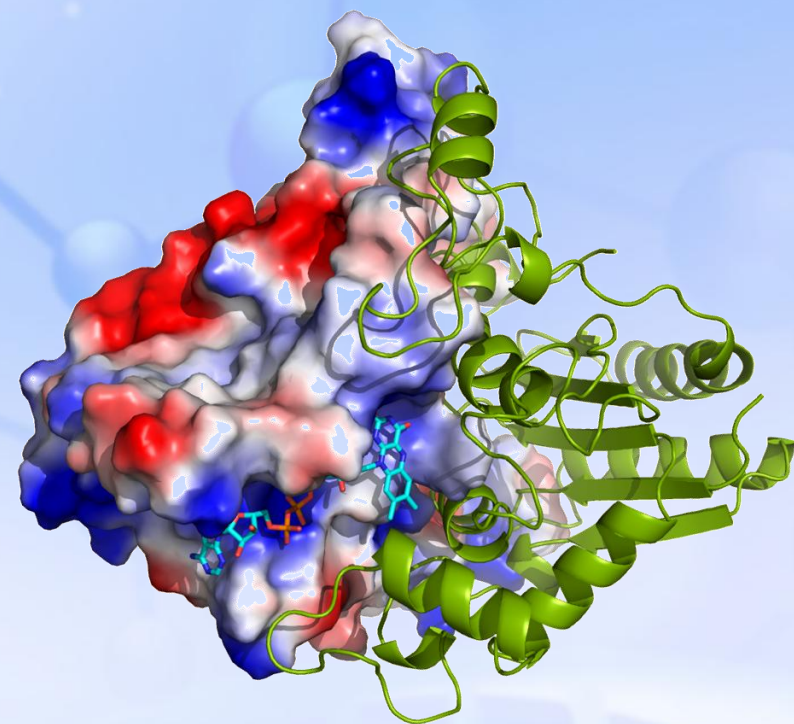


BIORTUS

Hit Discovery & Lead Generation



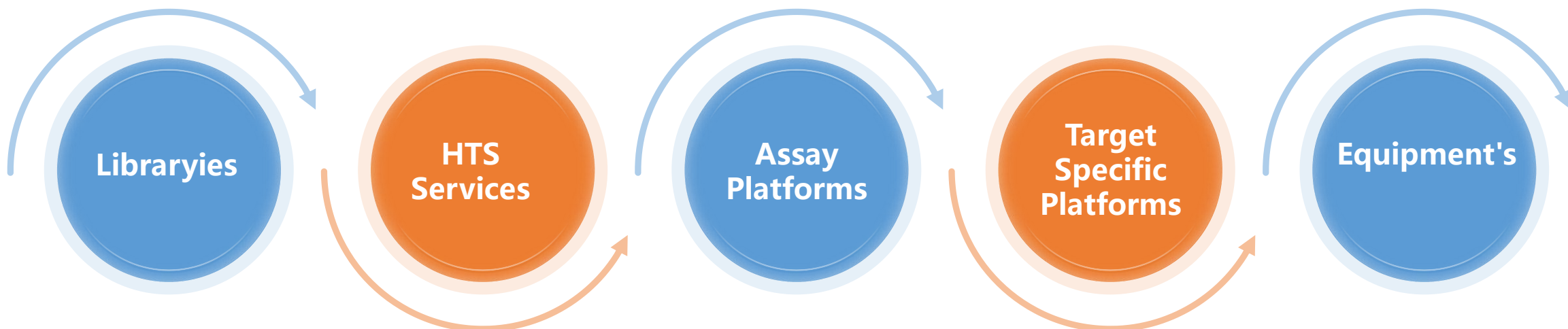
Web: en.biortus.bio



Hit Discovery



Lead generation



Multiple libraries to chose:

- Lead-like
- Covalent
- Fragment
- DEL through collaboration

Comprehensive HTS triage strategy for your target:

- Design, development, validation of primary screening assay
- Design and develop secondary/counter/orthogonal assays
- Screening condition and triage path recommendations

Multiple formats:

- Biophysical assays
- Biochemical assays
- Cell-based assays
- Label free
- High Content Analysis

Mature experiences on target analysis :

- GPCR panel assays
- Kinase panel assays
- DUBs panel assays
- Target Degradation Assays

Well equipped for QC of reagents, binding affinity test, and functional test in low, medium, and high through-put formats.

Fragment Libraries

- 3 Commercially Available (Maybridge, Key Organics, LifeChemicals)
- 1 Proprietary Library
- SDF files available upon request
- Automation Equipment for multiplexing

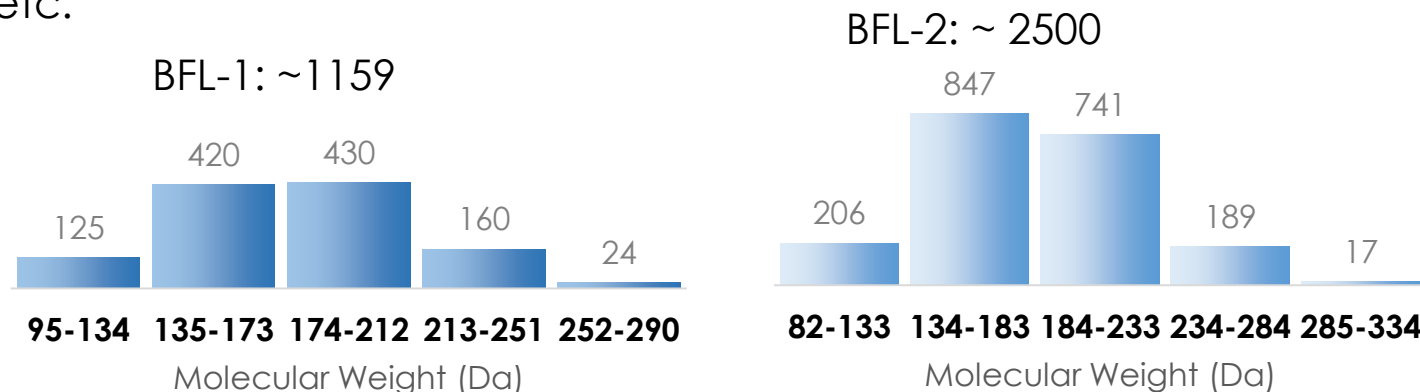
For your pipeline

- 2 Covalent Libraries
- 2 Non-covalent Libraries
- Lead like libraries
- Screening platforms can also use client provided libraries

BIORTUS

Noncovalent Libraries: 100mM DMSO Stocks. Ro3 compliant

Screening Methods: SPR, DSF, Crystallography based, etc.



Covalent Libraries:

Screening Methods: Intact MS

BFL-3: Functional groups targeting key amino acids
(Lys, Cys, Ser, Asp, Glu, His, Tyr)

BFL-4: Cysteine focused Acrylamide Warheads (**Unique to BiorTus**)

Automation Equipment

Echo 650



Apricot S2



Multidrop combi

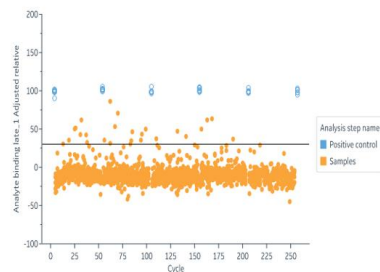
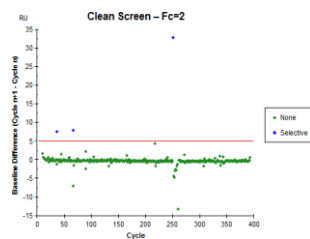


Dragonfly

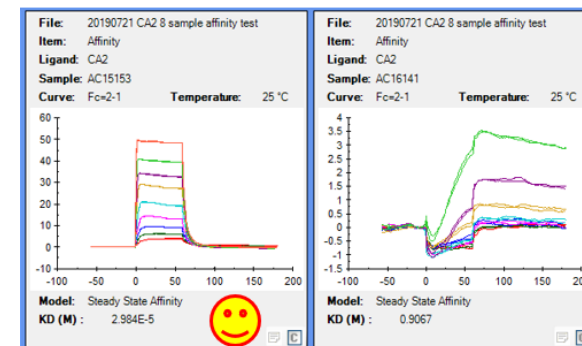
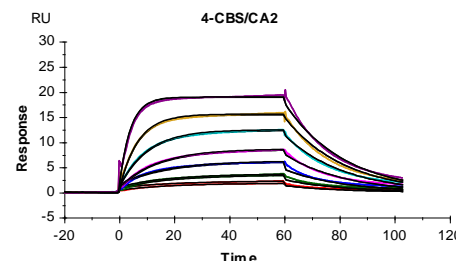


Fragment library Screen Using SPR

Fragment Library, 764



SPR screening campaigns

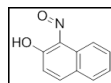
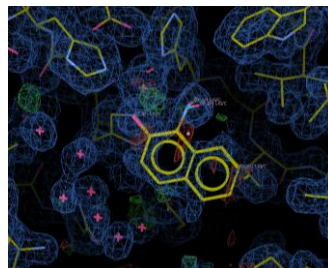


759 after Clean Screen tested in SPR

14/759 CRC for Binding Screen

SPR platform

- ✓ Protein-protein interaction
- ✓ Antibody - Antigen
- ✓ Protein - Small Molecule
- ✓ Protein - Nucleic Acid
- ✓ Nucleic Acid - Small Molecule
- ✓ GPCR - Small Molecule

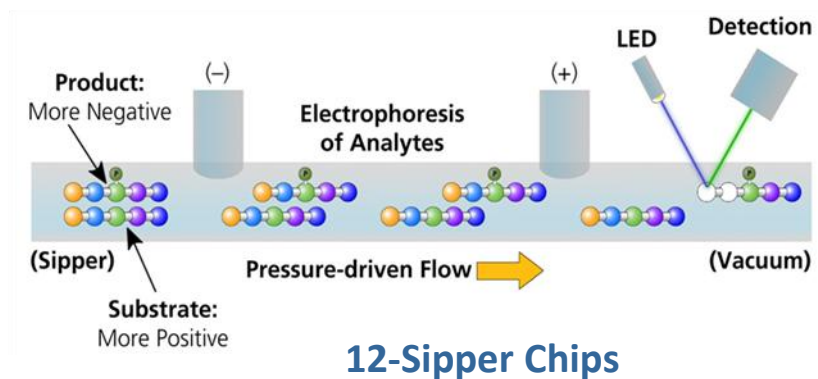


5/5 Co-crystallized with CA2 (1.44-1.52Å)

