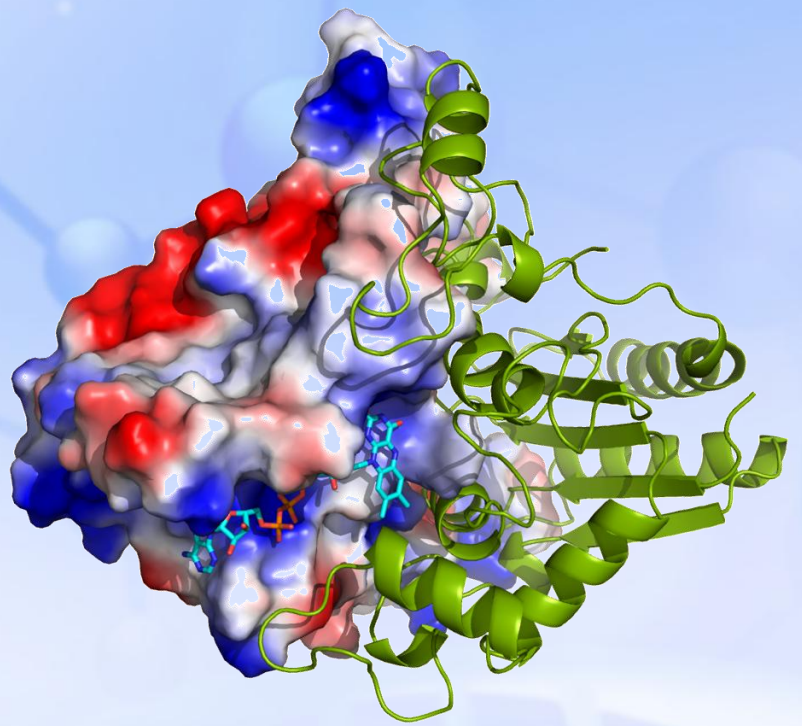
Intro of Biortus Crystallography

2025

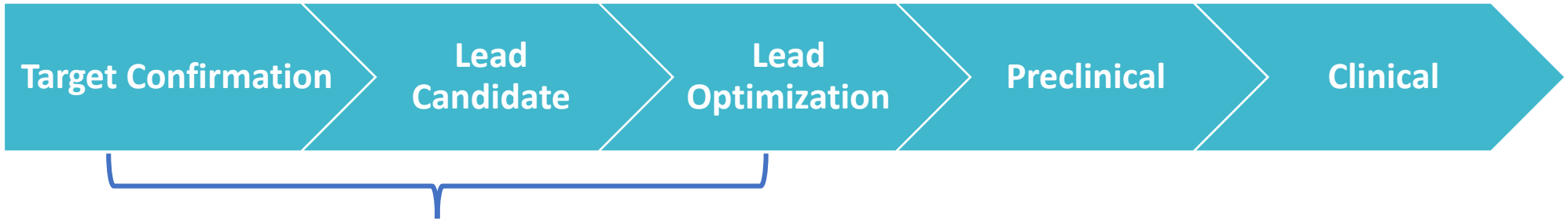


Web：en.biortus.bio

Table of Contents

Drug Discovery and Structural Biology

Drug discovery: long time ~10-15 years and high cost >$1 billion



Structural biology can help by different methods

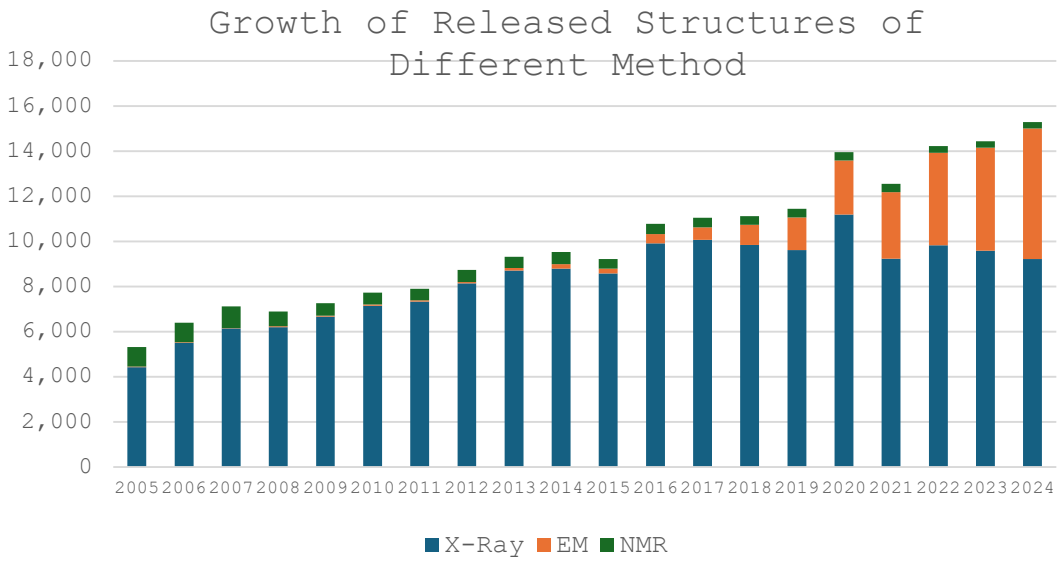
Target Identification
Helps identify binding sites and active sites on the target protein

