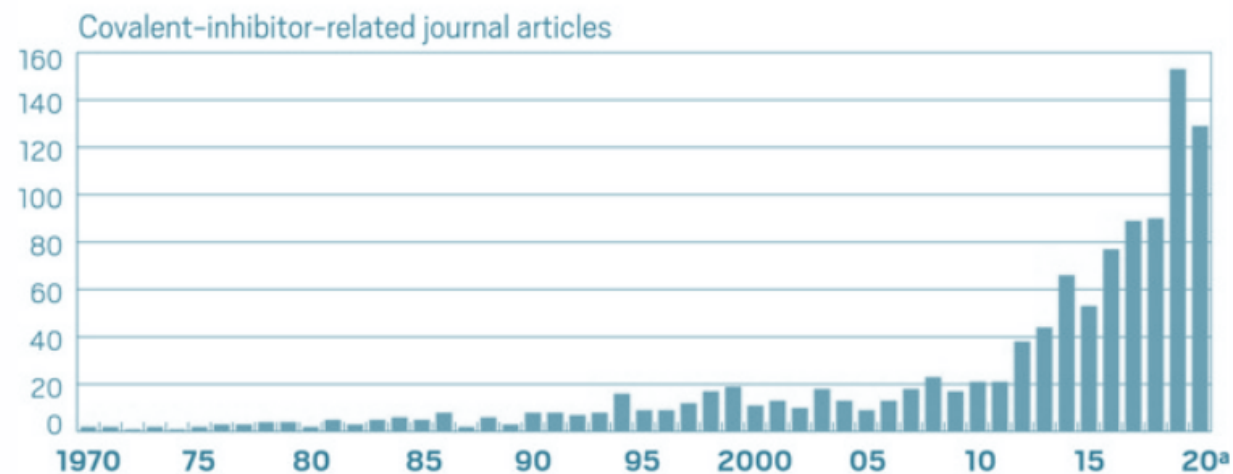
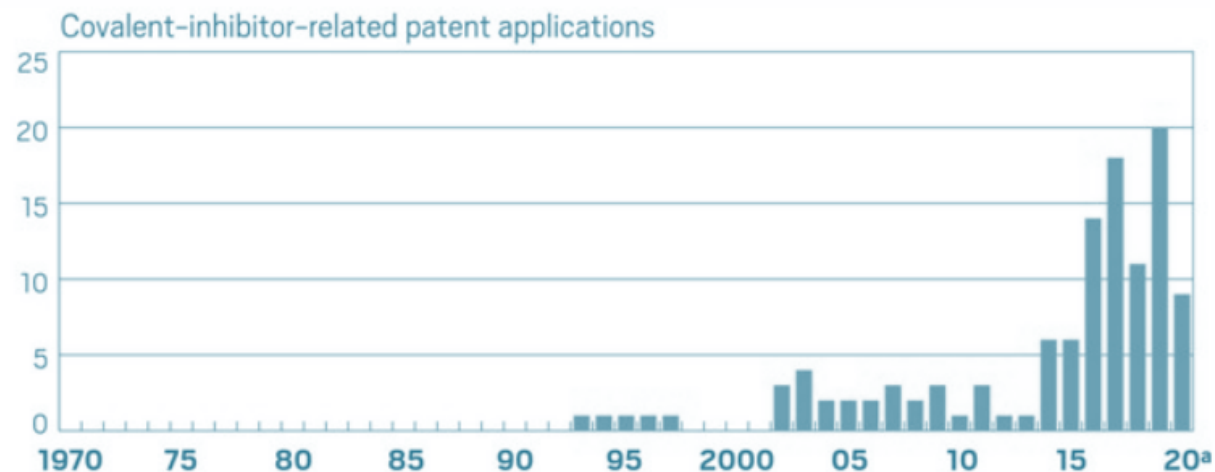
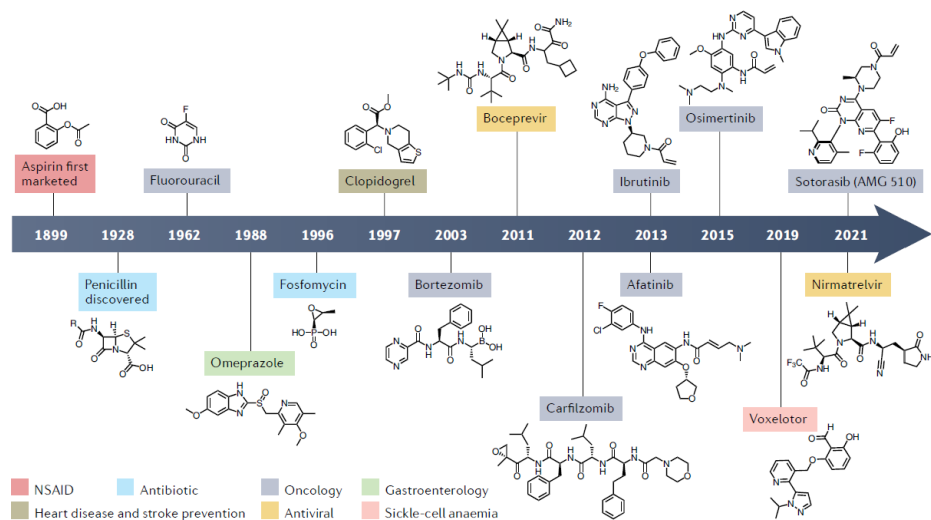
29a
Kd = 0.027 μ M
LE = 0.34

J. Med. Chem. 2019, 62, 7210–7232

The Resurgence of Covalent Drugs

“ If you look back over 100 years, some of the most important medicines advanced have a covalent mechanism of action.

— *Juswinder Singh, founder and chief scientific officer, Ankaa Therapeutics*



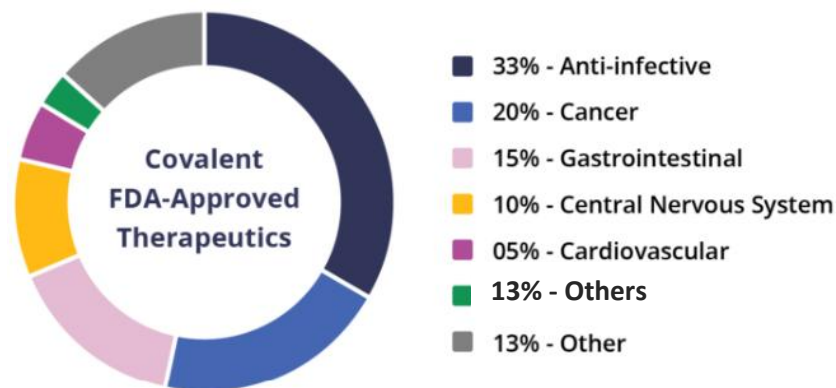
Source: CAS.a Data for Jan. 1 through Oct. 25, 2020.

<https://cen.acs.org/pharmaceuticals/drug-discovery/Covalent-drugs-fringe-field-fashionable/98/i43>

Boike, L., Henning, N. J., & Nomura, D. K. (2022). Advances in covalent drug discovery. *Nature Reviews Drug Discovery*, 1-18.

Boom in Covalent Drugs

Approved covalent inhibitors by therapeutic indication



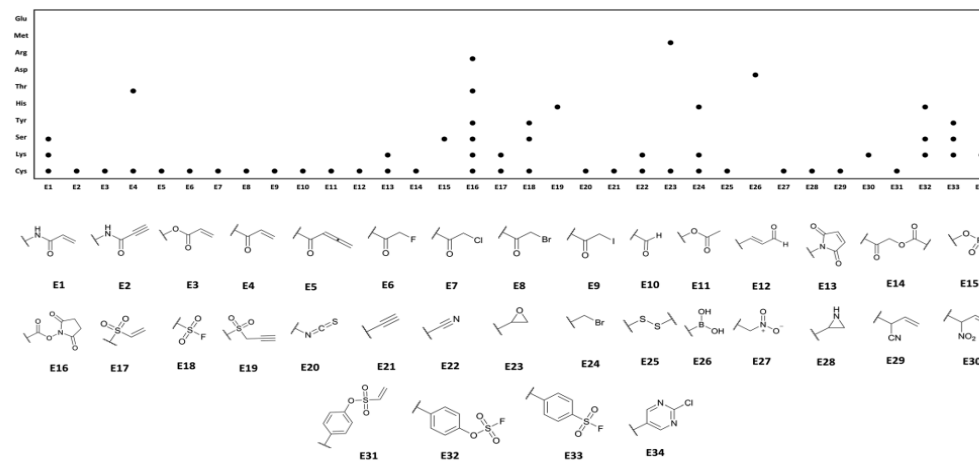
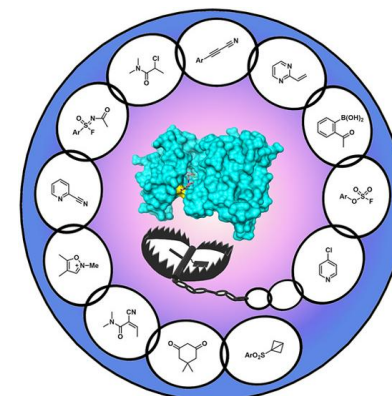
Advantages