Selected top 5/14 to co-crystal

Start to finish: 1-2 months

Fragment Screening Using Caliper Assay



1338 fragment screened using Caliper assay, single point

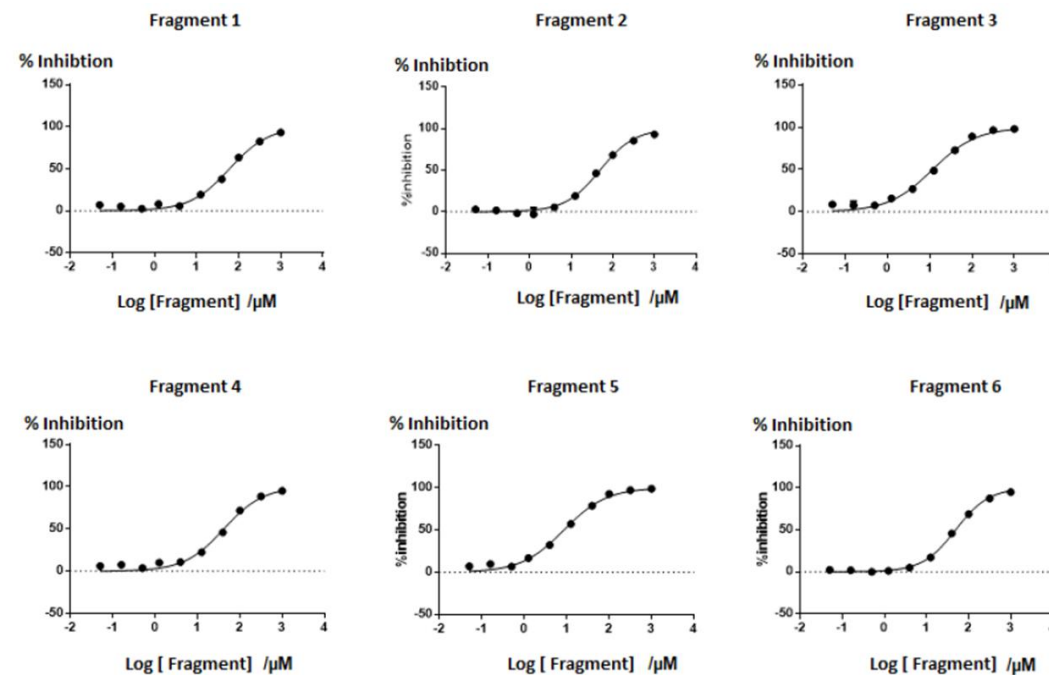
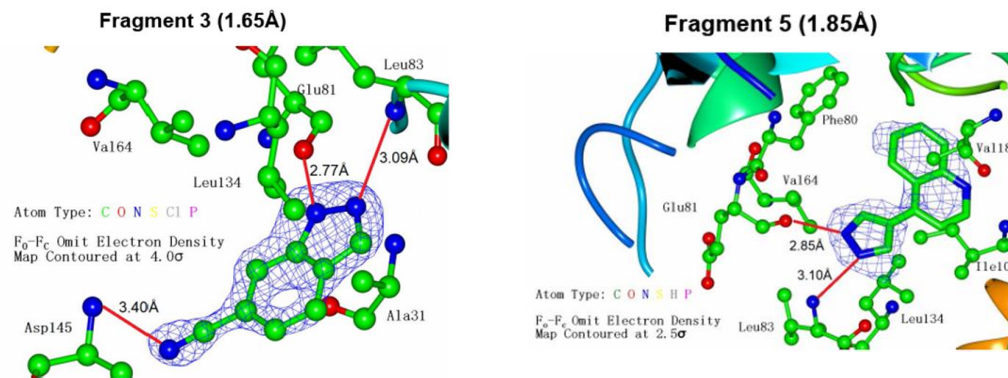
4.8% Hit rate

65 tested in concentration response

97% Confirmed

63/65 confirmed in CRC

4 selected for co-crystal



Fragment Screening Using Biochemical Assays

FP Assay

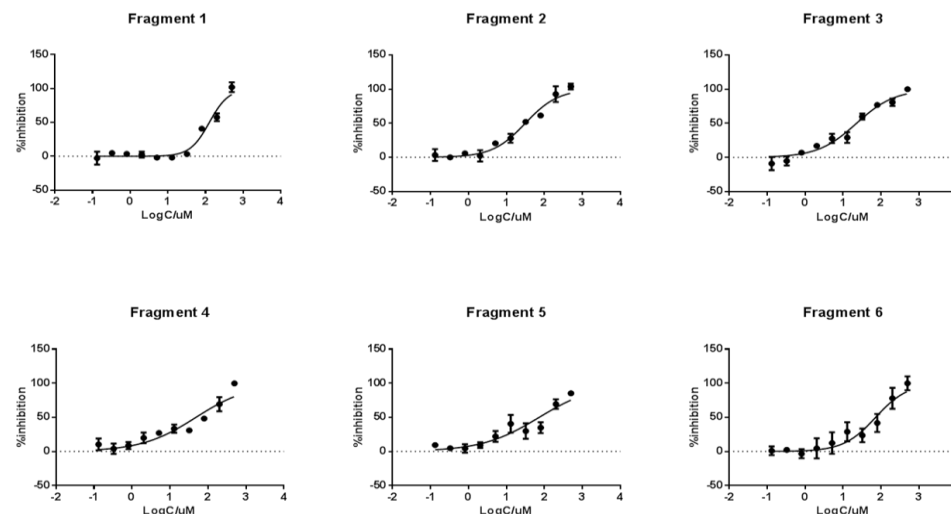
1338 fragment screened with FP assay, single point

1.2% Hit rate

14 tested in concentration response

100% Confirmed

14/14 confirmed in CRC



FI Assay

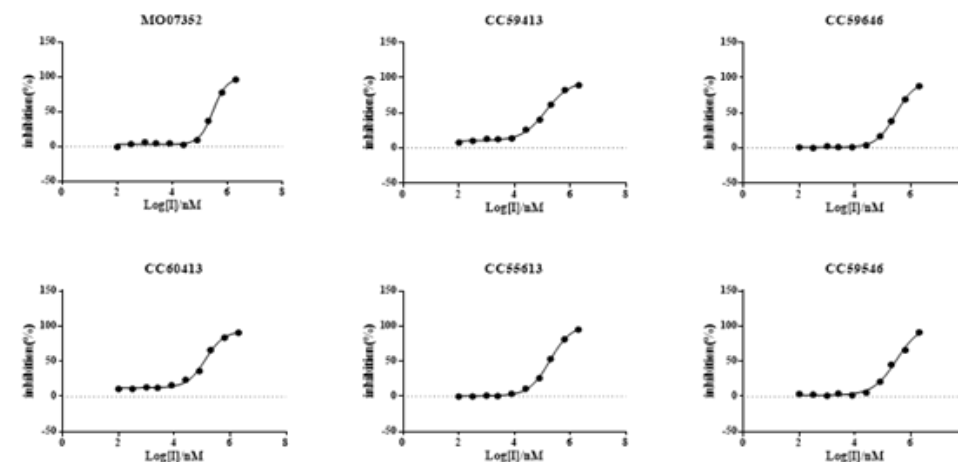
3659 fragment screened with FI assay, single point

1.2% Hit rate

45 tested in concentration response

71% Confirmed

32/45 confirmed in CRC



Fragment Screening Using LC-MS

BIORTUS

Covalent BFL- 4

1259 fragment screened with LC-MS assay, single point

175 hits with >20% modification ↓ 13.9% Hit rate

175 for confirmation & testing in counter screens

98/175 confirmed ↓

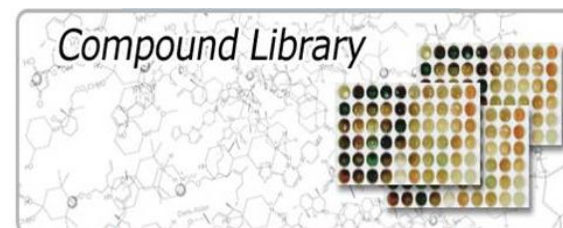
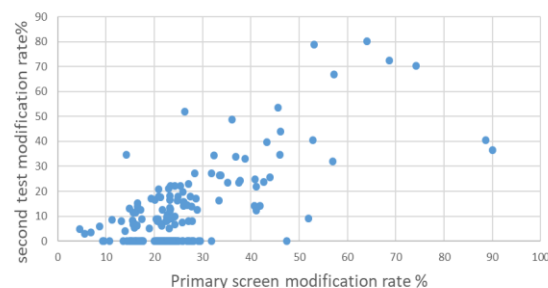
Selected 20 to test in 3 protein (wt & mutants)

7/20 selected for TSA

8/20 selected for peptide mapping

2 positives for co-crystal

modification rate of 175 compounds primary screen and re-test



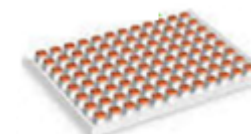
BFL-4: ~1200 covalent fragments



Echo 650



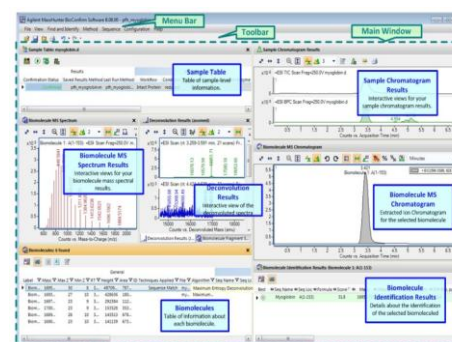
Dragonfly



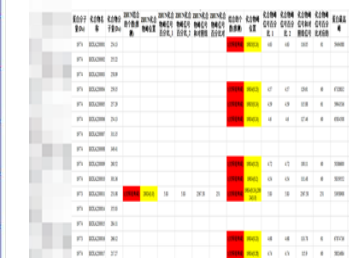
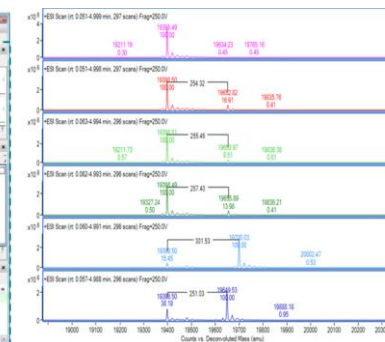
Compound-protein reaction



Agilent 6230B TOF



Masshunter BioConfirm 10.0 software



Biortus self-developed data analysis software

General Workflow for FBLG at Biortus

Reagent production

- ✓ Protein generation
- ✓ Stable cell line generation
- ✓ Reagent labeling

Screening

- Primary screening
 - Confirmation in CRC
 - Orthogonal assays

Structure Confirmaiton

- Co-Crystallization

Assay development & validation

~1 - 2 weeks

~ 4 -8 weeks

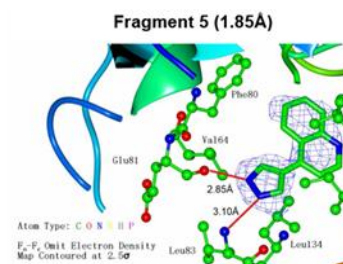
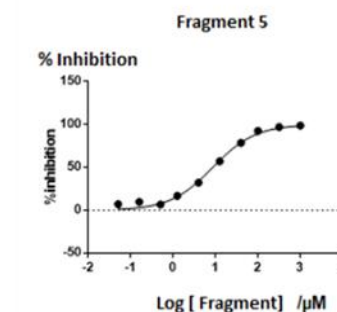
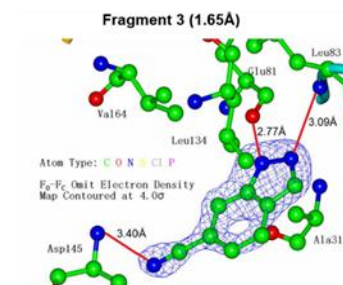
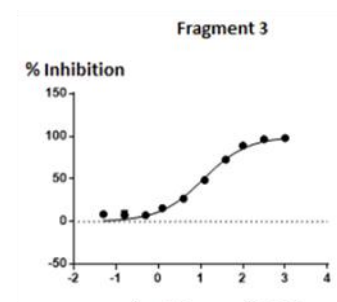
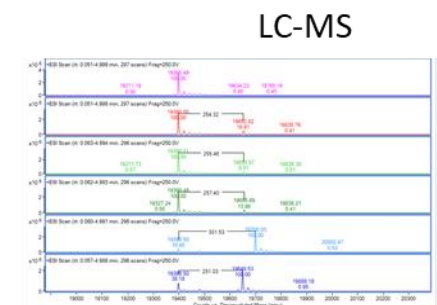
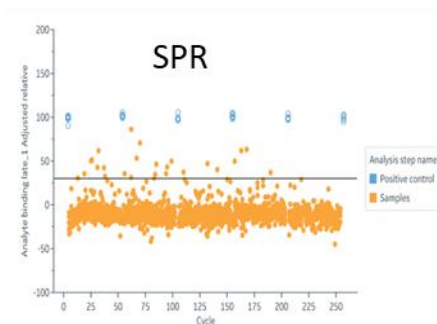
Screening Libraries

- ✓ Fragment libraries
- ✓ Covalent libraries
- ✓ Lead like diverse library
- ✓ Focused libraries

Assay formats for fragment screening:

- SPR
- LC/MS
- TRIC
- FP, FI
- Caliper
- TSA/DSF

>50 screens run



Partial Equipment List

Automation for routine and panel screening



Echo 650



Apricot S2



Multidrop combi



Dragonfly

Multiplate reader



TECAN M1000



TECAN F200



TECAN Spark



Envision



EZ Reader (x2)

Thermal shift assay



Prometheus
NT.Plex



7500 Fast



Light Cycler 480

SRP/TRIC for biomolecular interaction



Biacore S200



Biacore 8K+(x2)