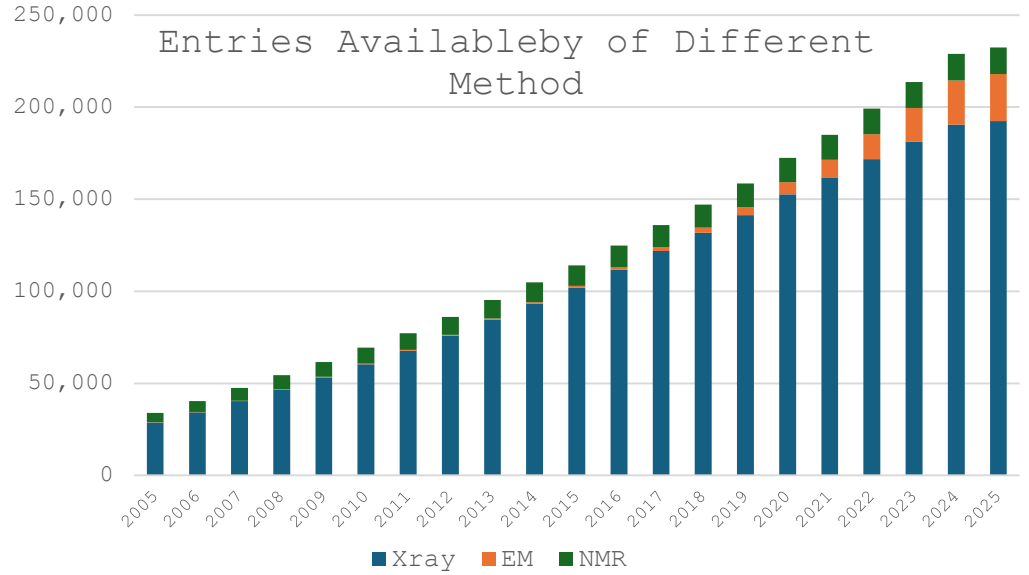
Fragment-Based Drug Discovery
Screen small molecular fragments and optimize hits

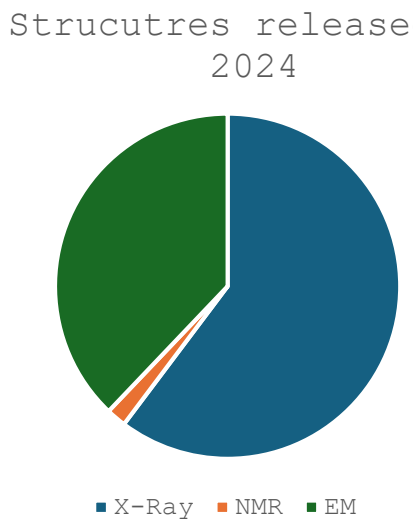
Structure-Based Drug Design
Design of drugs that fit precisely into the binding pocket of a target protein.

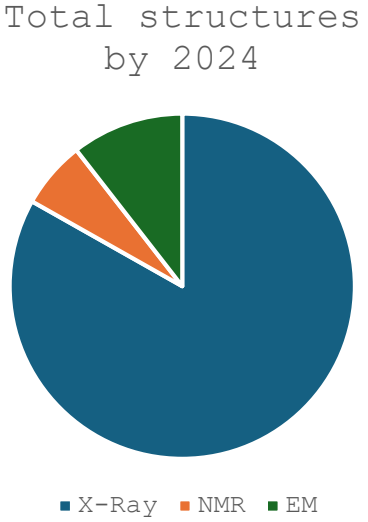
Lead Optimization
Modify drug candidates for improved binding affinity, specificity, and reduced side effects.

X-ray crystallography is the cornerstone of structural biology



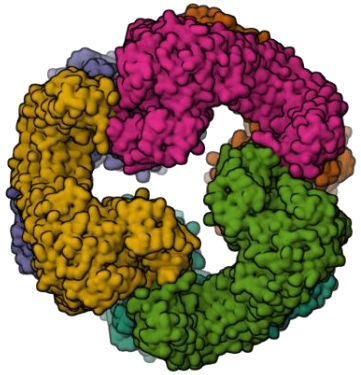




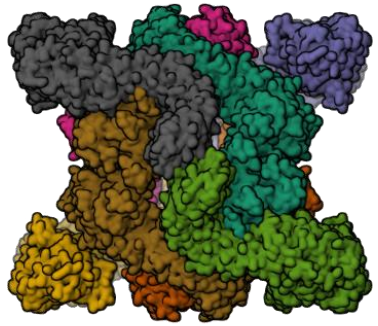


Method	Samples	Advantages	Limits
X-ray Crystallography (Well developed)	mg level amount, high purity, broad MW size	High resolution, low cost, high throughput	Need crystals, artificial in crystal packing, static state
Cryo-EM (rapid developing)	Very few amount, only large MW size(>65kDa at Biortus)	Structure in native state, short turn-round time at Biortus	Hard to obtain atomic-resolution map, dependent on sample preparation, high cost

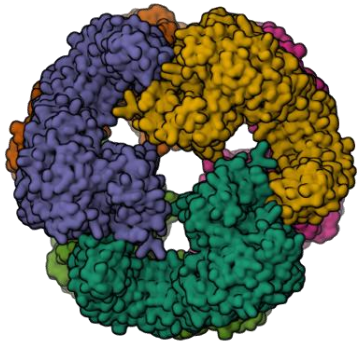
X-ray crystallography cooperates with Cryo-EM