- Increased potency, low effective dosing required
- Prolonged mechanism of action
- Complete inactivation of targets
- Opportunity for undruggable targets

Risks and challenges

- Uncontrollable selectivity and related toxicity
- Assessment of target-binder interactions
- PK/PD optimization of lead compounds
- Methods to discover selective inhibitors

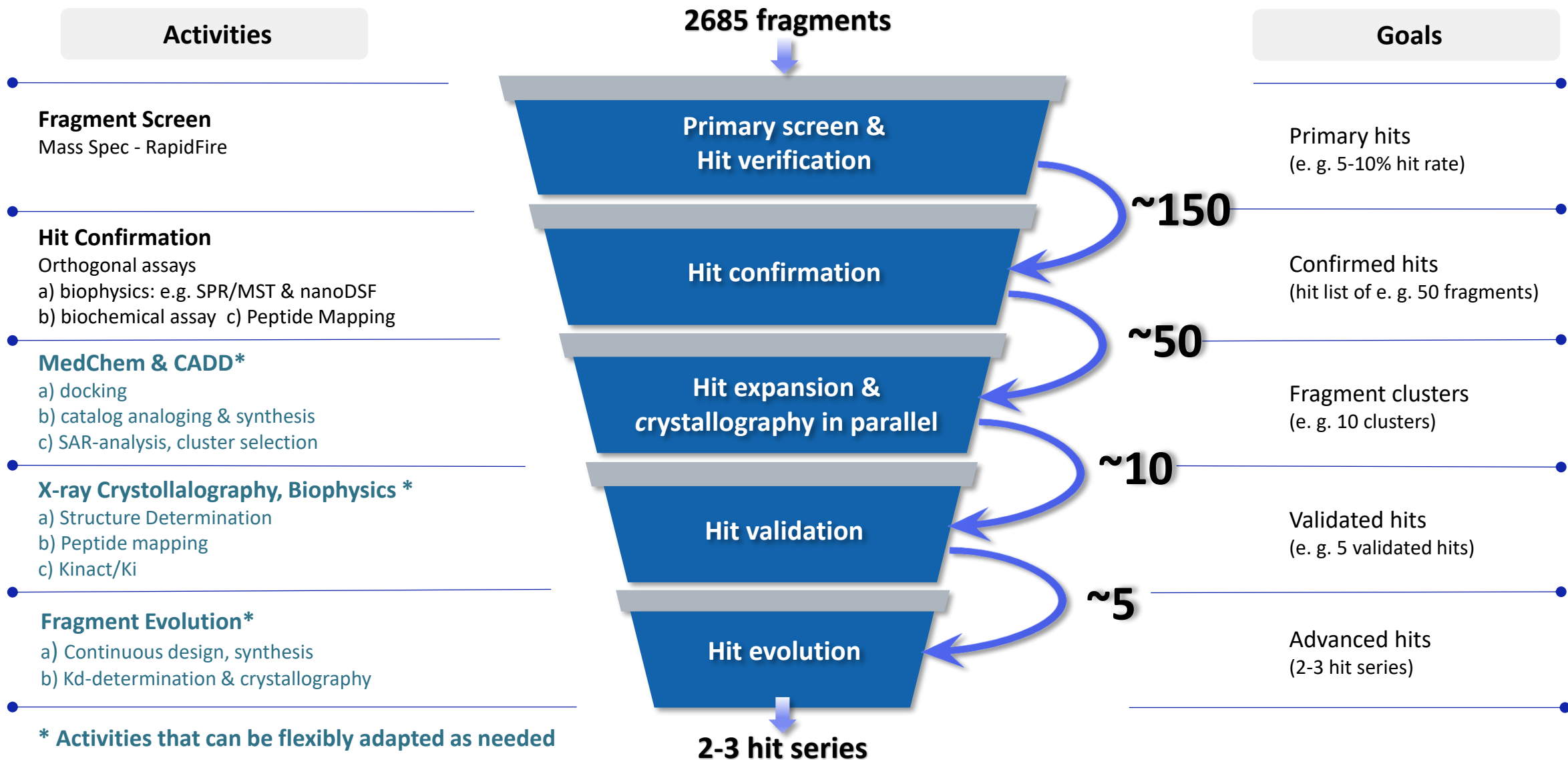
Emerging and re-emerging warheads



Amino acid selectivity of diverse electrophiles

<https://www.oxfordglobal.co.uk/resources/covalent-inhibitors-advancements/>
 Boike, L., Henning, N. J., & Nomura, D. K. (2022). Advances in covalent drug discovery. *Nature Reviews Drug Discovery*, 1-18.

Covalent FBDD Workflow and Compound Metrics



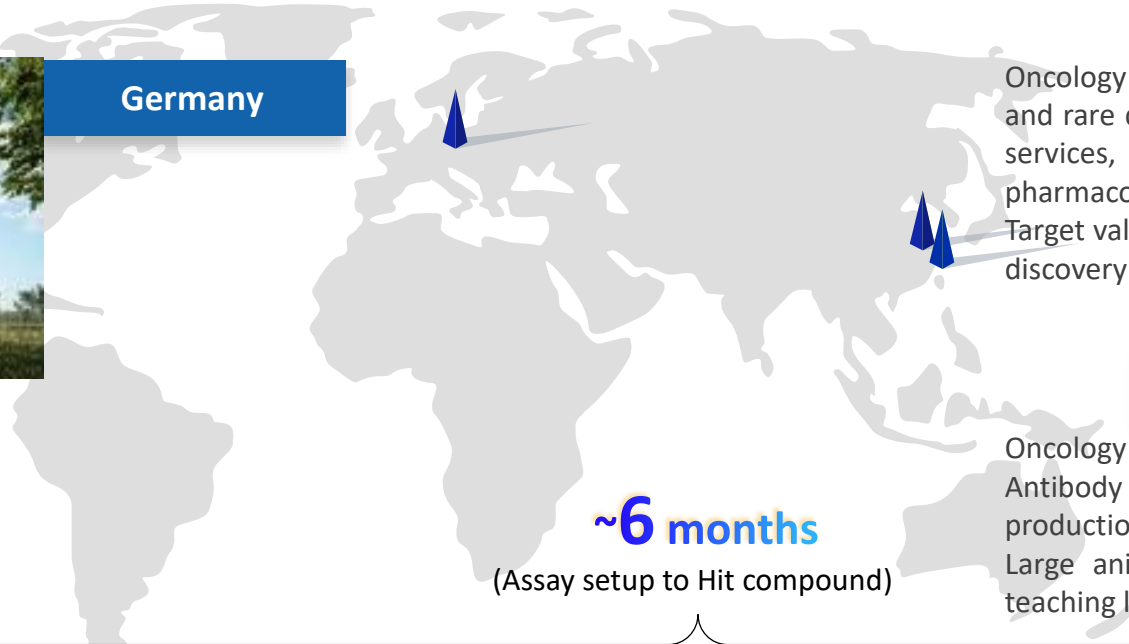
Fast Turn-around Time & Flexible Processing Sites



Gräfelfing, Munich

Protein production, FBDD, Crystallography, Biophysics, DEL

Germany



China

Oncology and immunology, GEMM and rare disease models, biomarker services, DEL, *in vitro* and *in vivo* pharmacology, Infectious diseases; Target validation, Hit to Lead & Lead discovery



Shanghai (waigaoqiao)

China

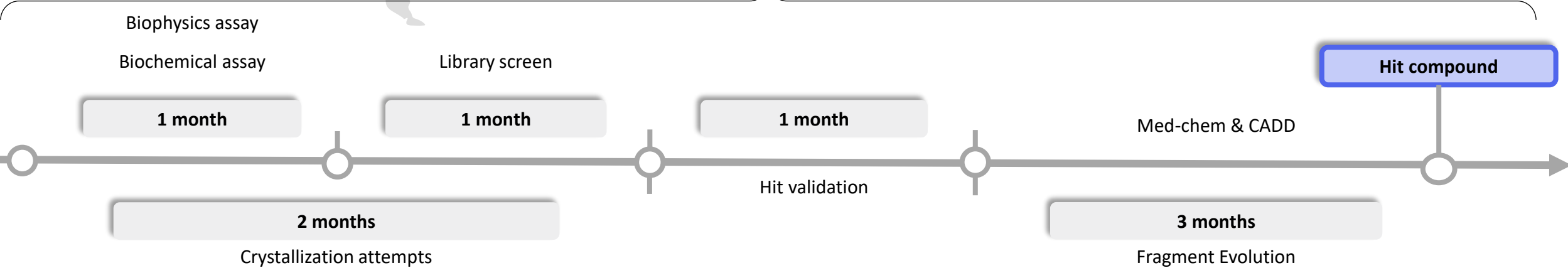
Oncology and immunology, Antibody production, Protein production, X-Ray crystallography, Large animal disease models, DEL teaching lab



Suzhou

~6 months

(Assay setup to Hit compound)



Topnotch Platforms for Covalent FBDD

Platforms and facilities

MS assay platform

- Intact-Mass analysis of protein and adducts (Waters-Xevo Qtof, Waters-BioAccord, Agilent-RapidFire)

Biophysical assay platform

- NanoDSF (Nanotemper Prometheus)
- MST (Monolith X)
- Dianthus-X

Biochemical and cellular assay platform

- Biochemical assay development and SAR screen: Abs, FI, FP, Lum, FRET, TR-FRET, AlphaLISA /Screen...
- Cellular assay: Proliferation, HCS, PPI, Cell line generation...