Octet
RED96



Dianthus NT.23 Pico Duo

Protein and compound analysis



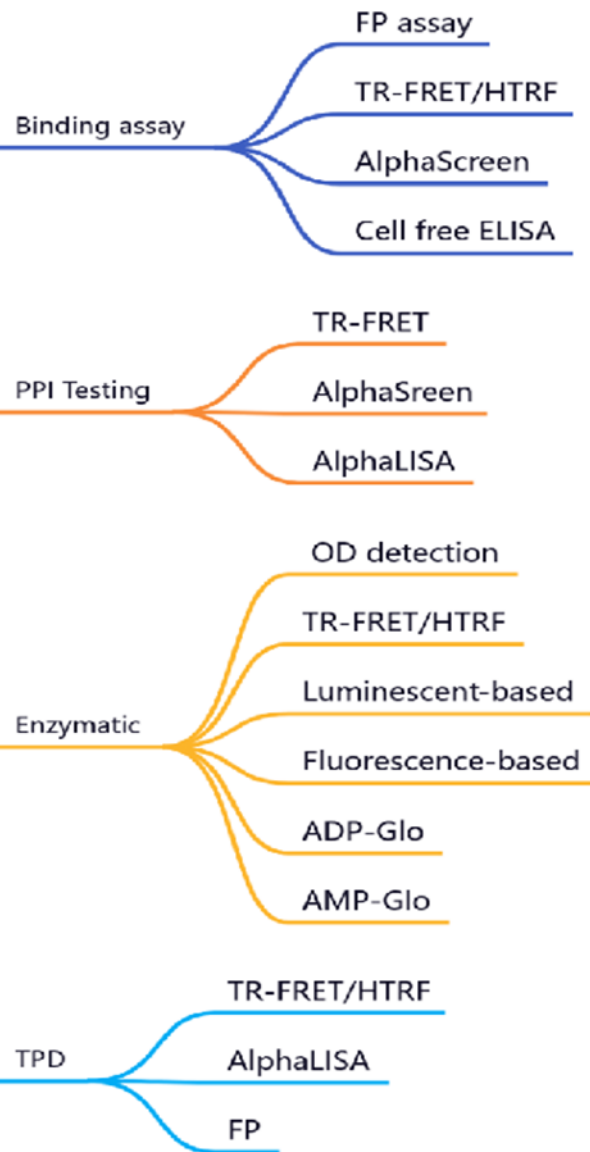
Agilent 6230 TOF



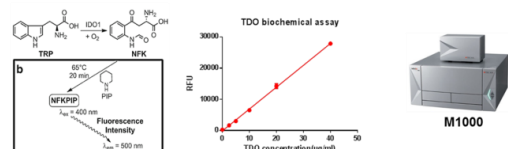
Refeyn TwoMP

Biochemical Assays

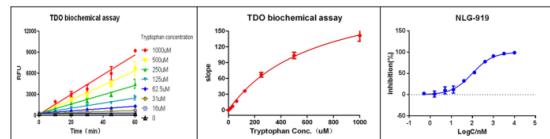
Biochemical Assays



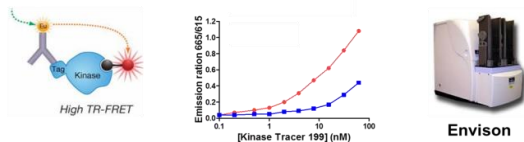
Fluorescence Intensity Assay



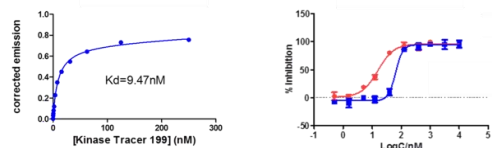
M1000



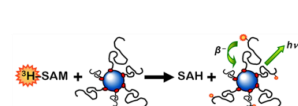
TR-FRET Assay



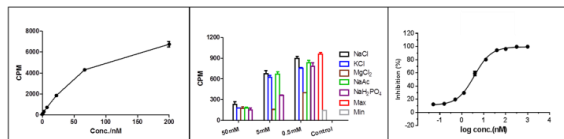
Envision



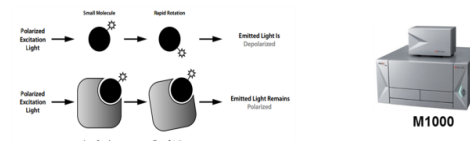
Radiometric Assay



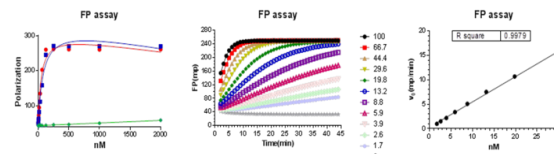
Top count



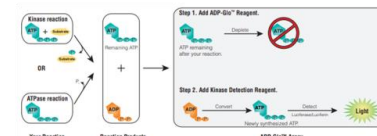
FP Assay



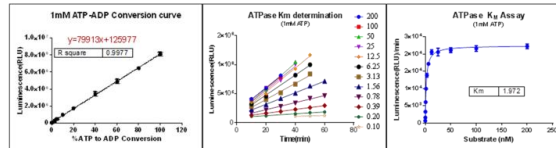
M1000



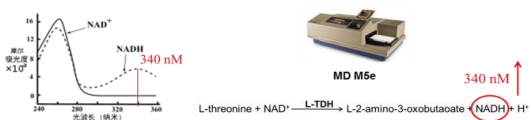
Chemiluminescent Assay



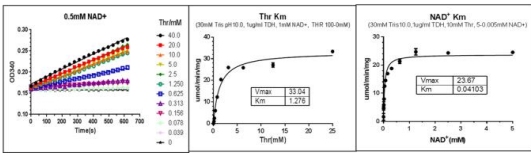
F200 pro



UV-Vis Spectra Assay



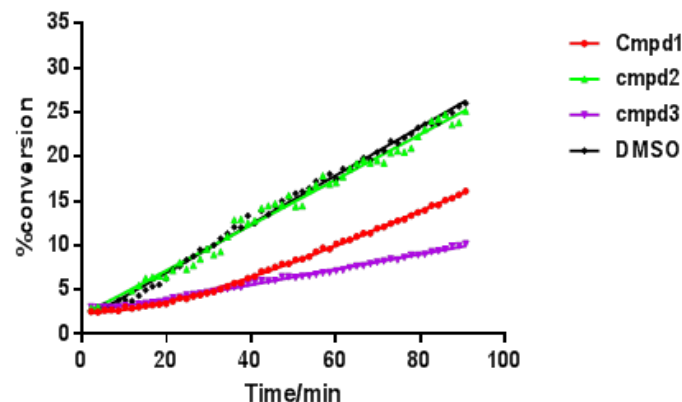
MD M5e



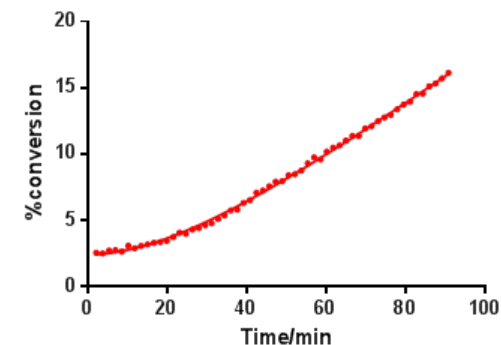
Drug Residence Time Evaluation

- The time a drug spends in contact with its biological target is known as residence time (the inverse of the kinetic constant of the drug-target unbinding, $1/k_{off}$).
- The drug (un)binding kinetics is thought to be correlated to in vivo efficacy.

Jump dilution



cmpd 1

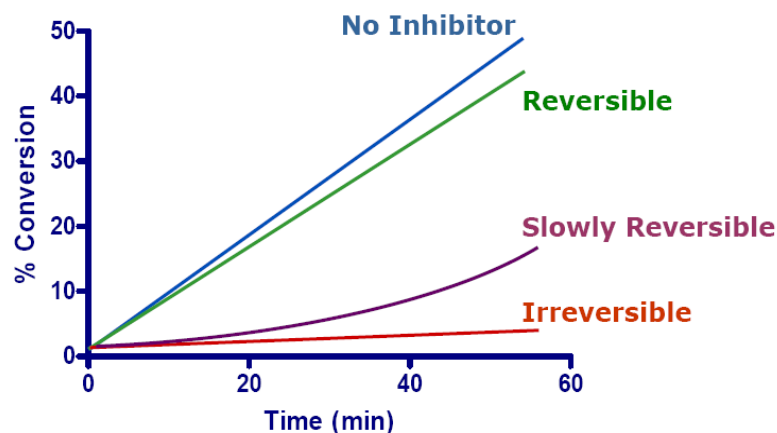


100 × Enzyme + Compound at 10 × IC₅₀ (91% Inhibition)

Incubate

Dilute 100-fold into reaction buffer containing 1 × substrate (9% Inhibition)

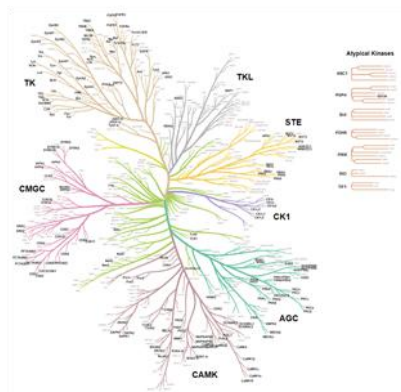
Generate progress curve



	K _{off} , Min ⁻¹	Tau, min	t _{1/2} , min
Cmpd1	0.03873	25.8	17.9

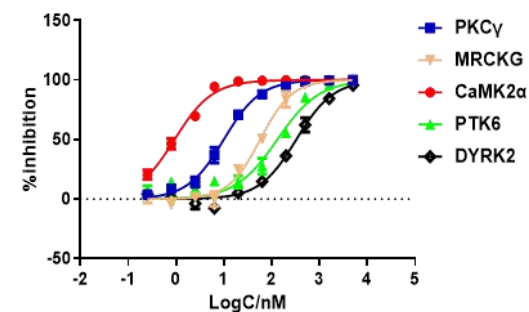
● **Conclusion:**
 Cmpd2: fully reversible
 Cmpd1: slow reversible
 Cmpd3: irreversible

Target specific assays/platforms – Kinase panel

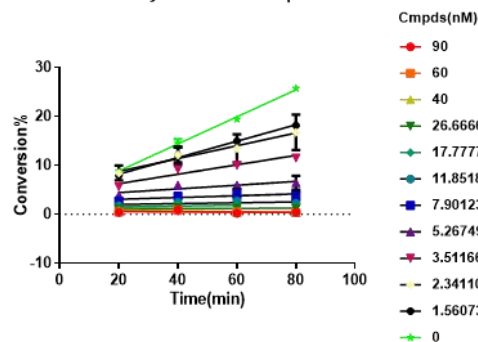


- A total of ~300 kinases available
- MSA (Caliper) assay ready for ~300 targets (wt/mutant)
- ADP-GLO assay ready for >100 targets
- SPR assay ready for >60 targets

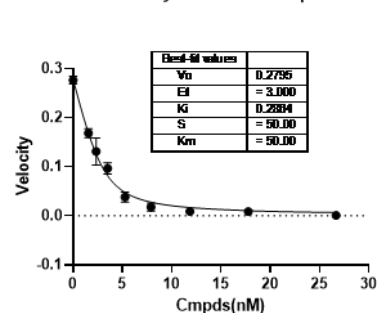
Reference compound selectivity on kinases