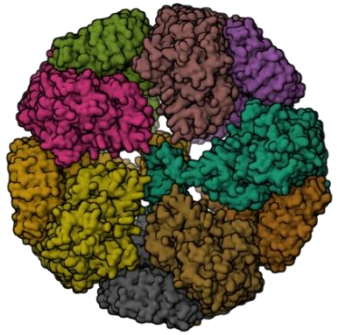
PYD-deleted NLRP3 hexamer



NACHT, LRR-NLRP3 open octamer

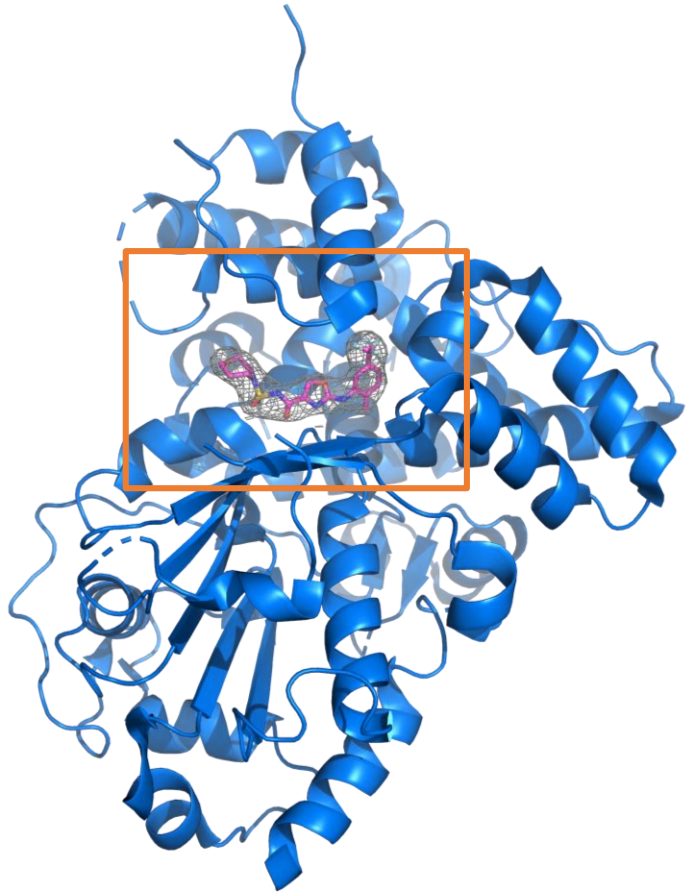


NACHT, LRR-NLRP3 closed hexamer



PYD, NACHT, LRR -NLRP3 decamer

NLRP3 oligomers by Cryo-EM shows how NLRP3 function



NLRP3 NACHT domain by X-tal clearly reveals the compound/protein interaction

Streamlined Process Enabling Avg. 3-Month Turnaround

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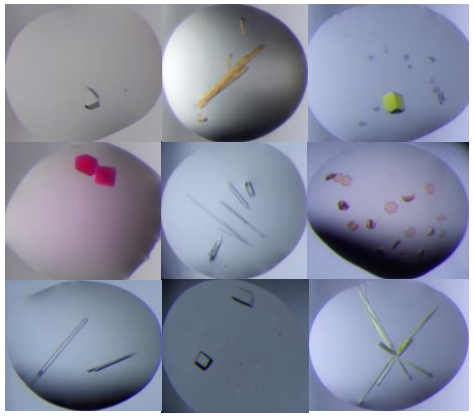
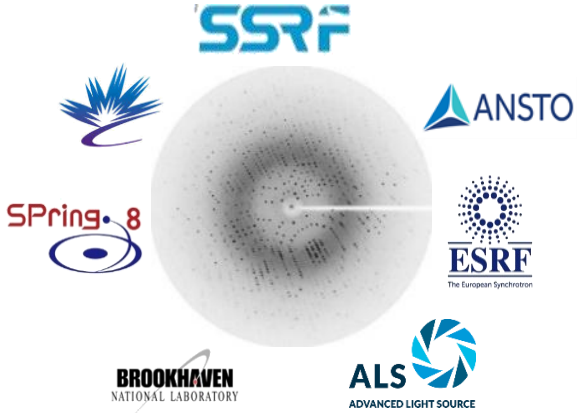
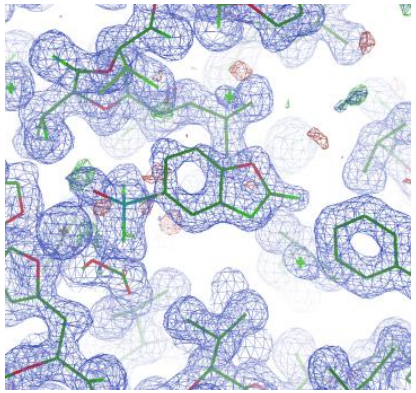
Crystallization	Data collection & processing, structure determination & refinement

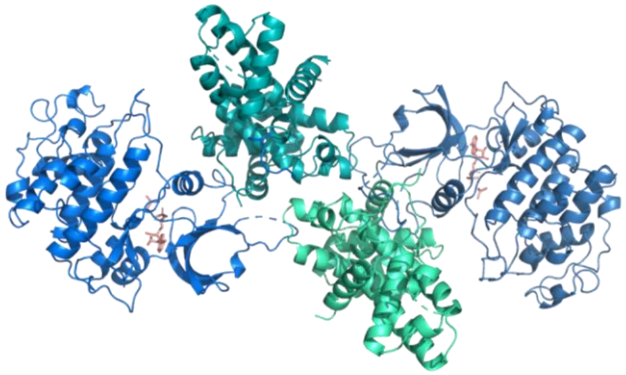
1-2 months





~1 month





- >1,000 conditions in one day
- 33+3 screening and additive kits
- Crystal growth mostly within two weeks

- Synchrotron source: **available almost every week**

- MR (AF2), SAD/MAD (when required)
- XDS, imosflm, CCP4i, Coot

- Diffraction files: .sca & .log & .mtz
- Structure files: .mtz & .pdb & .mmcif
- Ligand file: .cif

State of the Art Crystallography Facilities

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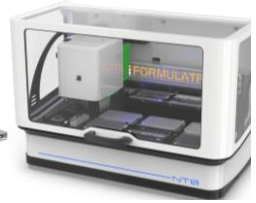
Crystallization | Data collection & processing, structure determination & refinement



ROCK IMAGER 2

ROCK IMAGER 1000

Instruments:

- **HT crystallization screening**: TTP LabTech Mosquito LCP, NT8, Rock Imager 1000, Rock Imager 2
- **Crystallography software:** XDS, CCP4i, Coot

Synchrotron Sources:

- SSRF (Shanghai, China)
- SPring-8 (Kawaguchi, Japan)
- CLS (Saskatoon, SK, Canada)
- MX2 (ANSTO, Australia)

} Long-term collaboration, cover full-year usage

- ESRF, APS, NLS, etc., Others synchrotrons in the world

Foundation is High Quality Proteins

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Sample requirements

- >90% purity
- Soluble & Homogeneous
- Concentration: 10mg/ml**
- Volume: 400-500ul**
- Protein: 5mgs total for 12-16 96x screens and optimization
- Generally avoid Glycerol, high concentration salt, etc. **

**rough guidelines

Standard QC Package

SDS-PAGE

Purity
Stoichiometry

LC- MS

MW verification
Protein Validation
PTMs

Analytical SEC

Solubility
Multimerization
Complex Integrity

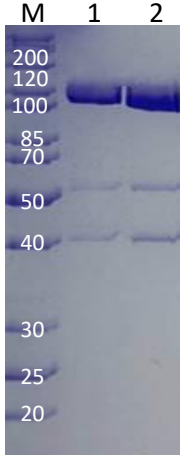
Custom QC Options

- Functional biochemical & biophysical assays
- SEC-MALS: oligomerization and solubility
- De-glycosylation
- Complex formation (gel-shift)
- Neg-stain EM
- Mass photometry
- DSF: Tm determination/stability

Cross-functional QC Improves Productivity

- High-throughput TSA for screening buffer conditions optimal for protein stability & crystallization
- Negative staining with CryoEM is a standard step in evaluating protein integrity & homogeneity

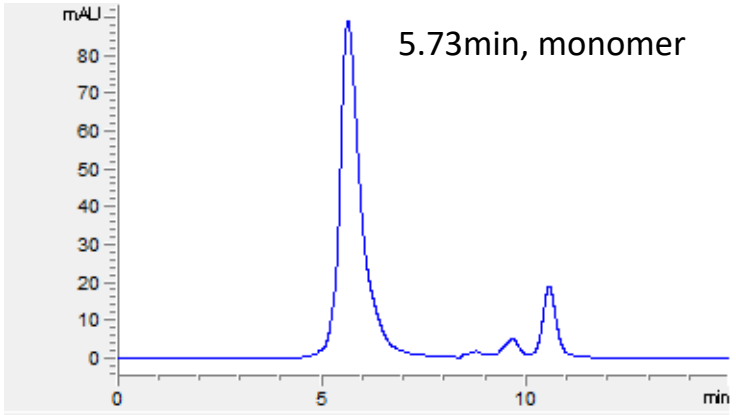
SDS-PAGE



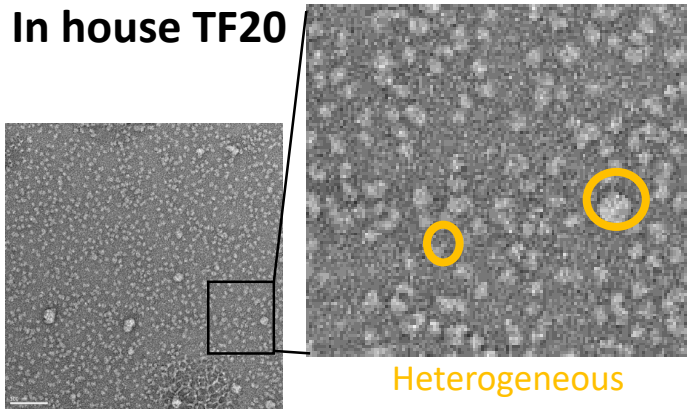
M = Marker
Lane 1 = 5 µg
Lane 2 = 10 µg

Conc. : 10.9 mg/ml
Purity: >90%
A_{260}/A_{280}: 0.61

HPLC-SEC



In house TF20



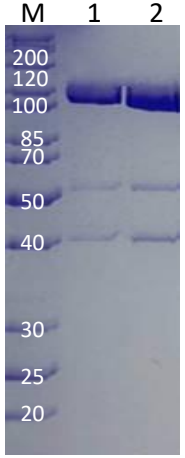
Heterogeneous

No Crystal !

Cross-functional QC Improves Productivity

- High-throughput TSA for screening buffer conditions optimal for protein stability & crystallization
- Negative staining with CryoEM is a standard step in evaluating protein integrity & homogeneity

SDS-PAGE



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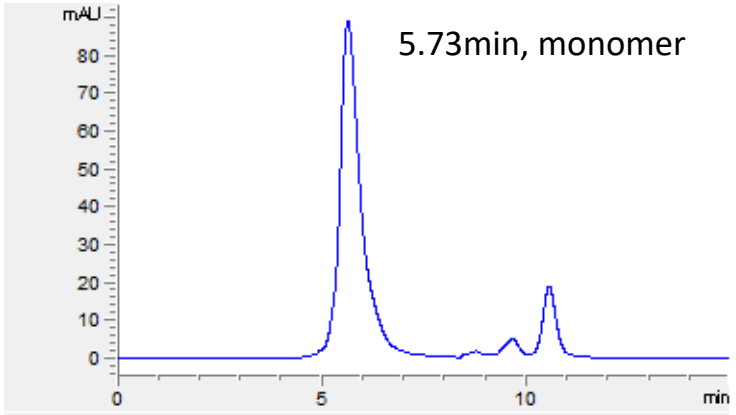
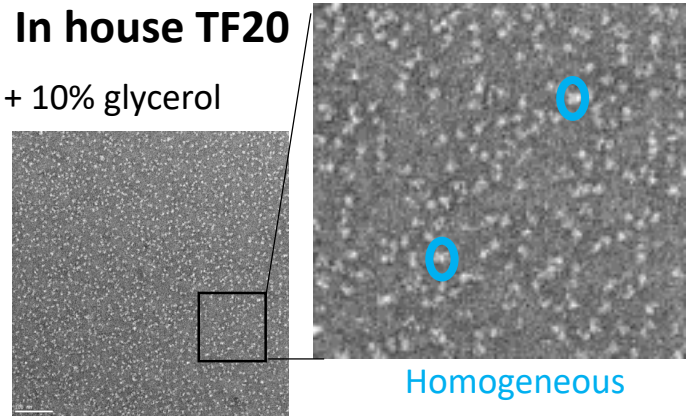
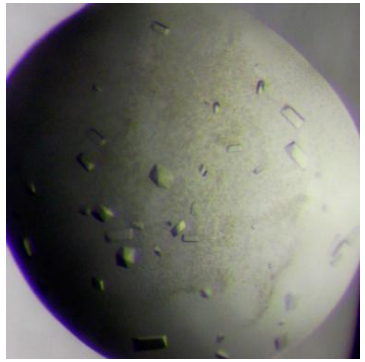