Crystallization

- Comprehensive structural generation (Bruker MetalJet)

Ready to go assay

- Ready to Go target list is continuous increasing, follow us to get more popular target list <https://hits.wuxiapptec.com/ready2go>



Agilent RapidFire



Waters BioAccord



Nanotemper Prometheus



Monolith X



Dianthus-X



Waters Xevo QToF



Bruker MetalJet

Covalent Fragment Library (2685 Fragments)

Library Features

Fragment design rules:

- ✓ Ro3: MW ≤ 300 , clogP ≤ 3 , HBD & HBA ≤ 3
- ✓ Additional rules: NRB ≤ 3 , PSA ≤ 90 , stereo ≤ 2 , charged groups ≤ 1
- ✓ PAINS, Lilly-filters, REOS, Kazius
- ✓ Free from chemical reactivity and stability issues
- ✓ Synthetically accessible (2~3 weeks quickly available)

Structure diversity:

- ✓ Warheads with different set of Ligands
- ✓ Tanimoto similarity analysis

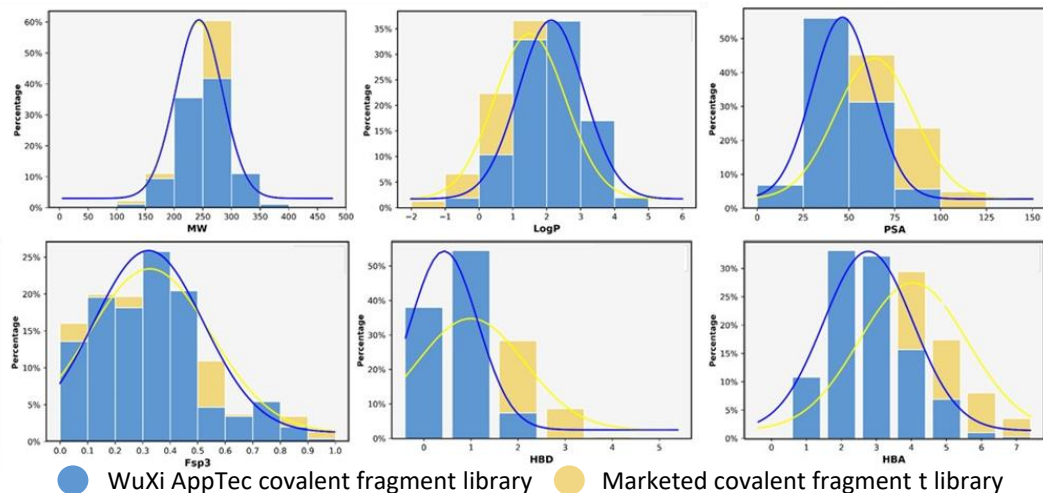
Library QC:

- ✓ LC-MS + NMR, with purity $\geq 90\%$

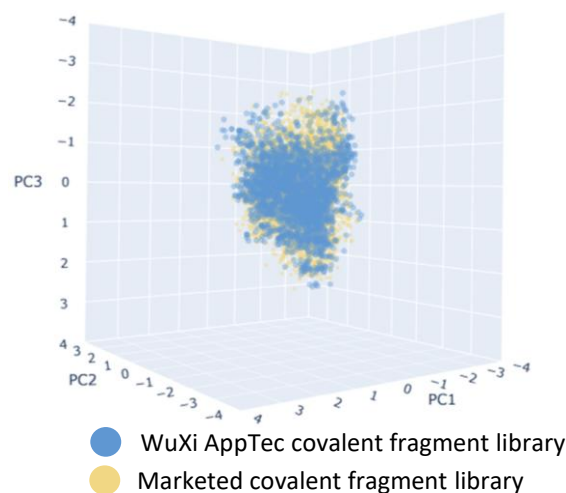
Warhead features:

Warhead type	Warhead structure No.	Cmpd No.	Target AA			
			Cys	Lys	Tyr	Ser
Activated Alkene	10	1315	✓	✓	✓	✓
Activated Alkyne	2	411	✓	✓	✓	✓
Activated Alkyl Halide	1	156	✓	✓	✓	✓
Activated Aromatic Halide	2	206	✓	✓		
Activated CN	2	221	✓	✓		✓
Active ester	1	142		✓		
Ketoamide	1	163	✓			✓
Beta-lactam	1	2	✓	✓		✓
Strain Release Motif	1	20	✓			
Bz-oxazinone	2	12	✓	✓		
Chromenone	2	23	✓	✓		
Ketoaldehyde	2	14	✓	✓		

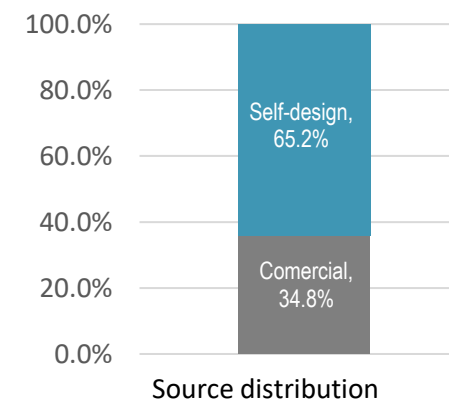
Physicochemical property distribution



Chemical space analysis

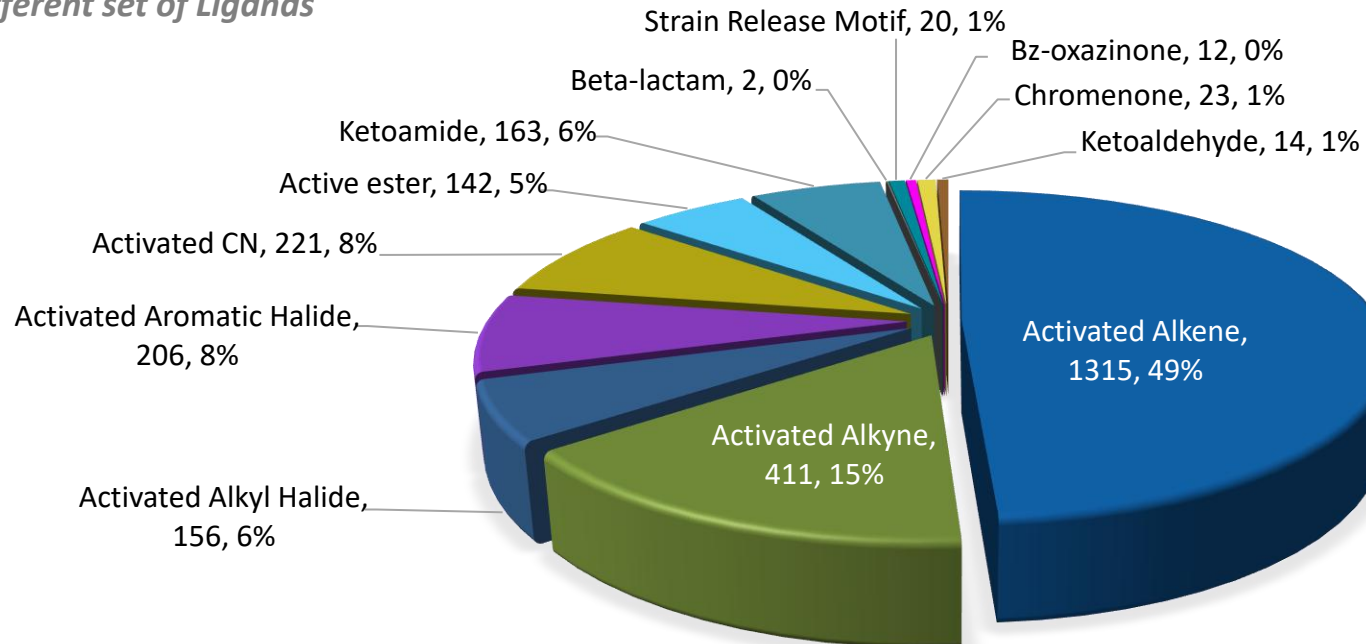


Library composition

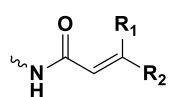


Covalent Fragment Library (2685 Fragments)