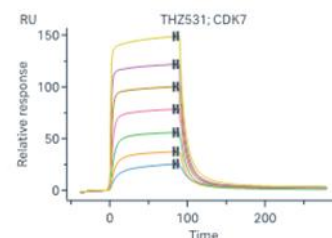
CDK7/Cyclin H/MAT1 K_i Test



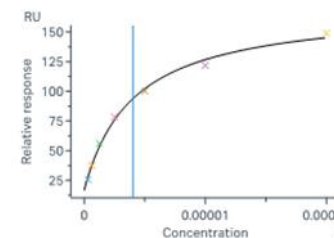
CDK7/Cyclin H/MAT1 K_i Test



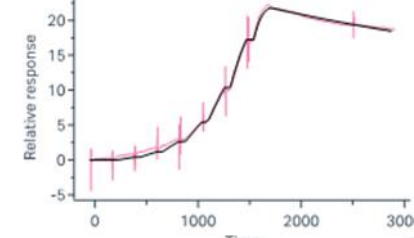
Steady State Affinity



Steady state affinity with fitted Rmax; KD=4.03e-06; Rmax=154.2



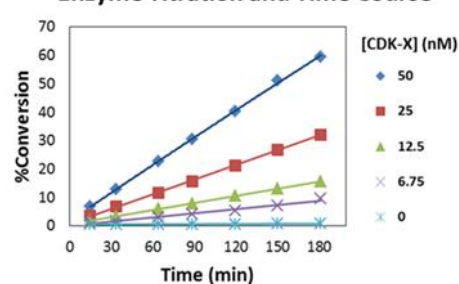
dasatinib; BMX; 1:1 binding



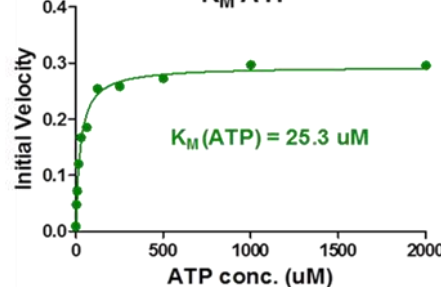
1:1 binding; ka=1.64e+06; kd=1.64e-04; Rmax=22.5; KD=9.95e-11

- CDK Kinase panel (30+)
- EGFR Panel
- AGC (31)
- CAMK (38)
- CK1 (7)
- CMGC (49)
- STE (15)
- TK (108)
- TKL (3)
- Others (12)

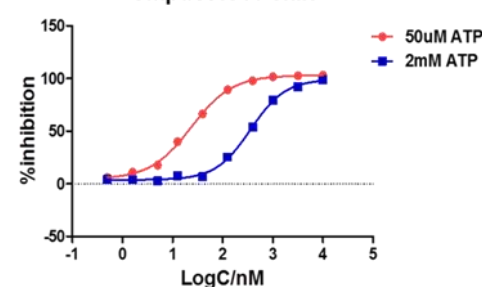
Enzyme Titration and Time Course



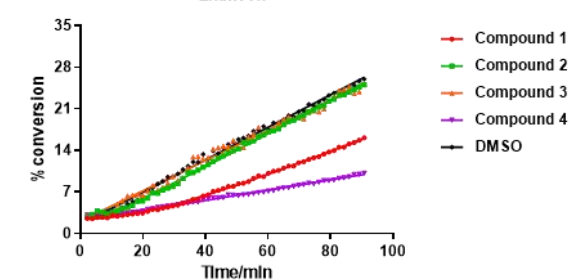
K_M ATP



Cmpd-X IC50 shift

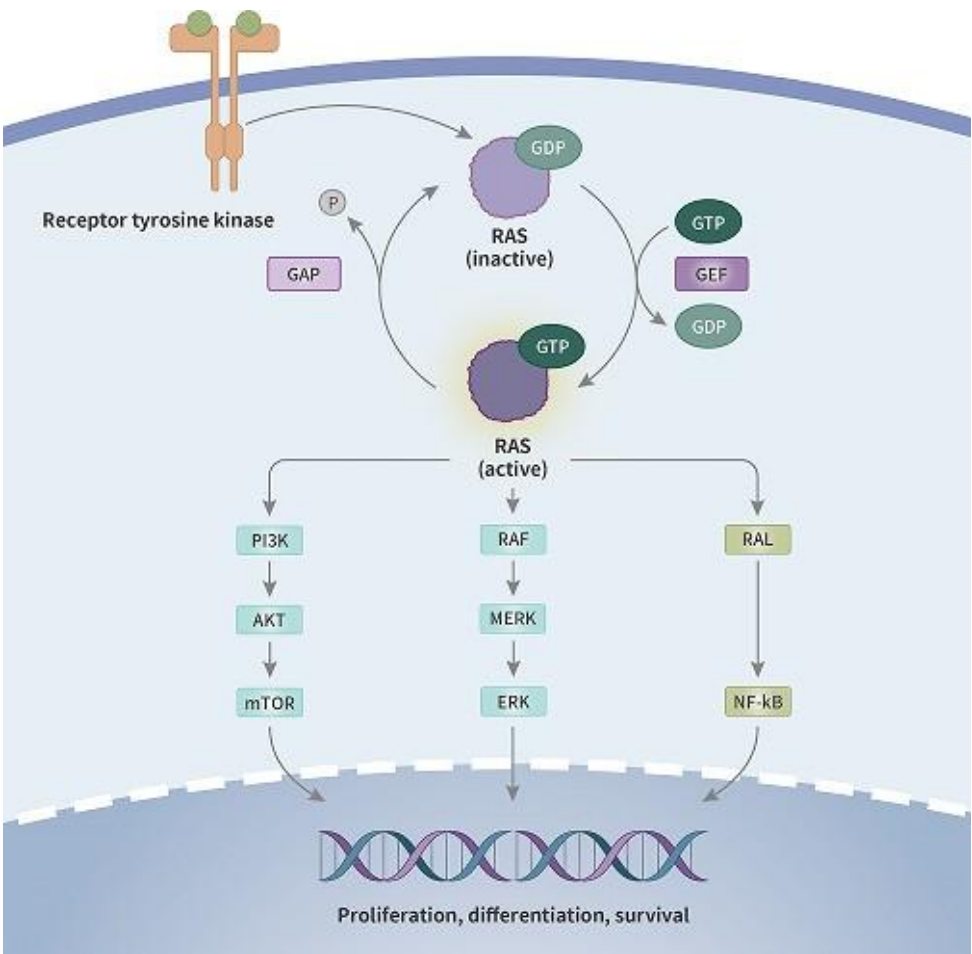


CDK7 Jump Dilution
2mM ATP





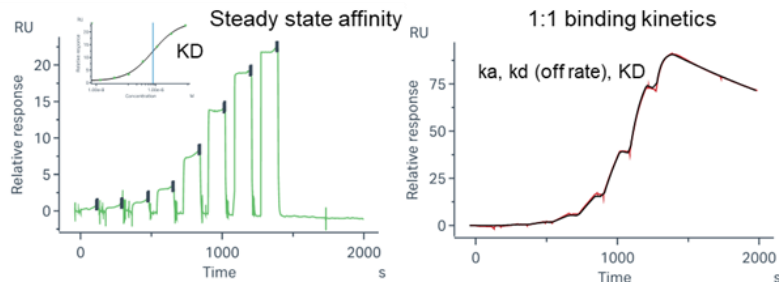
Ras Family



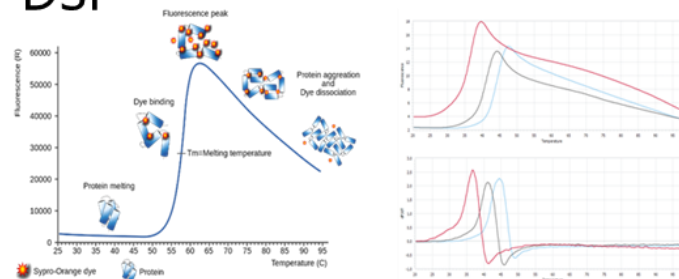
Biortus Protein		Uniprot ID	Modification	Protein	NanoDSF	LC-MS	SPR	Structure
KRas	WT	P01116	Biotinylated/ GDP loaded/ GMPPNP loaded	✓	✓	✓	✓	✓
	G12A			✓	✓	✓	-	-
	G12C			✓	✓	✓	✓	✓
	G12S			✓	✓	✓	-	-
	G12V			✓	✓	✓	-	✓
	G12R			✓	✓	✓	-	✓
	G12D			✓	✓	✓	✓	✓
	G13D			✓	✓	✓	-	-
	Q61K			✓	✓	✓	-	-
	Q61R			✓	✓	✓	-	-
	Q61H			✓	✓	✓	-	-
	H95L			✓	✓	✓	-	-
	H95R			✓	✓	✓	-	-
Nras	WT	P01111		✓	✓	✓	✓	✓
	G12C			✓	✓	✓	✓	-
	G12D			✓	✓	✓	-	-
	G13D			✓	✓	✓	-	-
	Q61L			✓	✓	✓	-	-
	Q61H			✓	✓	✓	-	-
	Q61R			✓	✓	✓	-	✓
	Q61K			✓	✓	✓	-	✓
	L95H			✓	✓	✓	-	-
	HRas			WT	P01112	✓	✓	✓
G12V		P01112		✓	✓	✓	-	

Binding Assays

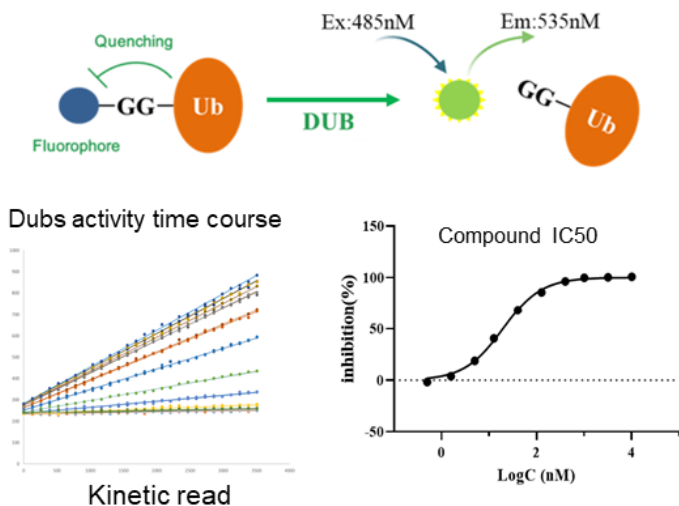
SPR



DSF

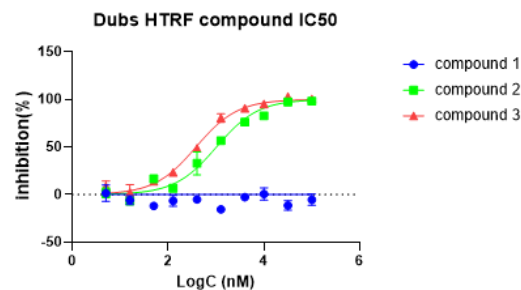


Functional Assay

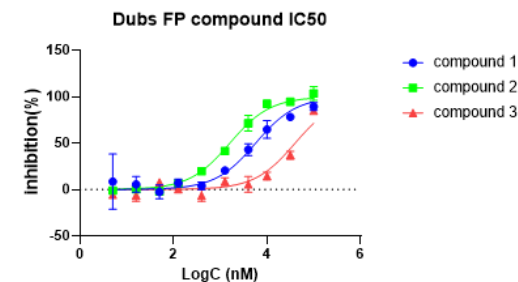


Displacement Assays

HTRF



FP



Active Deubiquitinase enzymes

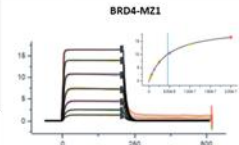
USP1	USP51
USP2	UHL1
USP3	UHL3
USP5	UHL5
USP7	BAP1
USP8	OTUD1
USP9X	YOD1
USP10	OTUD6A
USP15	OTUD7B (Cezanne-1)
USP16	ZRANB1
USP20	VCPIP1
USP21	ATXN3L
USP25	ATXN3
USP28	JOSD1
USP30	JOSD2
USP32	SEN2
USP37	SEN5
USP40	SEN6
USP48	SEN8

- 38 biologically active DUBs for Custom Assay Development and HTS.
- 18 ready to use assay kits for HTS and validation.