In house TF20

+ 10% glycerol



Homogeneous



Crystallized 2.8 Å

Commercial Screening Kits Available

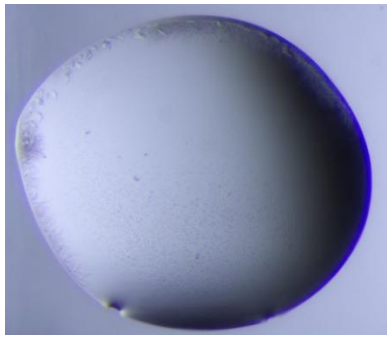
38 kits for broad screening and 3 additive kits for optimization

Soluble Proteins		Protein-Protein Complex	Protein-Nucleic Acid Complexes	Kinase Proteins	Membrane Proteins	Additive
PEG/Ion	SaltRx	PEGs	JBScreen Nuc-Pro	JBScreen Kinase	MemGold™	Additive Kit
PEGs	MultiXtal	PEGs II	Natrix	• Highly specialized screen formulated for kinase protein • Previously identified crystallization conditions from published structures	MemGold2™	Silver Bullet Kit
PEGs II	Structure Screen 1-2	ProPlex	Crystal Screen		MemGoldMeso™	Detergent Kit
Index	XP Kit	JBScreen Wizard 1-4	JBScreen Wizard 1-4		MemPlus™	
JCSGplus	Morpheus	LMB Crystallization Screen	Structure Screen 1-2		MemStart + MemSys	
JCSG I/II/III/IV	Morpheus Green	MIDASplus	MIDASplus		MemTrans™	
PACTpremier	Morpheus II		Crystal Screen Cryo			
Crystal Screen	Morpheus III					
LMB Crystallization Screen	MIDASplus					
3D Structure Screen	Crystal Screen Cryo					
JBScreen Wizard 1-4	TOP96					

Screening Principle:

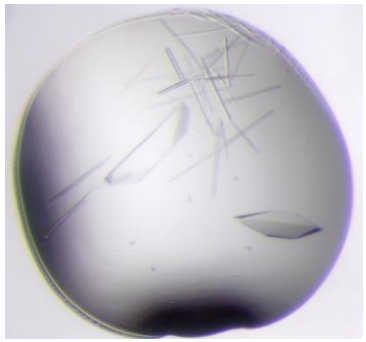
- Different protein samples, different screening kits
- Follow reference conditions, *e.g.* if PEG as precipitant, PEG series kit as first choice

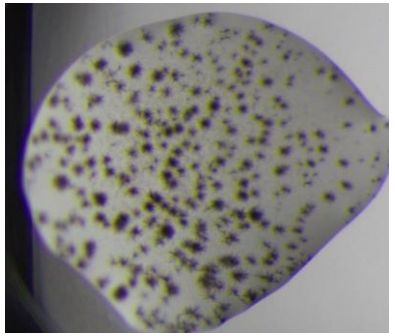
Representative Crystal Optimization Strategies



Optimization Condition

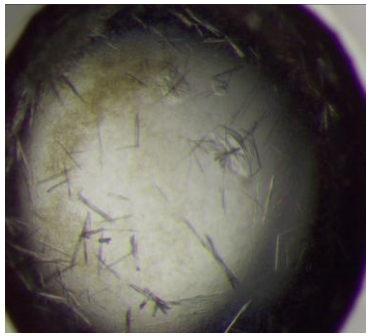
(Precipitate VS pH)

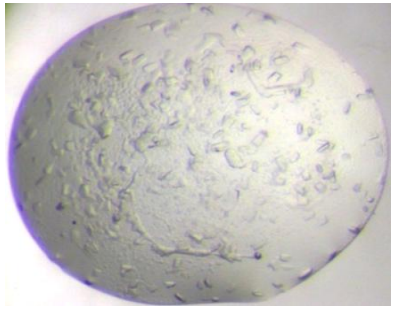




Additive screen

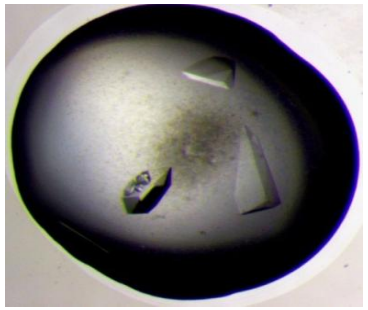
2.7 Å

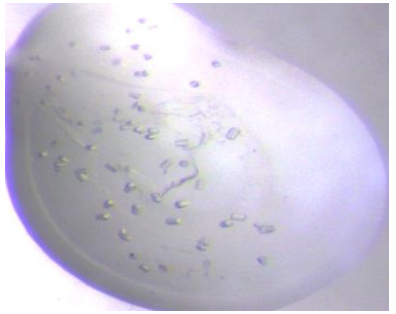




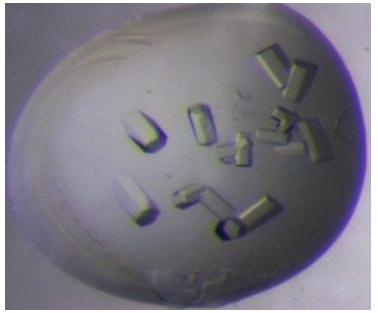
Optimized different cations

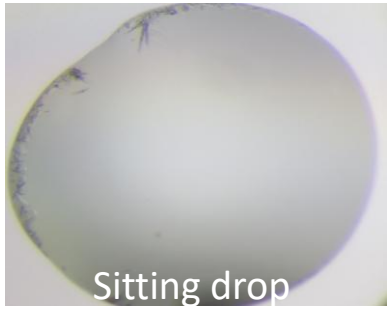
(Na^+, Li^+, K^+)