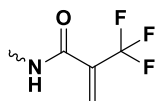
Warheads with different set of Ligands



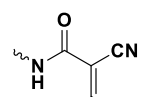
Activated Alkene



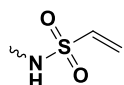
Acrylamide



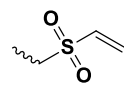
Trifluoromethylacrylamide



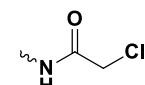
2-cyano-acrylamide



Vinylsulfonamide



Vinylsulfone



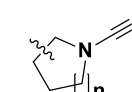
Chloroacetamide



Aromatic halide



Acetonitrile

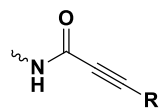


N-Nitrile

Activated Halide

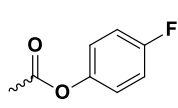
Activated CN

Activated Alkyne



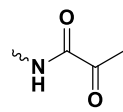
Ynamide

Active Ester



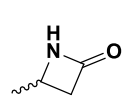
Active ester

Ketoamide



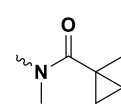
Alpha-ketoamide

Beta-lactam



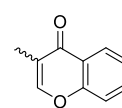
Beta-lactam

Strain-released



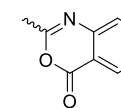
BCB amide

Chromenone



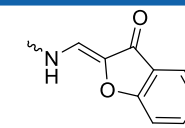
Chromenone

Bz-oxazinone

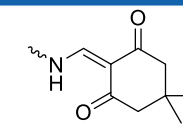


Bz-oxazinone

Ketoaldehyde



Methylene benzofuranone

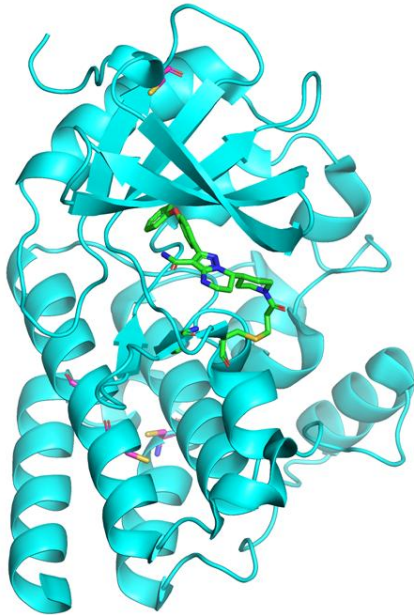
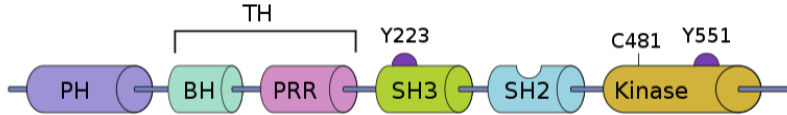


Dimethylcyclohexanedione

Case Study: BTK

Protein Information

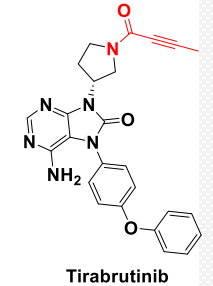
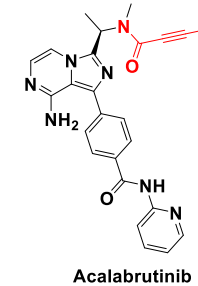
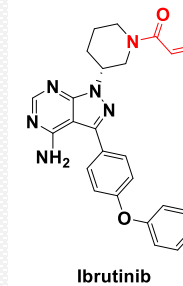
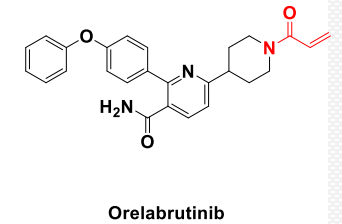
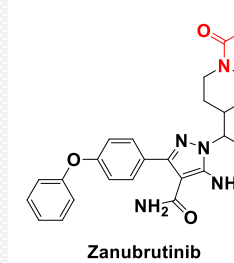
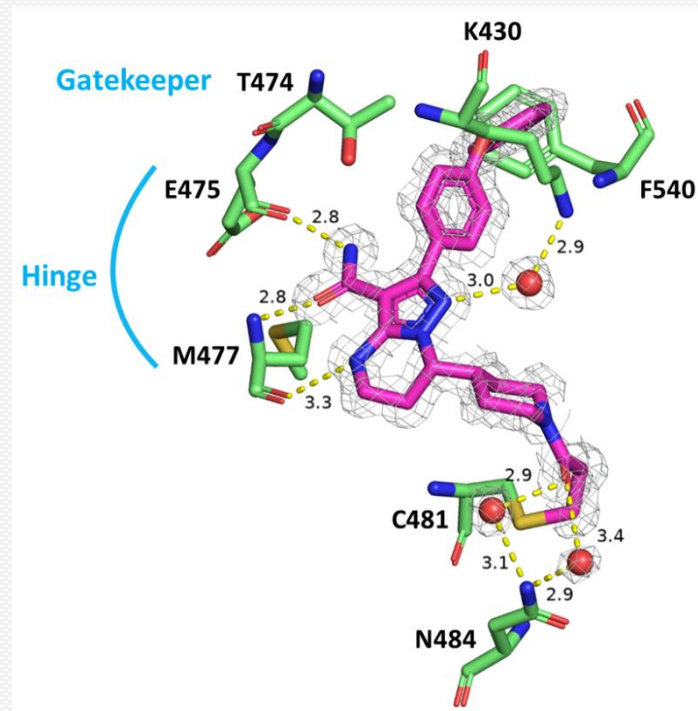
Protein



The kinase domain contains 6 Cys residues with 2 Cys on solvent-accessible surface.

Bruton's tyrosine kinase (BTK) is a key component of B cell receptor (BCR) signaling and functions as an important regulator of cell proliferation and cell survival in various B cell malignancies.

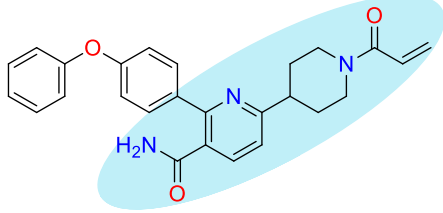
Reported covalent inhibitors



BTK Assay Setup: Reference Fragment

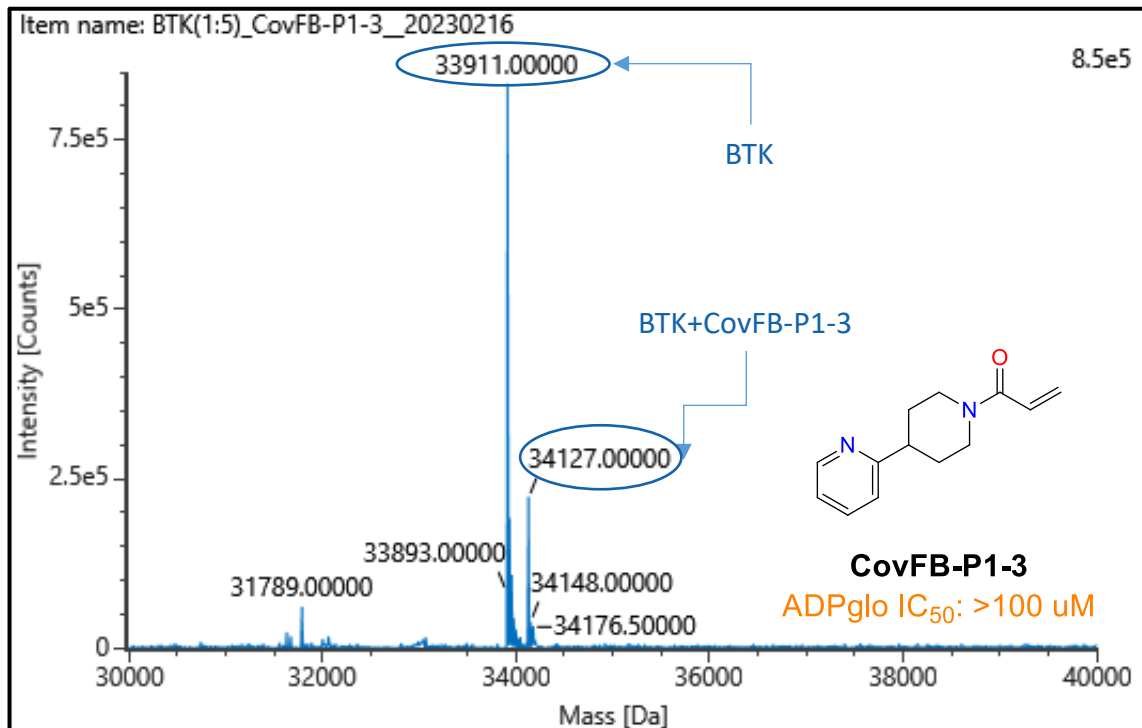
Intact Mass

Fragmentation of Orelabrutinib:

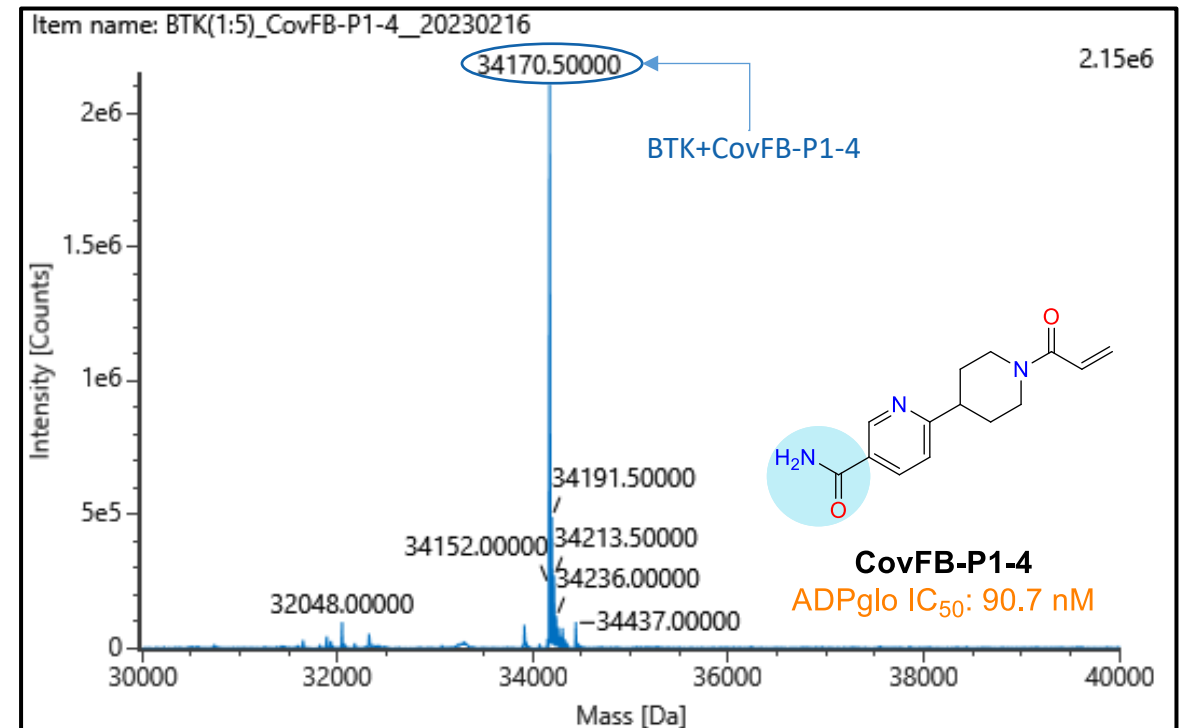


Protein			Fragment			Incubation condition
Name	Conc.	Theo. Mass (Da)	Name	Conc.	Theo. Mass (Da)	
BTK	1 μ M	33911.0	CovFB-P1-3	5 μ M	216.13	RT, 6h
			CovFB-P1-4		259.13	

1. BTK + CovFB-P1-3



2. BTK + CovFB-P1-4



Covalent Fragment Primary Screening

Intact Mass

2612 covalent fragments were screened with intact protein RapidFire MS

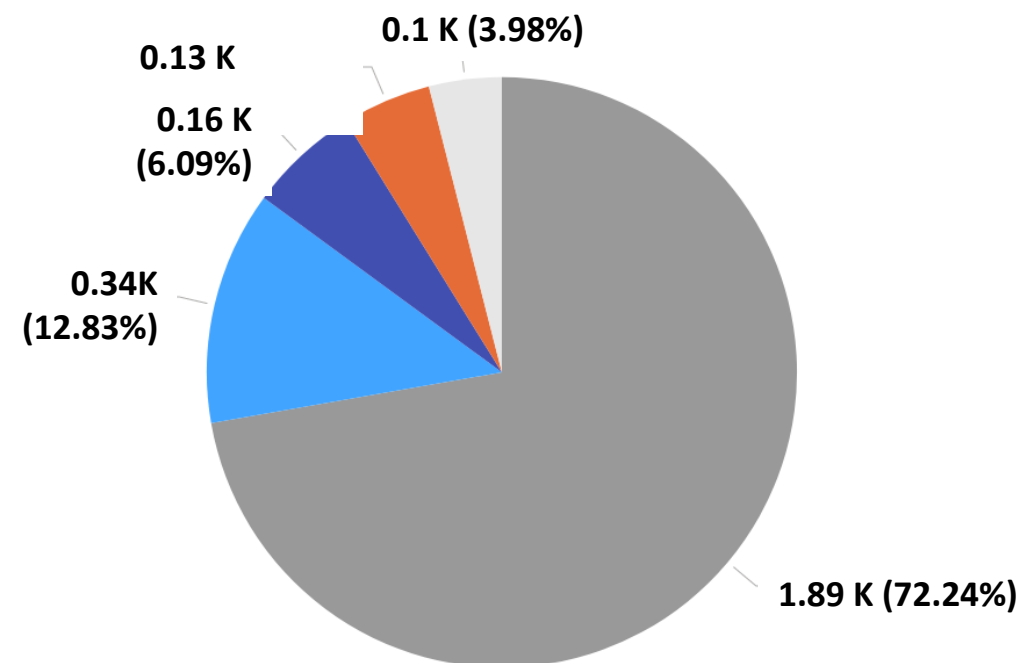
- 1 fragments/well
- 1 μM protein and 5 μM of each compound (molar ratio protein to compound = 1:5) incubated for 6h at RT
- Covalent binders with different intensity were discovered for BTK

Class (Occupancy*)	Count of class	Share
Binder (90-100)	127	4.9%
Partial binder (50-89.9)	159	6.1%
Weak binder (0.1-49.9)	335	12.8%
Non binder (0)	1887	72.2%
No data [†]	104	4.0%
	2612	

*Calculation of occupancy (conjugation efficiency) based on signal intensities in the deconvoluted MS spectrum.

$$\text{Occupancy} = \frac{\text{Signal intensity of bound protein}}{\text{Signal intensity bound protein} + \text{Signal intensity of apo protein}} \times 100$$

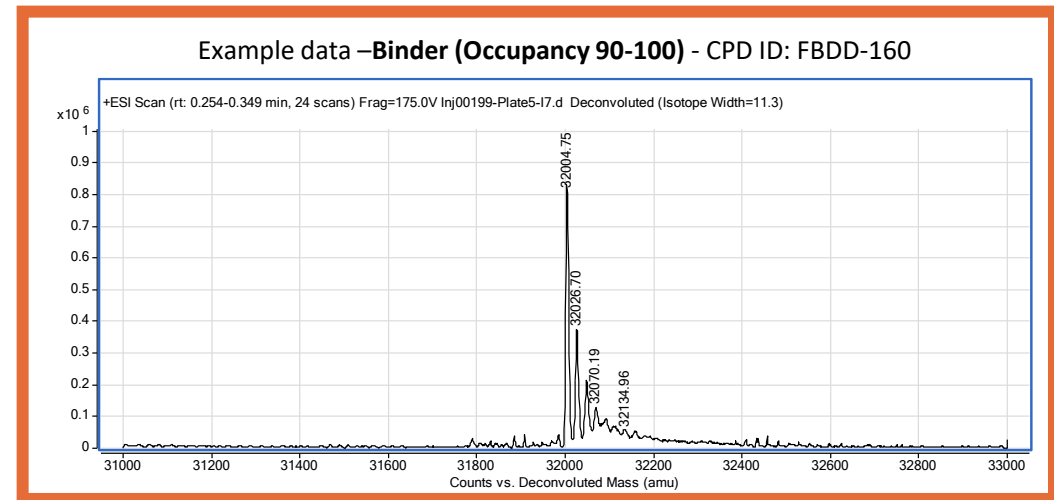
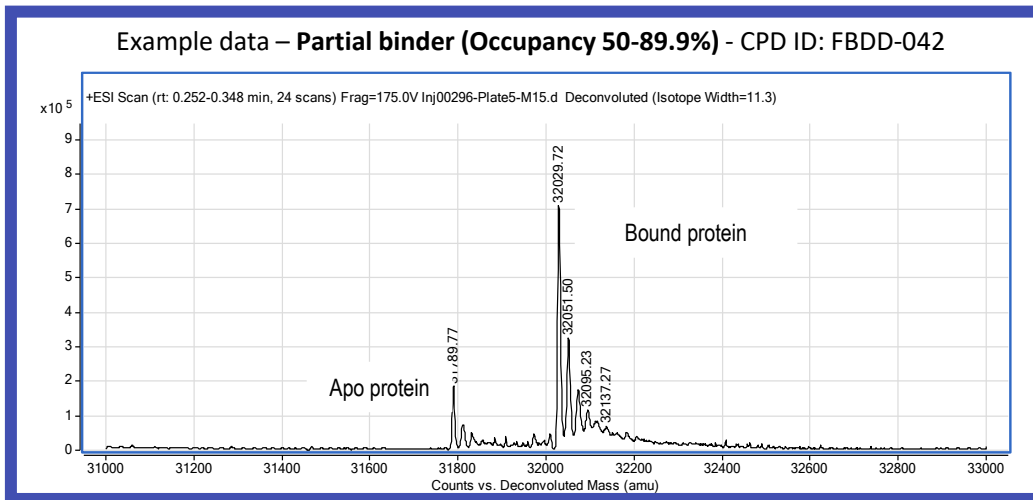
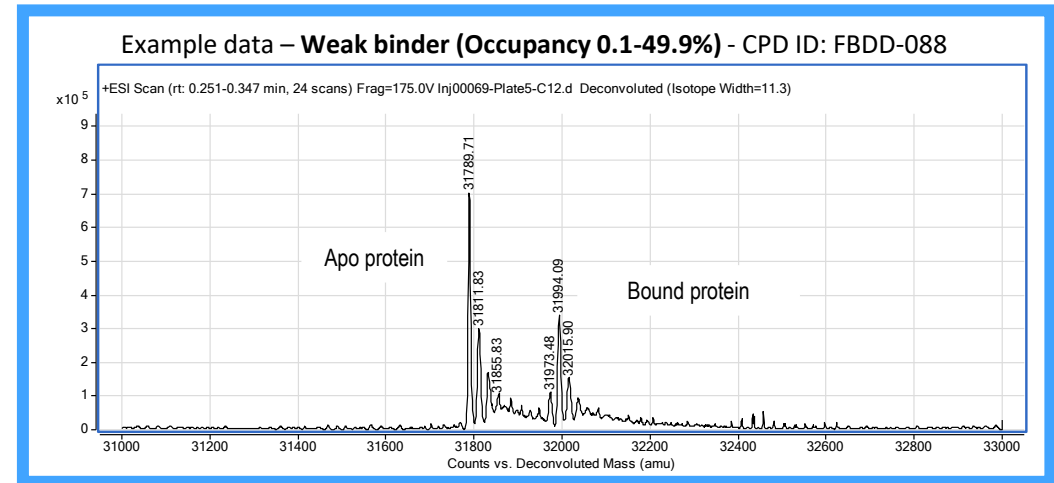
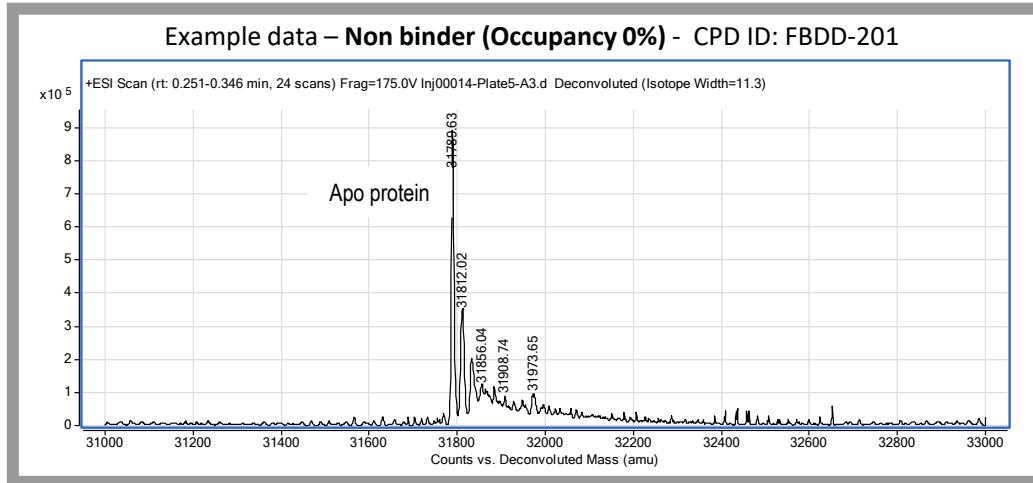
[†]No data comprises all samples that did not show a signal for mono conjugated protein and/or apo protein (only mono-conjugation adducts were considered for the classification).