Assays for PROTAC Drug Discovery

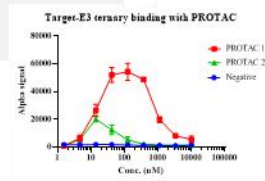
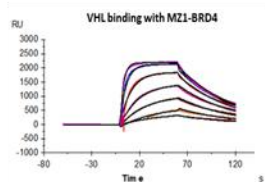
Binary binding assay:

- SPR
- TR-FRET
- ELISA
- FP
- DSF

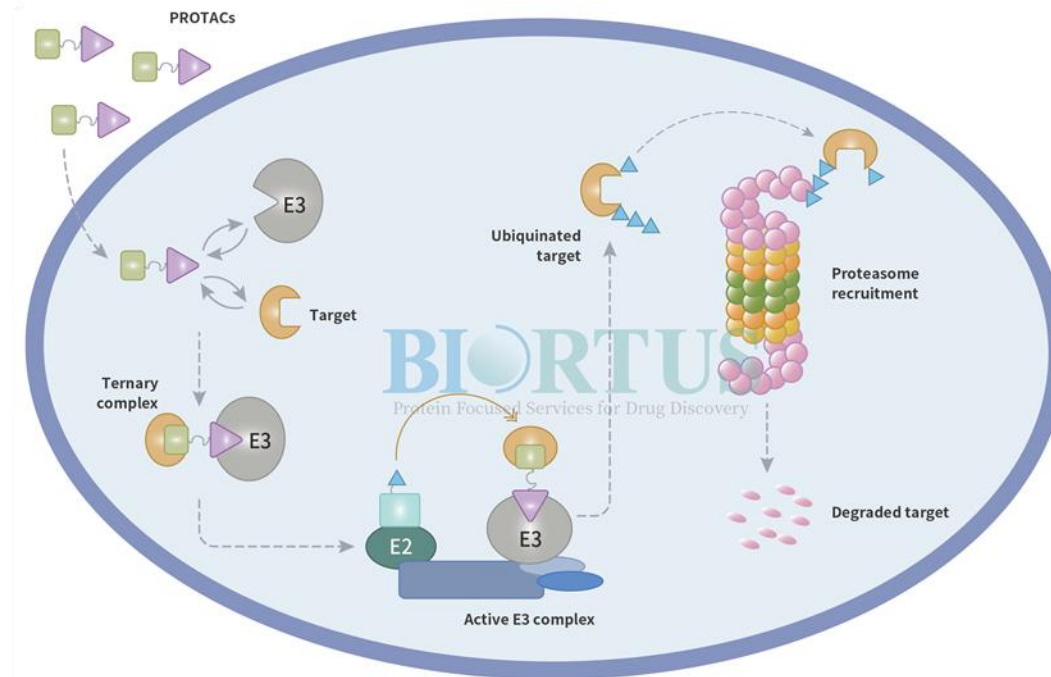


Ternary binding assays:

- AlphaLisa / AlphaScreen
- SPR
- TR-FRET



- 8 E1 (ubiquitin-activating enzyme)
- 36 E2 (ubiquitin-conjugating enzyme)
- 178 E3 (ubiquitin-protein ligase)

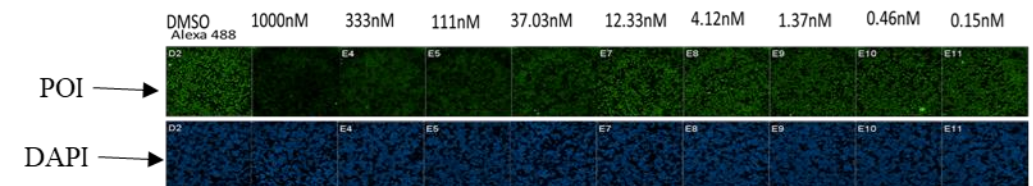
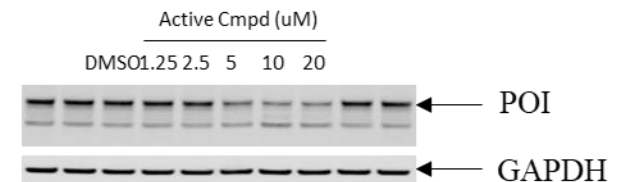


Target ubiquitination assays:

- WB
- TR-FRET
- AlphaLisa / AlphaScreen
- NanoBRET

Cellular assays:

- HCS, WB for target degradation
- qPCR for mRNA level detection
- NanoBRET & NanoLuc for PPI and ubiquitination
- CETSA (split NanoLuc)



➤ Assay types

01

NanoDSF/Thermal Shift Assay (TSA)

02

Surface Plasmon Resonance (SPR)

03

Octet Fortebio (BLI)

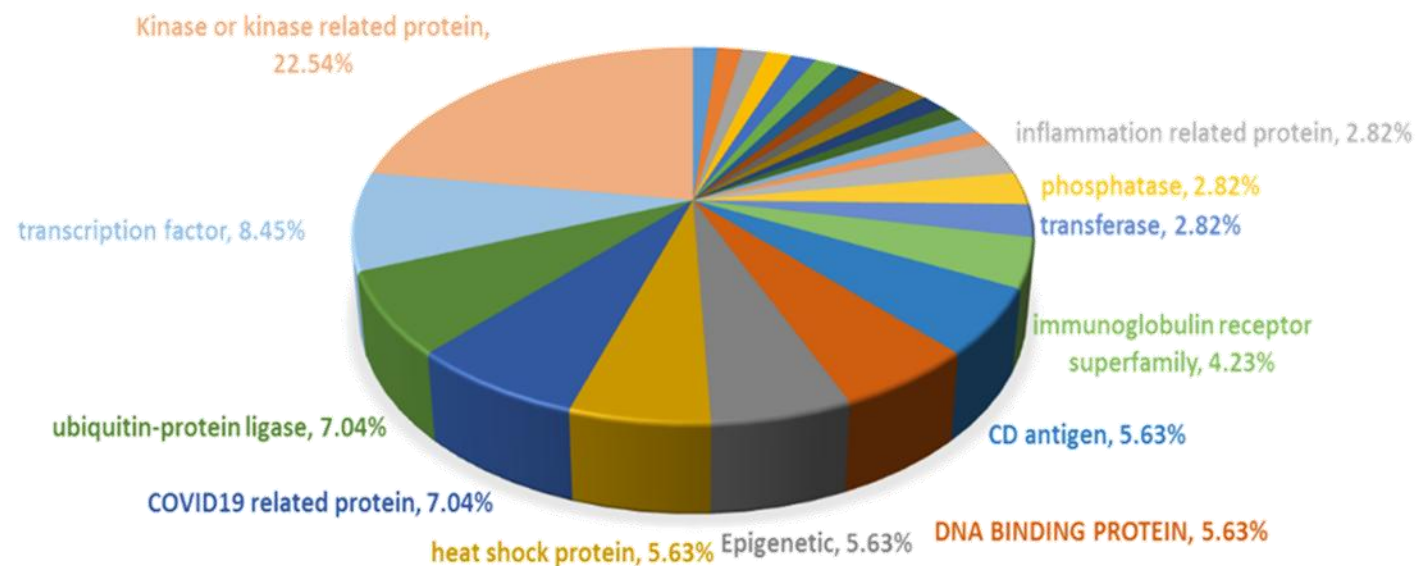
04

Temperature Related Intensity Change (TRIC)

05

Mass Photometry

➤ Types of proteins tested



Light Cycler® 480 II



Prometheus NT.Plex



Octet RED96



Biacore S200



Biacore 8K+



Refeyn Two^{MP}



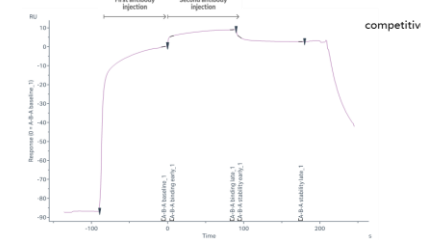
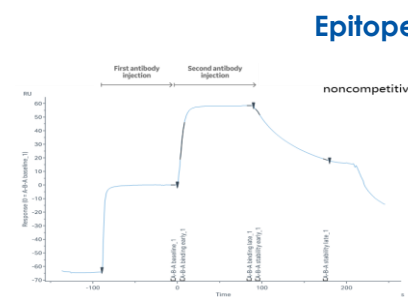
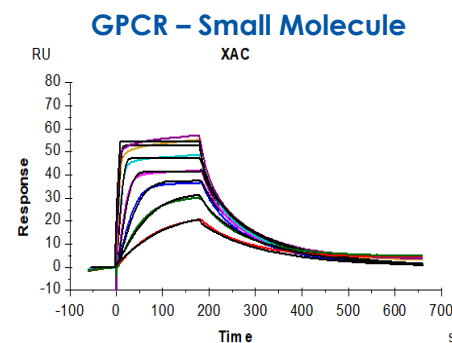
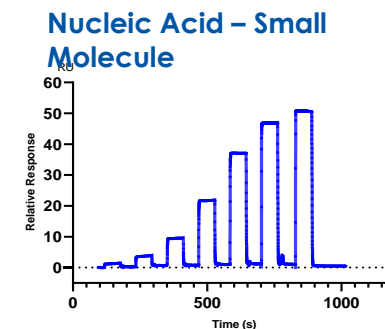
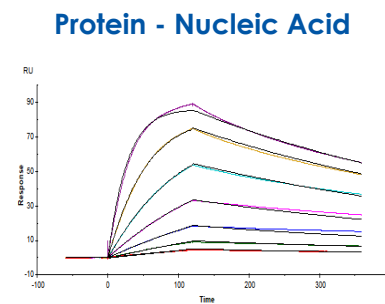
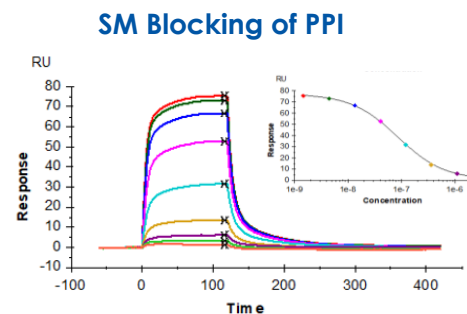
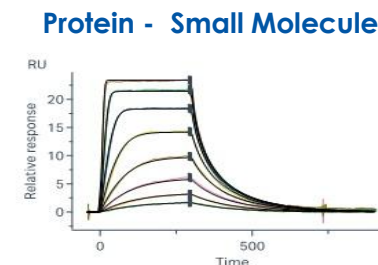
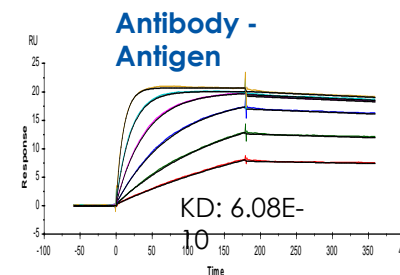
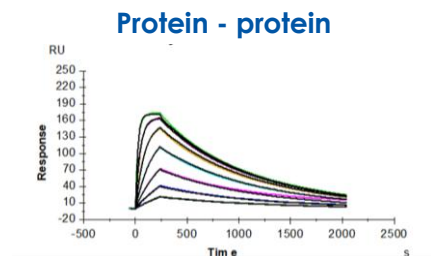
Dianthus NT.23 Pico Duo

SPR

- Affinity and Kinetics determinations
- Competition Assays
- 2 – Biacore 8k+
- 1 – Biacore S200
- CM5, SA, CAP, Protein A/G, NTA chips

For your pipeline

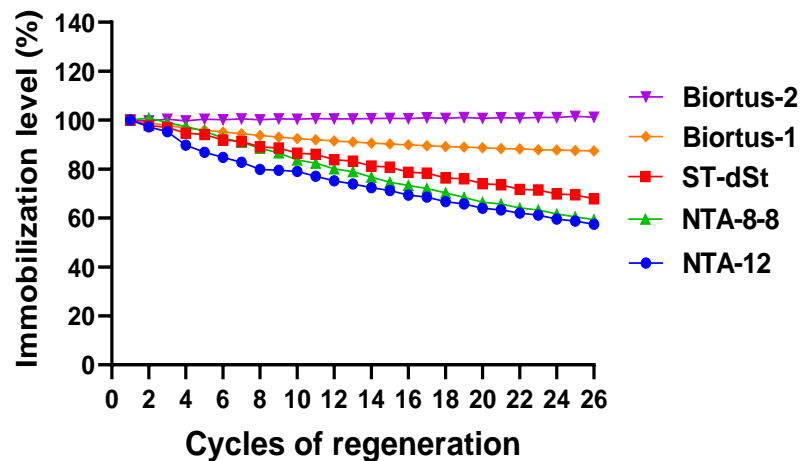
- Experienced in wide range of targets
- Fragment Screening Capability
- Assay Transfer or *De novo* Development