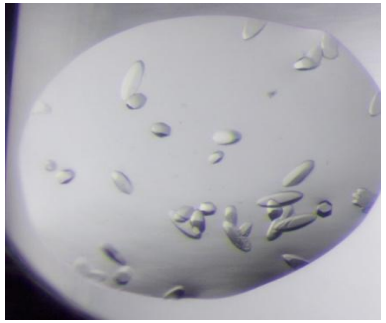
Serial seeding





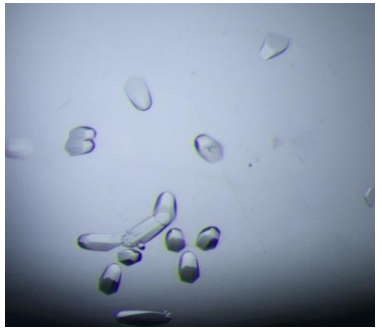
Drop Size

Sitting/Hanging



Dehydration

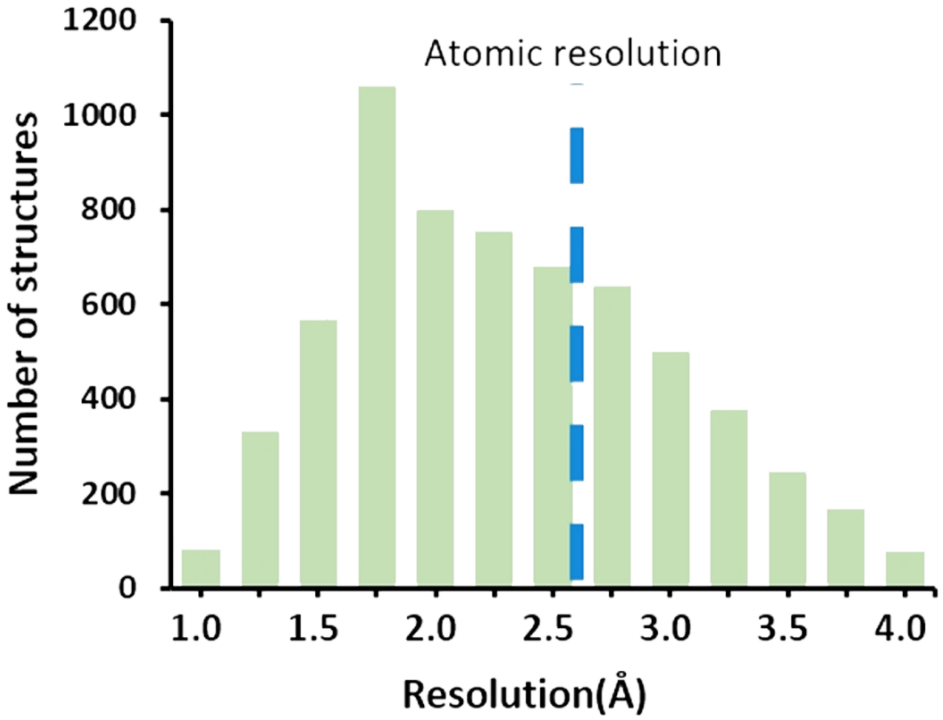
6.5Å→3.75Å



Variety of Applications for Crystallography

- Novel protein structures for publication and/or target characterization
- Protein / ligand structures for Structure Activity Relationship (SAR) analysis
- Antibody/Antigen structures to support structure-guided antibody maturation and epitope mapping
- PROTAC & Molecular Glue structures to support E3 based drug degradation system
- Crystal Fragment screening for fast identification of fragment hit to support fragment-based drug design (FBDD)

Resolution Distribution of Structures (2020-YTD)