*Screening with Waters-Xevo Qtof or Waters-BioAccord is also available

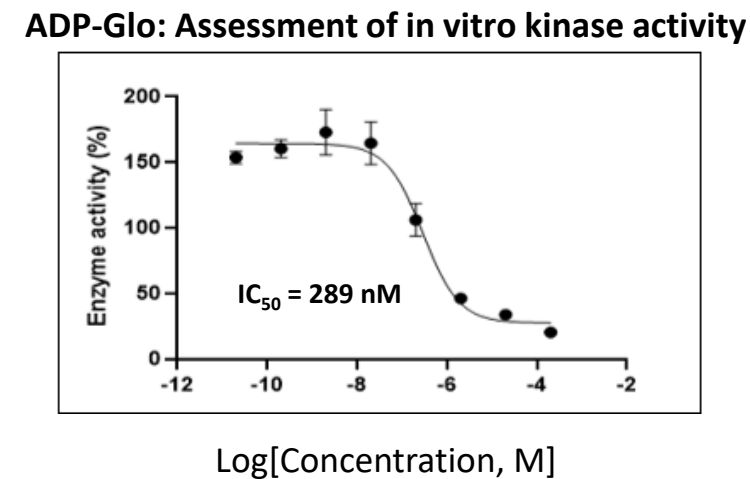
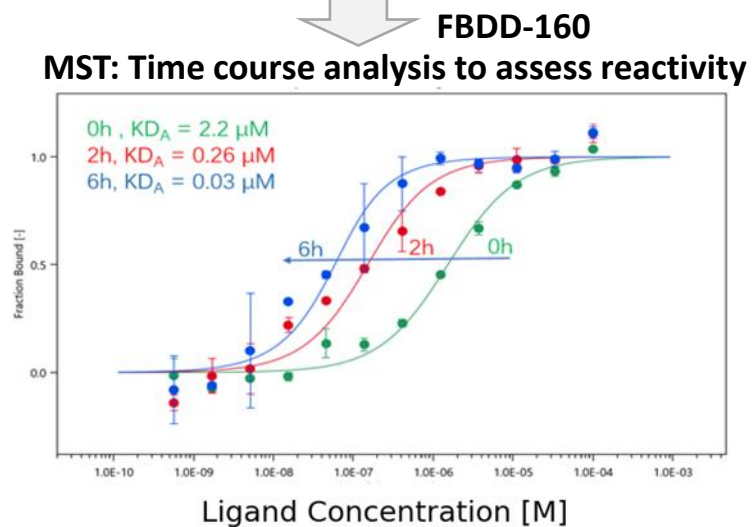
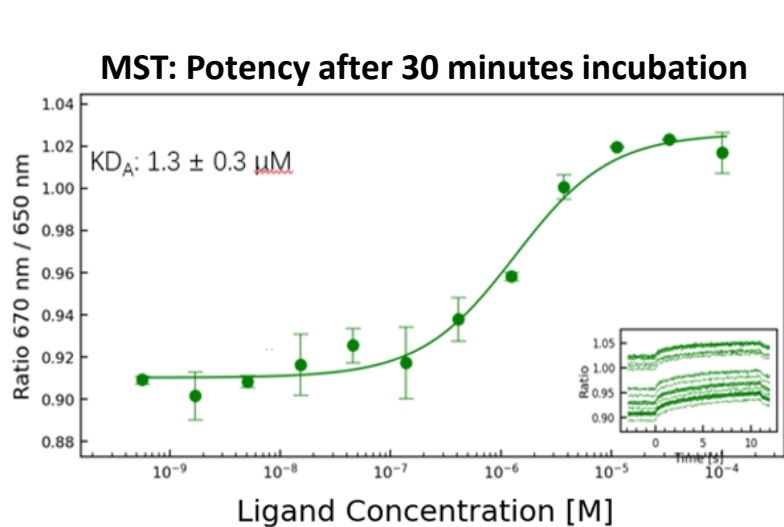
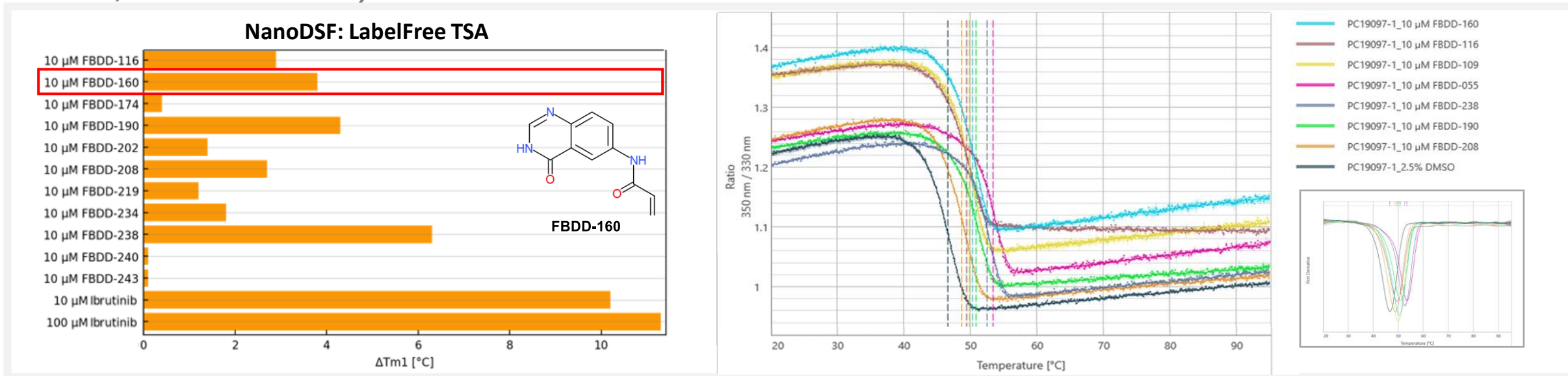
Example Data - Deconvoluted MS Spectra

BTK – Covalent fragment screening



Hit Confirmation: Orthogonal Validation

NanoDSF, MST and ADP-Glo Assay



Binding Site Identification: Peptide Mapping

BTK + FBDD-160_Trypsin-Mix

BTK (Boundary: 387-65z9)