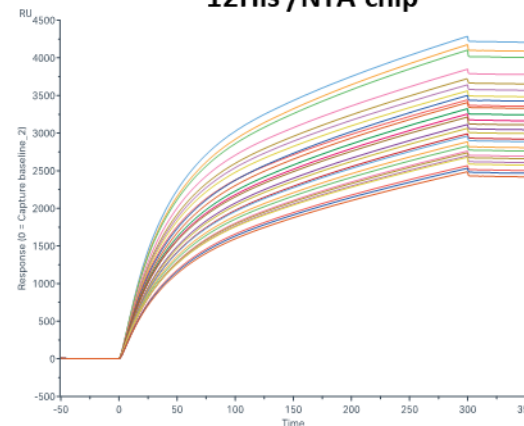
Biortus Reusable SPR Chip



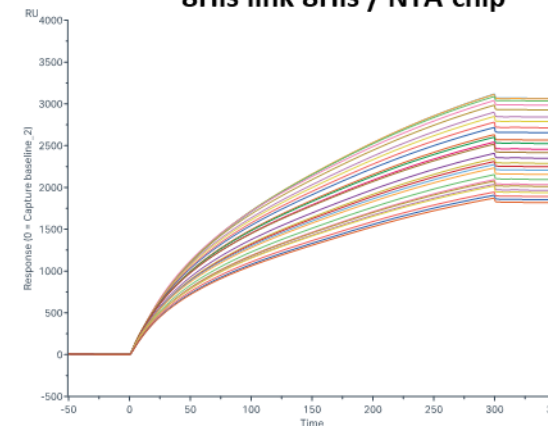
Regeneration stability of Biortus Chip



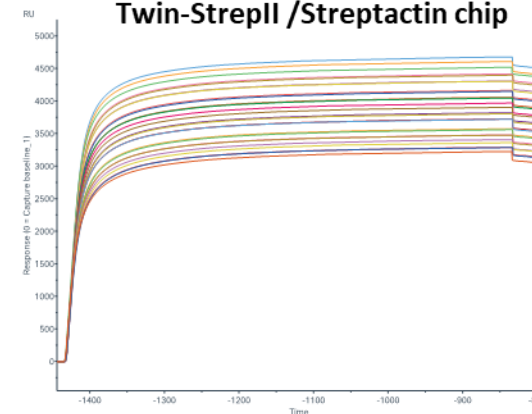
12His /NTA chip



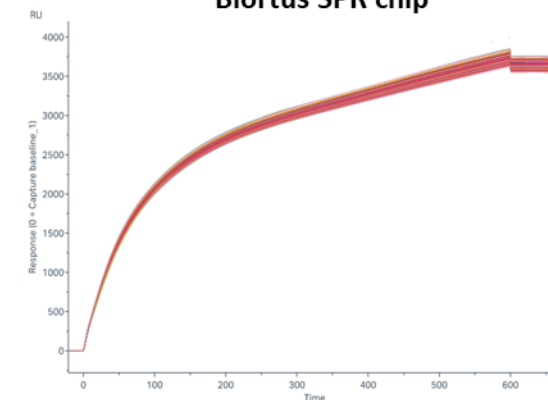
8His link 8His / NTA chip



Twin-StrepII /Streptactin chip



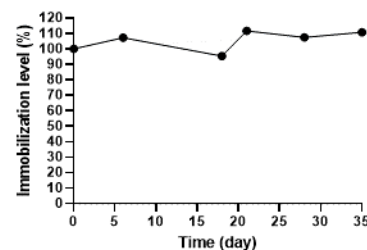
Biortus SPR chip



Advantage of Biortus SPR Chip:

- Reusable for >25 cycles
- Stable for > 1 month storage
- Cost effective
- Feasible for unstable target
- Patent covered

Biortus SPR chip is stable after >1 month storage in ethanol

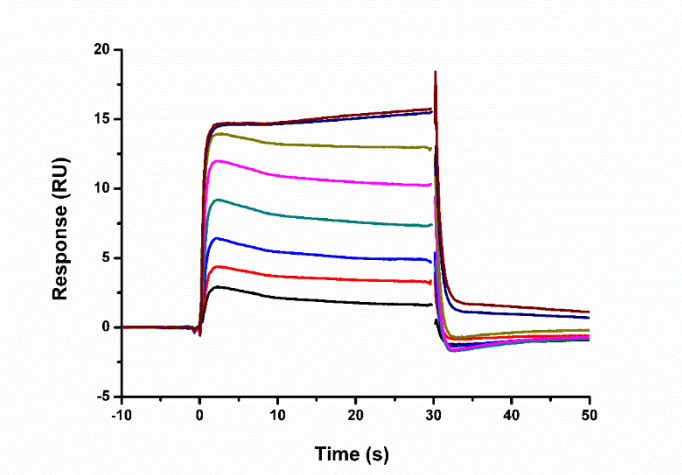
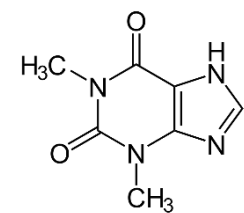




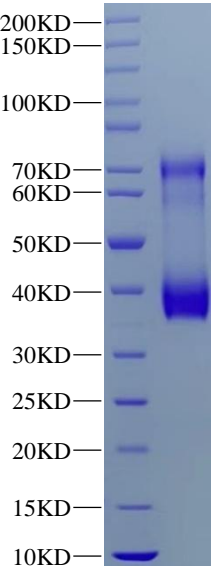
Biophysical assay-SPR

- SPR assay for A2AR

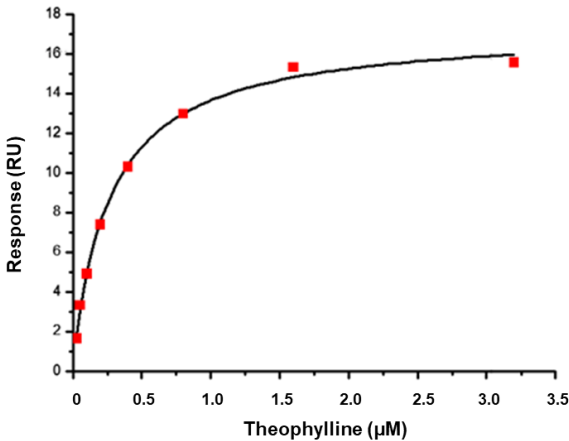
Theophylline
180 Da



M A2AR



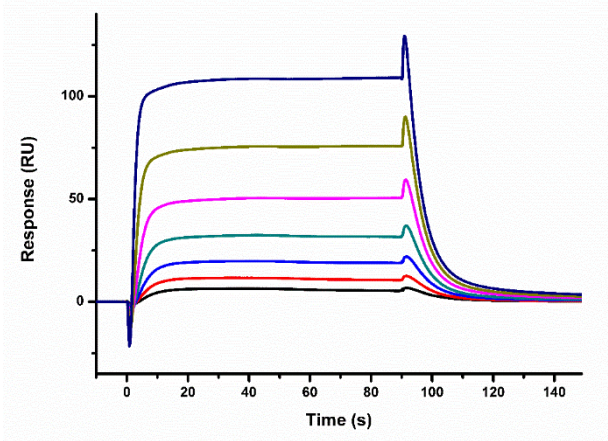
Kinetics: very fast



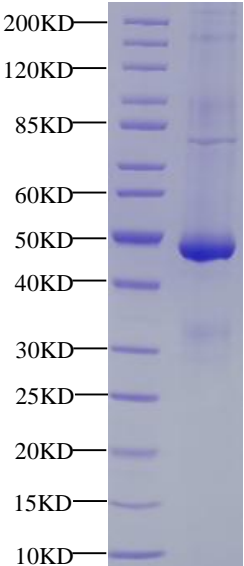
Affinity: ~2.7 μM

- SPR assay for APJR

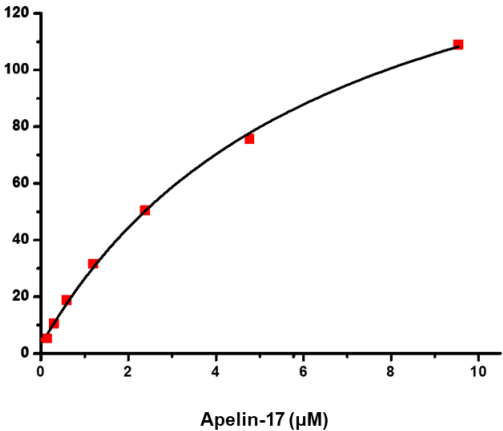
Apelin-17



M APJR



Kinetics: very fast



Affinity: ~6.89 μM

Biophysical assay-TRIC

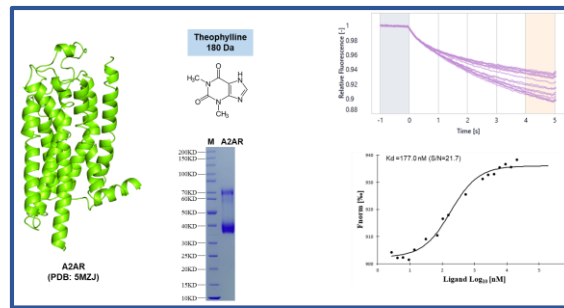
- TRIC (Temperature Related Intensity Change) assay



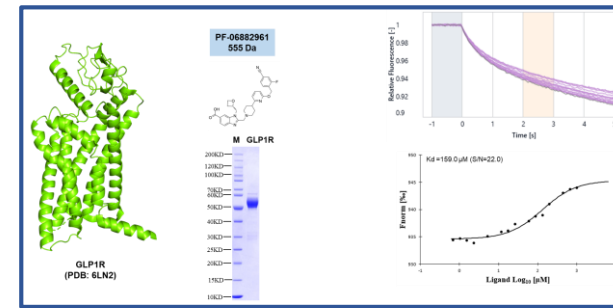
Dianthus NT.23 Pico Duo

Compared to Traditional MST,
TRIC:

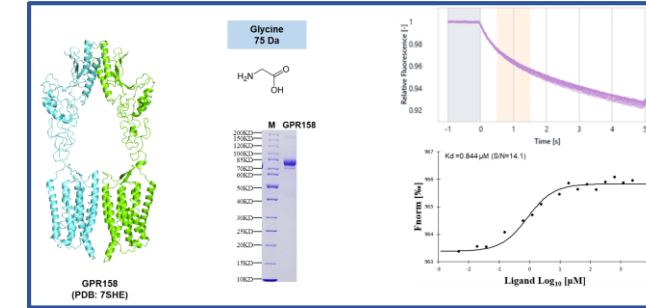
- Uses less protein
- Allows more conditions (384wells)
- Higher throughput
- Done in as little as 30 min
- Less background



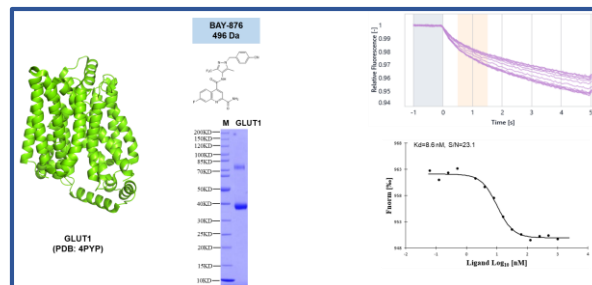
A2AR (Class A GPCR)



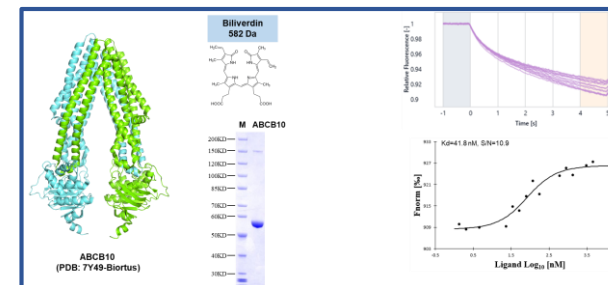
GLP1R (Class B GPCR)



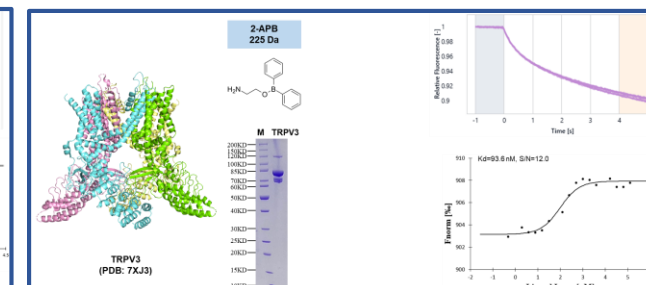
GPR158 (Class C GPCR)



GLUT1 (SLC)



ABCB10 (ABC transporter)



TRPV3 (Ion channel)

Fragment Screening of A2AR

Virtual Screening (2 days)

1. Docking of Biortus Fragment Library, 3,659 fragments.
2. 60 top ranking fragments were selected for TRIC assay.

Assay development (1-2 weeks)

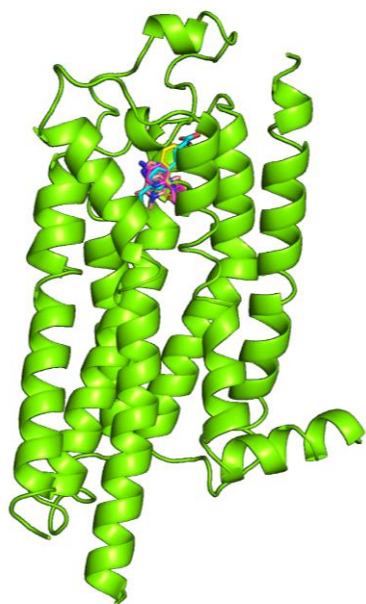
1. Affinity test: Test the affinity of positive control towards labeled target.
2. Buffer optimization: Select suitable buffer for fragment screening as needed.