Sequential Rounds of Optimization

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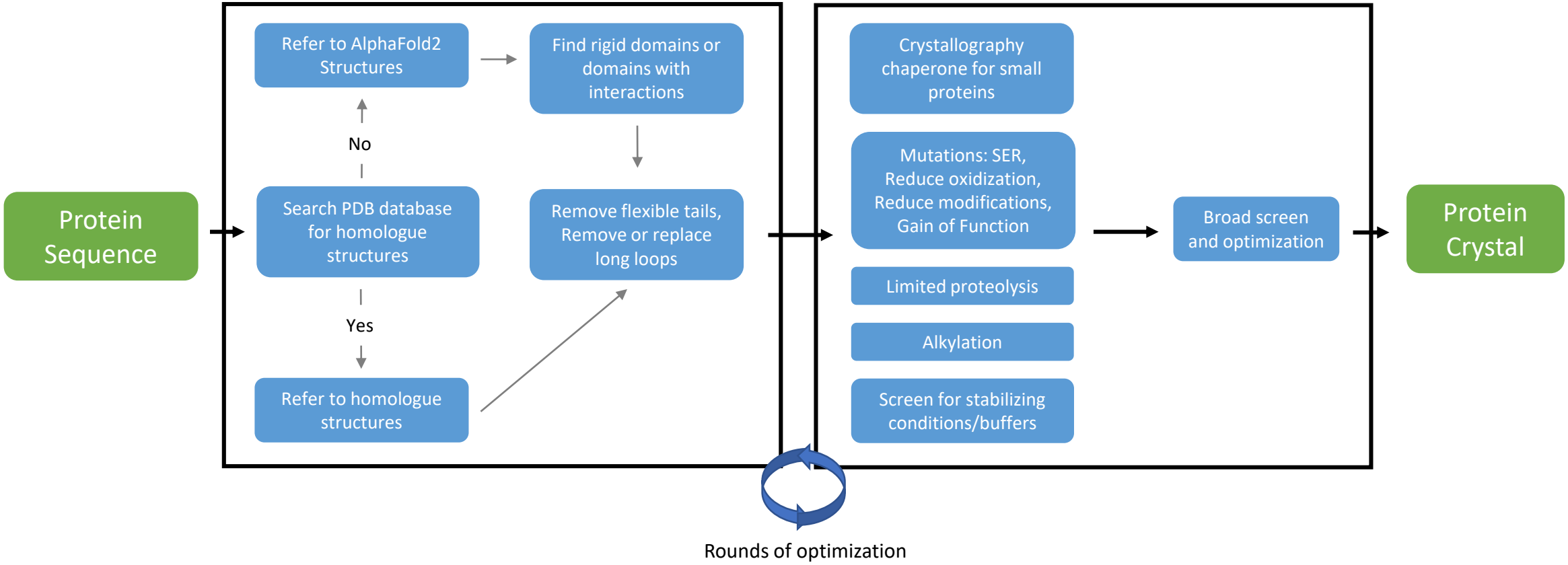
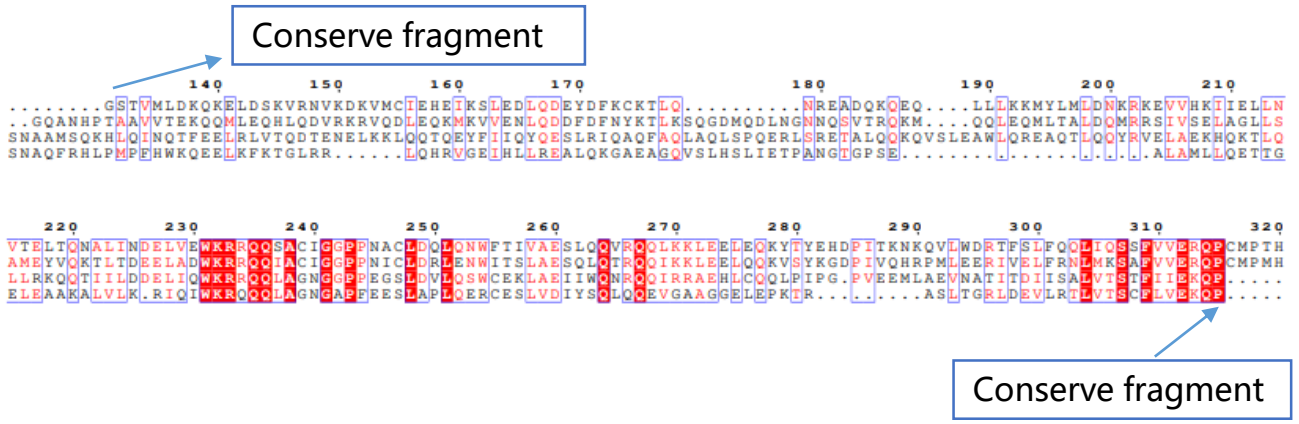
Initial construct design based on Bioinformatic and/or structure prediction