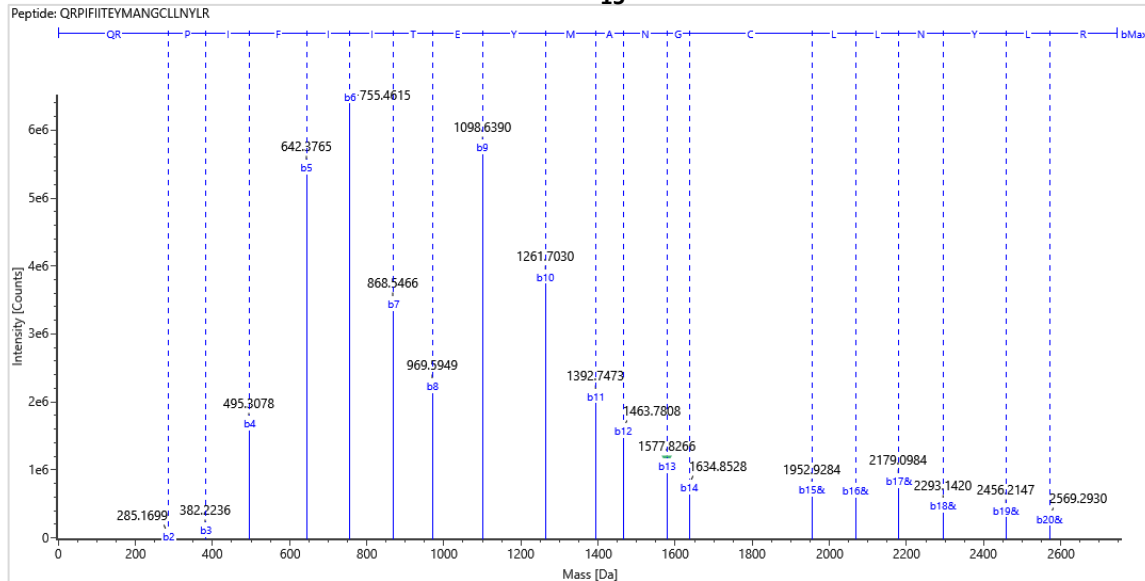
Coverage: 100%

1: 1 to 80
1: 81 to 160
1: 161 to 240
1: 241 to 292

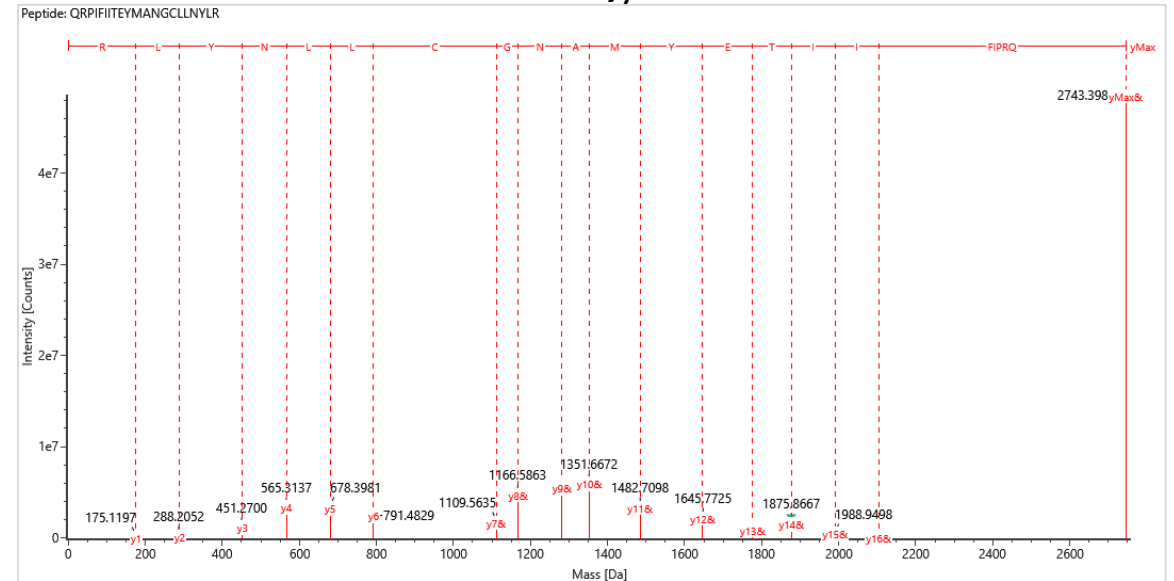
GSSHHHHHHS SGENLYFOGT AGLGYGSWEI DPKDLTFLKE LGTGQFGVVK YGKWRGOYDV AIKMIKEGSM SEDEFIEEAK
 VMNLSHEKL VQLYGVCTKQ RPIFIITEYM ANGCLLNYLR EMRHRFQTQQ LLEMCKDVCE AMEYLESKQF LHRDLAARNC
 LVNDQGVVVK SDFGLSRVYL DDEYTSSVGS KFPVRWSPPE VLMYSKFSSK SDIWAFGVLM WEIYSLGKMP YERFTNSETA
 EHIAOGLRLY RPHLASEKVV TIMYSCWHEK ADERPTFKIL LSNILDVME ES

Protein Name	Mass Difference (Da)	Digestion Enzyme	Run Time (min)	Coverage	Modification Site	Comment
BTK + FBDD-160 (1:5)	215.2	Trypsin-Mix	45	100%	Acetylation: G ₁ FBDD-160: C ₄₈₁	N-terminal methionine excision & Acetylation & FBDD-160

N-Term Ions b₁₅:1737.8451

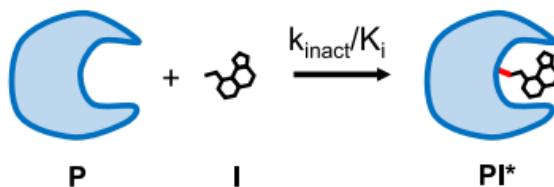


C-Term Ions y₁:894.4866



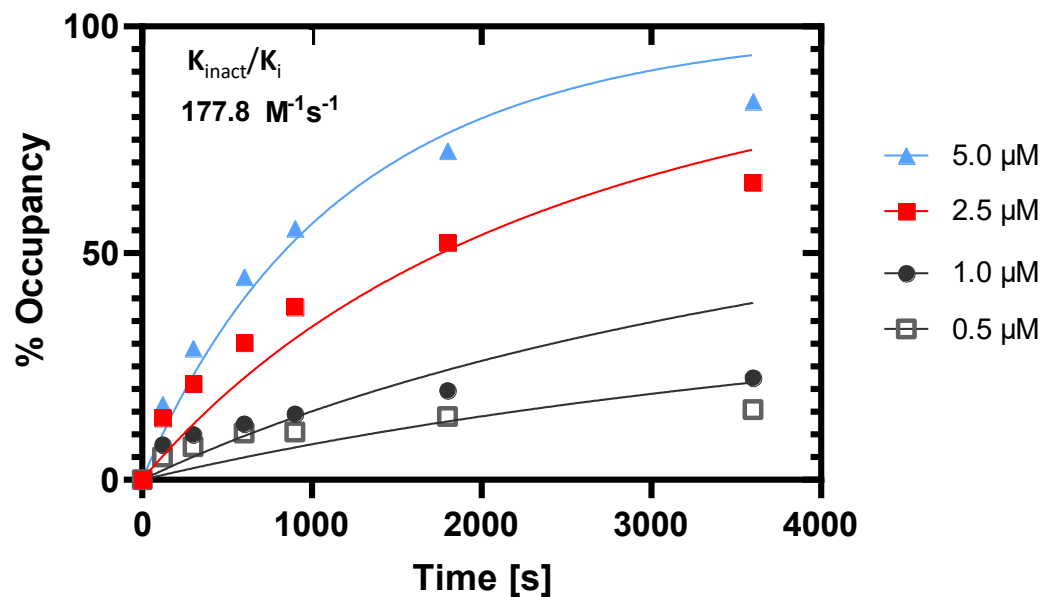
MS Based Kinact/Ki Determination

Measure the kinetics of covalent target inhibition by intact protein mass spectrometry