Preliminary Screen (1-2 weeks)

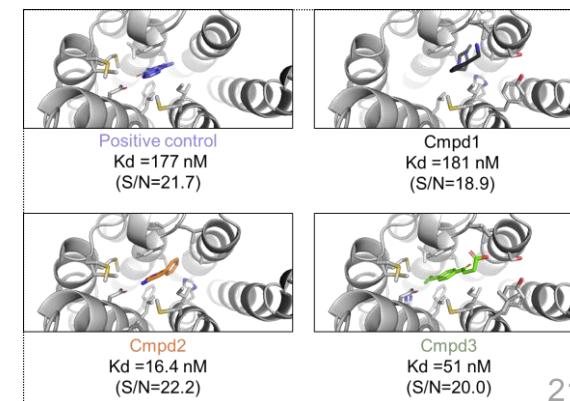
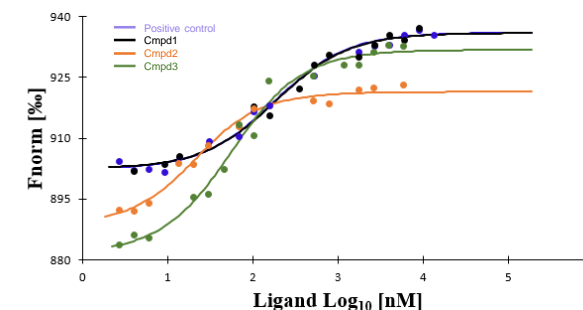
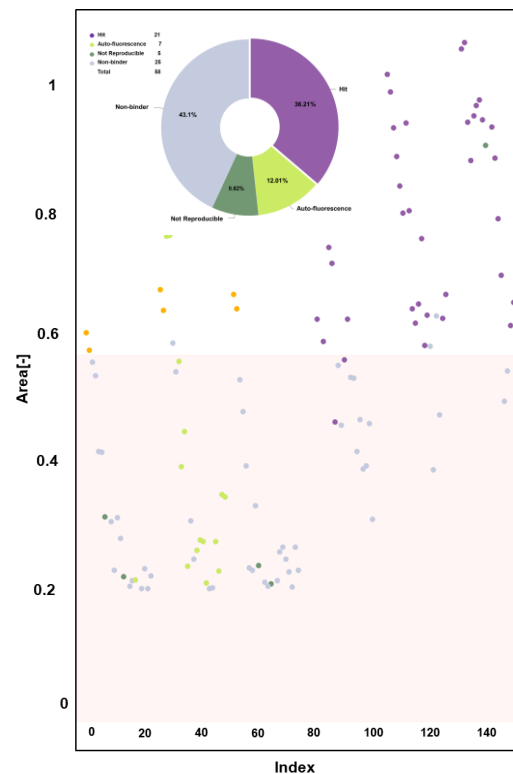
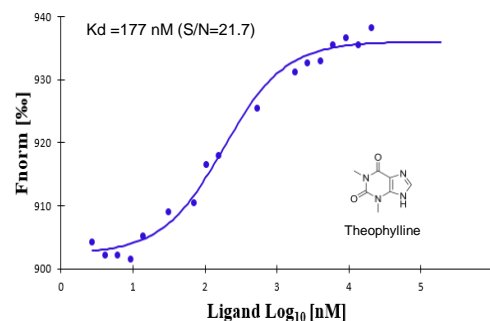
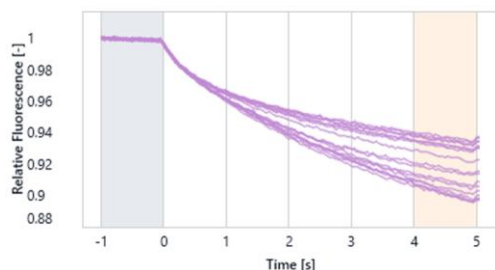
1. 60 top ranking fragments were used for TRIC assay.
2. Binding level screen: The fragments with significant response will be screened out for further dose affinity screen. (27 shows binding)

Dose affinity screen (1-2 weeks)

1. The fragments with $K_d \leq 1000 \mu\text{M}$ and good behavior (TRIC trace, S/N) will be screened out as potential hits.
2. Confirmation of Docked pose with structure



A2AR
(PDB: 5MZJ)





Light Cyclcr 480: A high-throughput TSA/DSF platform



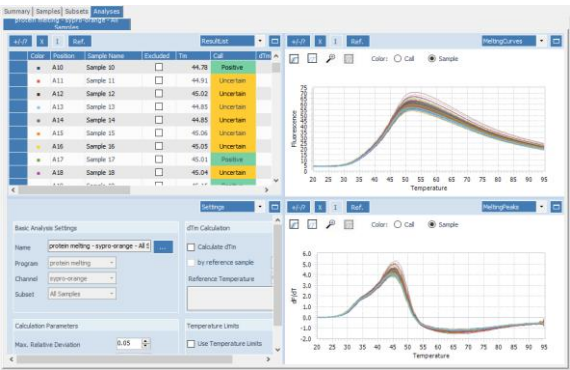
The LightCycler® 480 System is a proven high-performance, medium-to high-throughput PCR platform that provides various methods for gene detection, gene expression analysis, genetic variation analysis, and array data validation.

Applications:

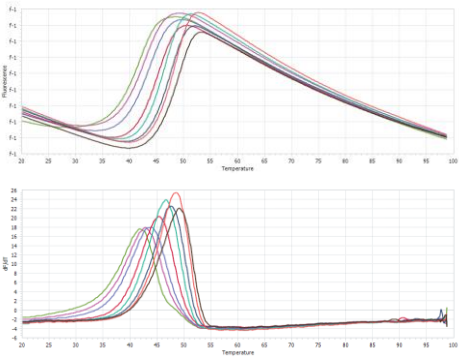
- Buffer screening
- Small molecule interaction test
- Fragment screening

Small molecule interaction test

Fragment screening



Color	Position	Sample Name
P17	250μM DMSO	
P18	2.5μM TNG-0241029	
P19	5μM TNG-0241029	
P20	12.5μM TNG-0241029	
P21	25μM TNG-0241029	
P22	50μM TNG-0241029	
P23	100μM TNG-0241029	
P24	200μM TNG-0241029	



Protein	Compound	Control-7% DMSO	2.5 μM	5 μM	12.5 μM	25 μM	50 μM	100 μM	200 μM	
Protein 1	Control	55.20	54.91	55.03	54.96	55.15	54.70	54.29	53.67	
	2.5 μM	55.29	56.08	56.55	57.54	58.70	59.88	60.88	61.86	
	5 μM	55.20	55.92	56.55	58.21	59.32	60.61	62.14	62.69	
	12.5 μM	55.46	56.22	57.37	59.67	60.54	60.75	61.32	61.74	
	25 μM	55.14	55.83	57.21	61.97	63.28	63.80	64.61	64.80	
	50 μM	54.99	55.53	56.20	55.96	57.33	58.06	59.21	58.89	
	100 μM	55.11	55.93	56.97	58.66	58.75	59.09	59.30	59.77	
	200 μM	55.05	56.05	56.54	57.89	59.23	60.58	60.87	61.52	
	Protein 2	Control	55.24	56.11	56.89	58.74	60.29	61.51	61.66	61.85
	2.5 μM	55.28	55.77	56.34	57.97	59.30	60.58	60.38	60.60	
Protein 3	Control	55.24	56.23	57.18	59.36	58.96	58.96	59.31	60.00	
	2.5 μM	55.34	56.08	56.98	58.65	60.23	61.60	63.02	63.89	
	5 μM	55.19	55.90	56.98	59.12	60.53	61.27	61.86	62.03	
	12.5 μM	55.01	55.69	55.51	55.81	56.10	56.62	57.33	58.39	
	25 μM	55.14	55.69	56.32	57.40	57.61	56.94	57.09	57.69	
	50 μM	55.14	55.10	55.70	56.93	56.56	60.60	61.51	62.18	

Prometheus: Dye free thermo shift assay instrument

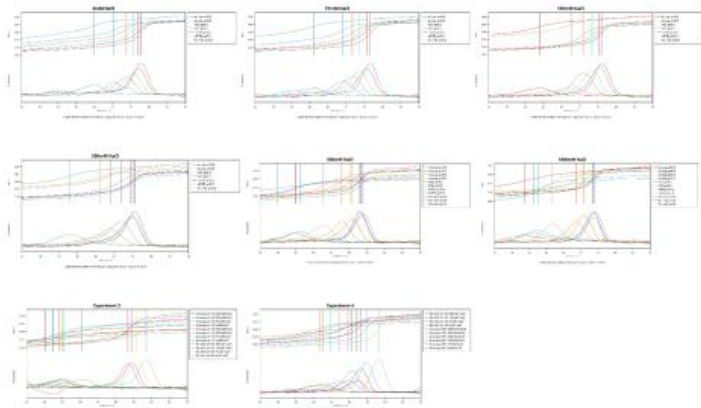


The Prometheus NT.Plex is a high-performance nanoDSF instrument that provides high-quality protein unfolding curves, melting temperatures (T_m).

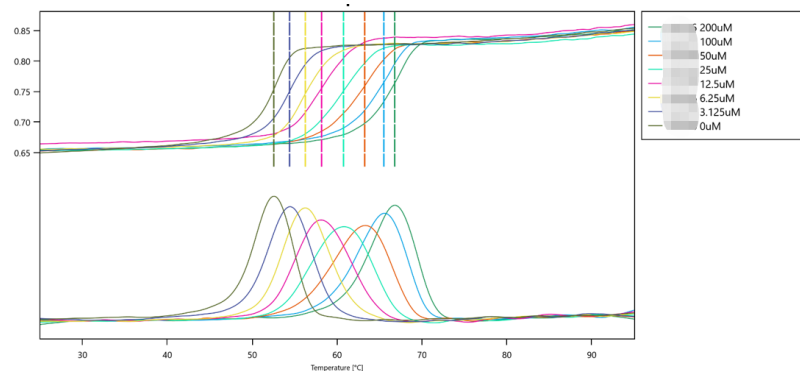
Applications:

- Buffer screening
- Small Molecule Interaction Test
- Membrane Protein

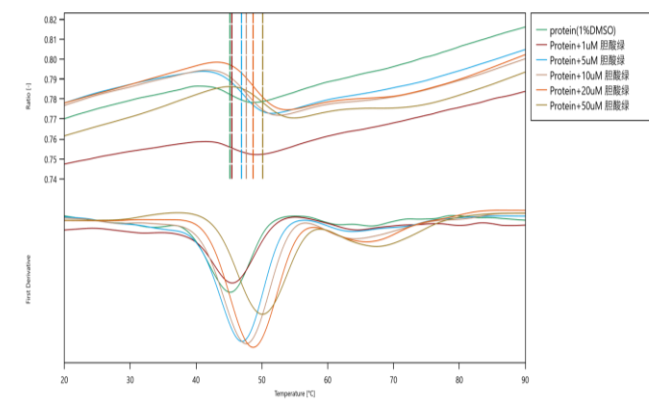
Buffer screening



Small Molecule Interaction Test

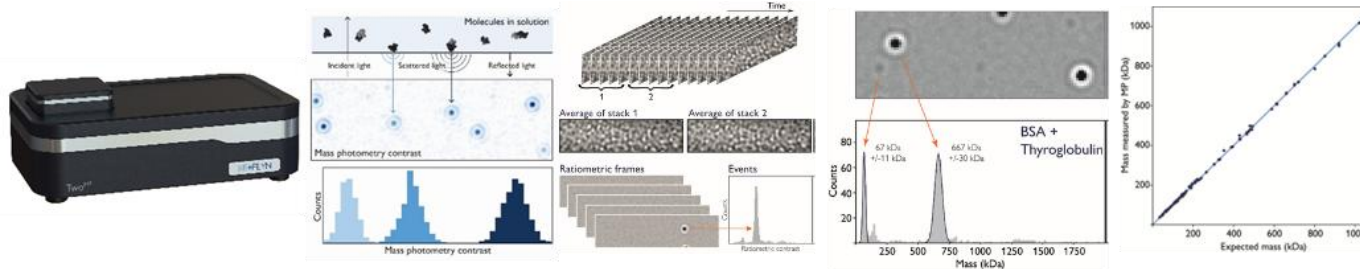


Membrane Protein



Mass photometry: A rapid detection of protein particle distribution

Mass photometer from Refeyn and sample data



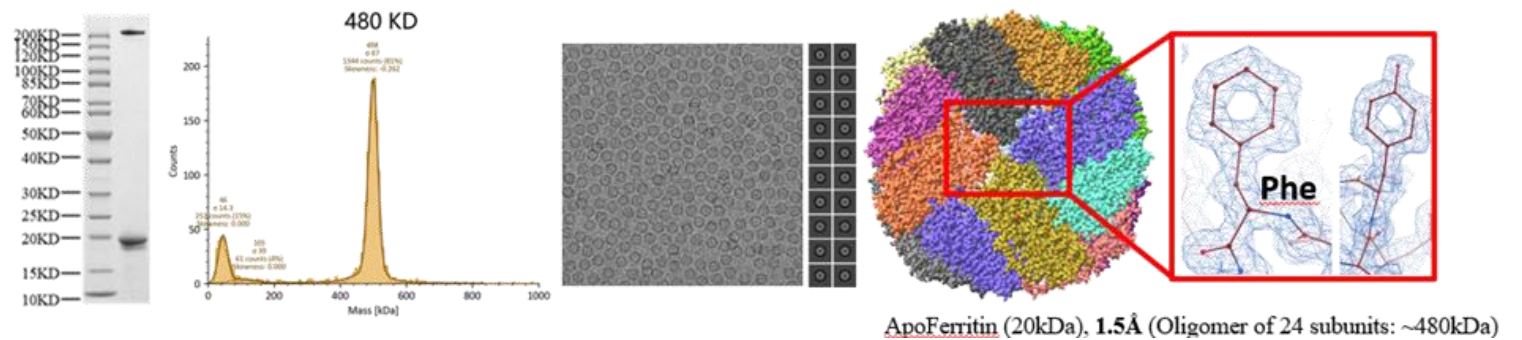
Benefits

- measures true molecular mass
- shows molecular heterogeneity
- works in solution
- uses minimal amounts of sample
- no modifications

Applications:

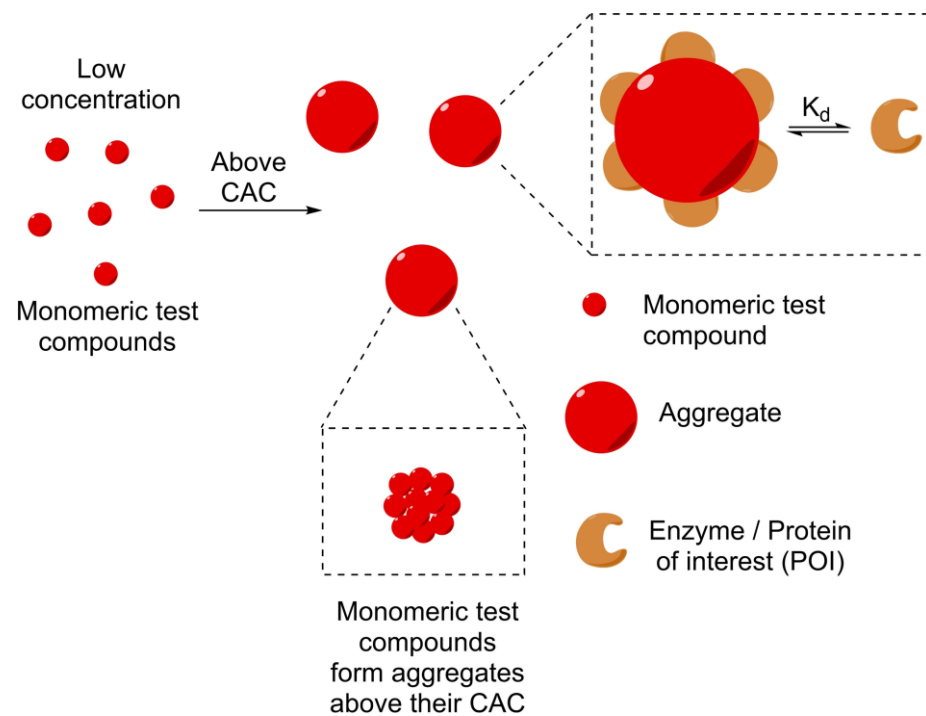
- Measuring the molecular mass of single biomolecules
- Measuring the oligomerization state
- Screening buffer conditions
- Characterizing sample heterogeneity
- Monitoring sample components' stability
- Studying biomolecular interactions

Mass photometry application in ApoFerritin gene to structure workflow

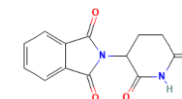


ApoFerritin (20kDa), 1.5Å (Oligomer of 24 subunits: ~480kDa)

Mass photometry: CAC detection (Critical aggregation concentration)

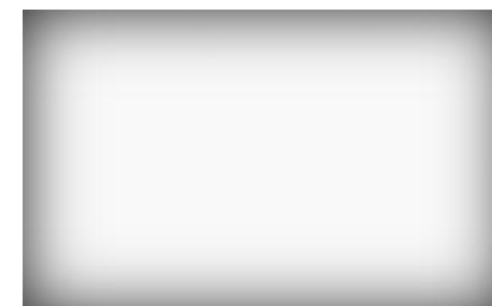


1000 μ M Thalidomide

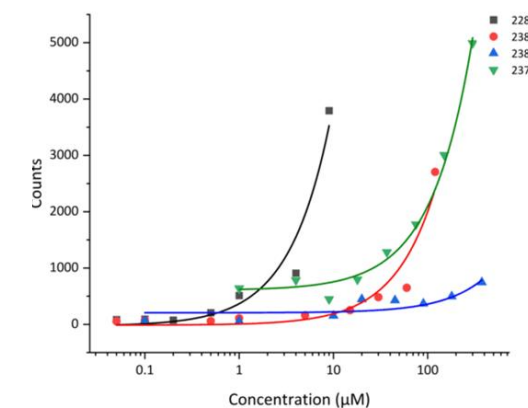
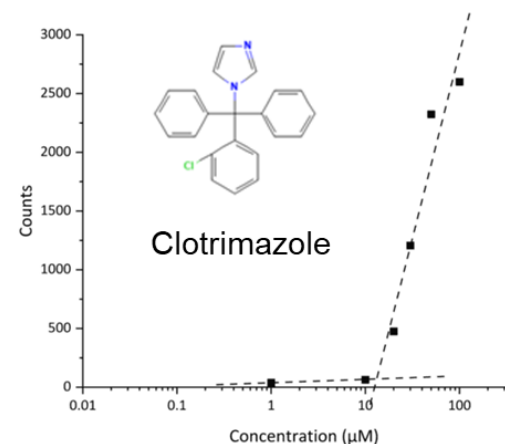
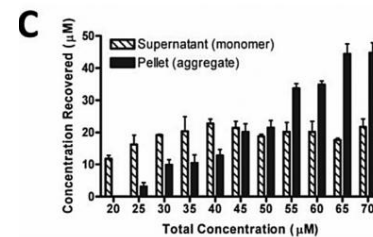
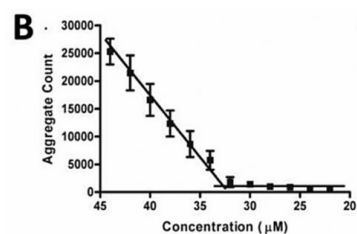
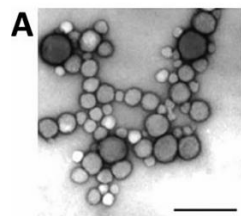


No aggregation

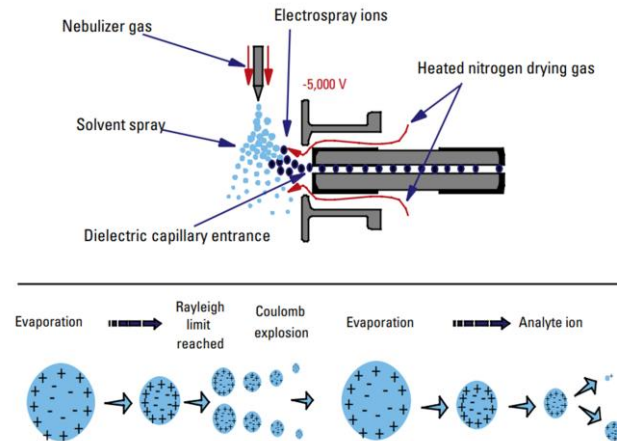
20 μ M Clotrimazole



Strong aggregation



Mass spectrometry: Accurate protein MW determination



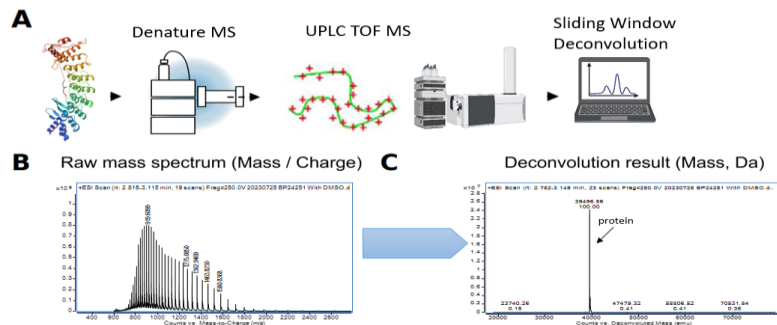
Benefits:

- Better than 1 ppm mass accuracy
- Characterize large biologic compounds with a broad mass range of up to 20,000 m/z
- Separate target compounds from interferences with high-resolution data

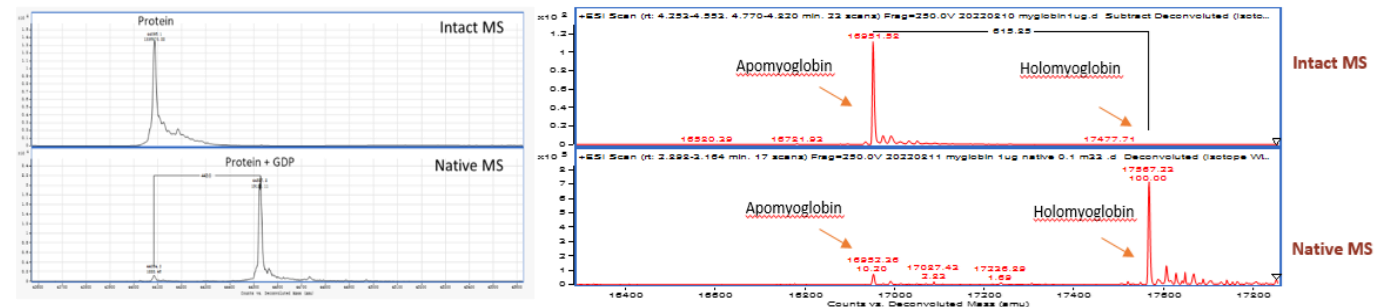
Applications:

- Intact protein characterization
- Native MS analysis
- Covalent library screening

Standard QC: Intact protein MS



Native MS: RAS protein nucleotide binding





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For off-the-shelf: order@biortus.bio