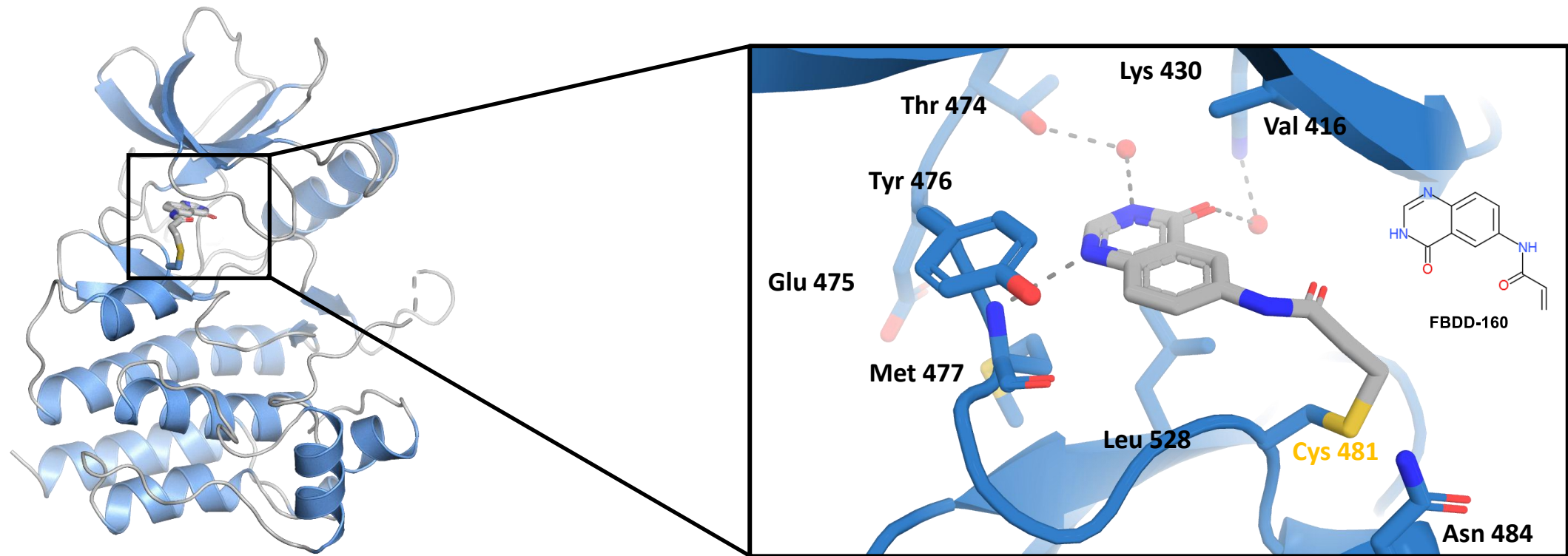
Graphic adapted from <https://doi.org/10.1021/acs.analchem.1c04463>

Conjugation of FBDD-160 to BTK in a time- and concentration dependent manner analyzed by intact mass analysis.



Binding Site Identification: X-Ray

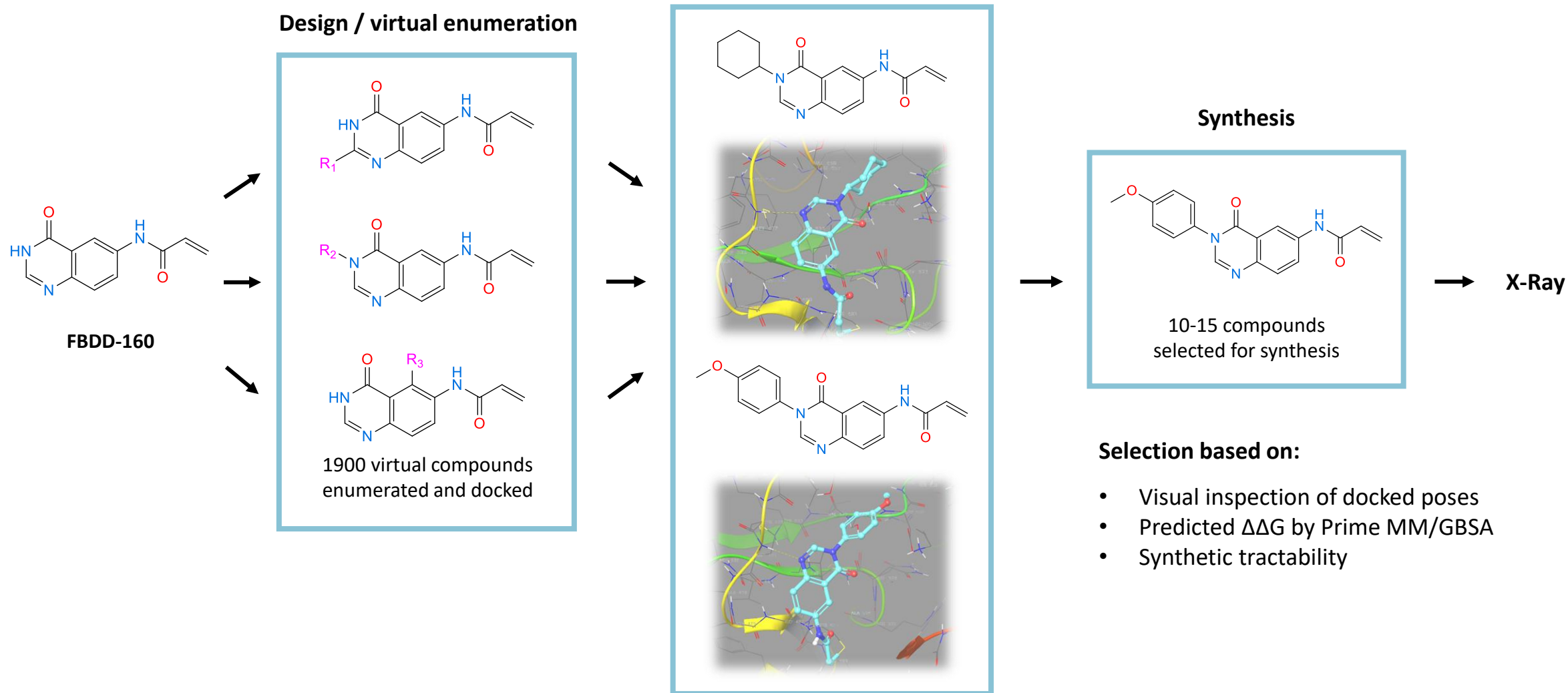
FBDD-160 binds BTK on Cys-481

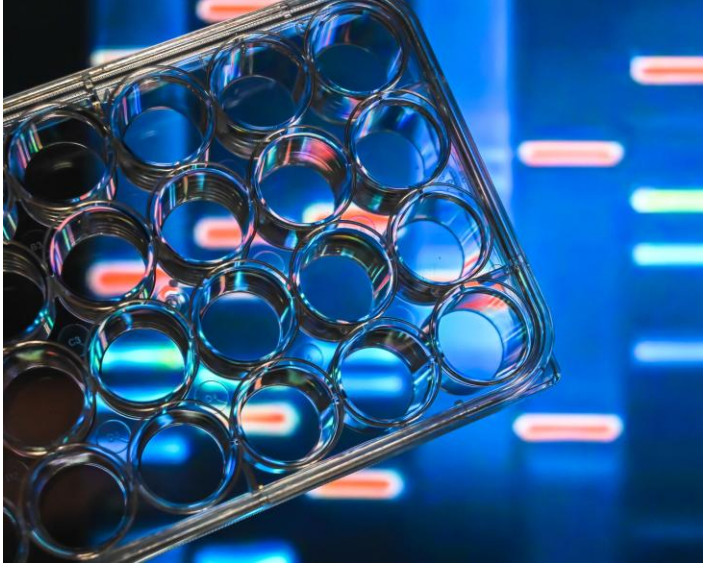


Crystallization by Protein Science and Biophysics team – WuXi AppTec In Vitro Biology Unit

Resolution: 1.6 Å

Fragment Evolution: Medicinal Chemistry





A comprehensive spectrum of biology services and solutions, supporting stand-alone and integrated projects, from target discovery to hit finding, lead optimization, candidate selection, and beyond.

- **Comprehensive** discovery and translational biology centers, with **~3,000** experienced scientists and global footprints in 9 sites
- Early discovery screening platform, providing diverse **hit finding solutions** and **high throughput hit optimization solutions** such as DEL/HTS/HCS/ASMS/FBDD*/Display/virtual screening and Direct-to-Biology, supported by informatics and data sciences
- **Thousands** of validated, 'ready to go', *in vitro* assays and *in vivo* models enabling discovery biology for comprehensive target classes, therapeutic areas and modalities
- **Extensive Oncology, Immunology, Infectious Disease, Inflammation, Neuroscience, Rare disease and Metabolic Disease indication**, offering an end-to-end service from discovery, through optimization and into clinical development
- **AAALAC** accredited and **BSL-2** certified on multiple sites
- Pathology and **CAP-certified** FACS capabilities supporting clinical biomarker services

Modalities	Early Discovery	Lead Optimization	<i>In Vivo</i> Pharmacology
<ul style="list-style-type: none"> • Small molecules • Peptides • Oligonucleotides • Bi-functional molecules, e.g. ADC/PDC/POC/TPD* • Vaccines • Cell Therapy 	<ul style="list-style-type: none"> • New target discovery and mechanistic study • Protein production & structural biology • Screening & hit identification: DEL/HTS/HCS/ASMS/FBDD/Display/VS and libraries for small molecules, covalent, peptides, TPD & macrocycles • High throughput hit optimization: D2B platform 	<ul style="list-style-type: none"> • <i>In vitro</i> biochemistry and cell biology • Cell panel screening • MOA studies • Radiometric assays • Clinical biomarker development and validation 	<ul style="list-style-type: none"> • Comprehensive <i>in vivo</i> disease model collection • Targeted oncology and immuno-oncology • Drug resistance and other novel models of higher translational value • Tumor model database

*DEL: DNA-Encoded Library; HTS: High Throughput Screening; HCS: High Content Screening; ASMS: Affinity Selection Mass Spectrometry; FBDD: Fragment-Based Drug Discovery
 ADC: Antibody-Drug Conjugate; PDC: Peptide-Drug Conjugate; POC: Peptide-Oligonucleotide Conjugate; TPD: Targeted Protein Degradation


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Appendix

Examples Compounds at A Glance