Advanced design and sample handling in the late phase of crystallization

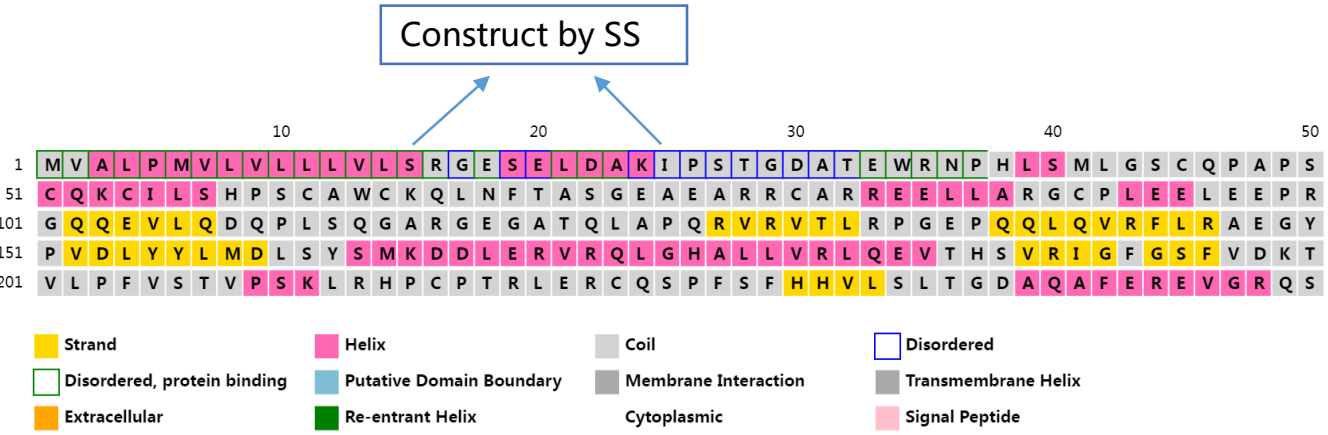


AlphaFold helps in construct design

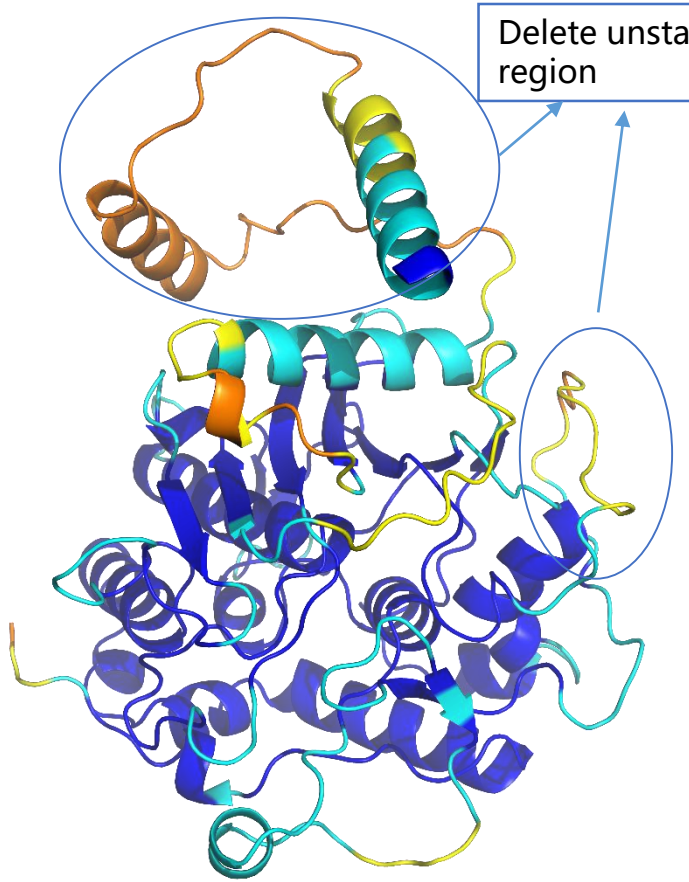
Sequence alignment



Second structure prediction



AlphaFold 2 prediction



Crystal Preparation for Protein/Compound Complex

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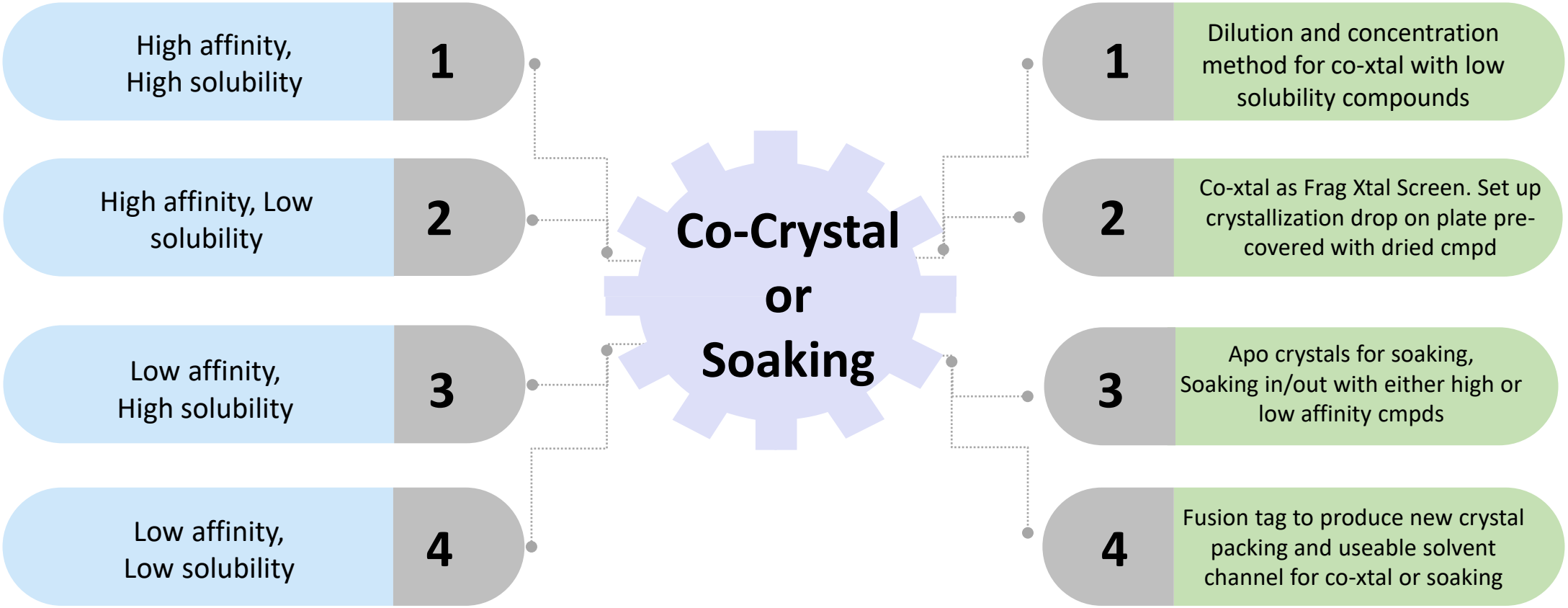
Co-Crystallization and/or Soaking Strategies per compound and public information



- High affinity: Kd value at nM or single-digit uM range;
- Good solubility: compound dissolved at 1mM or higher in 2%-5% DMSO.

Epitope Mapping via Crystallography

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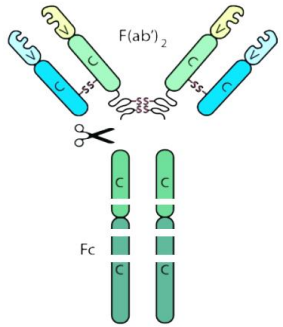
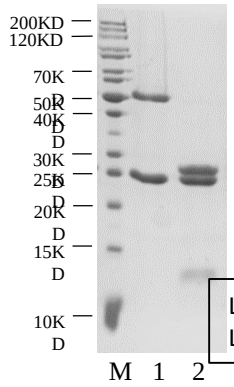
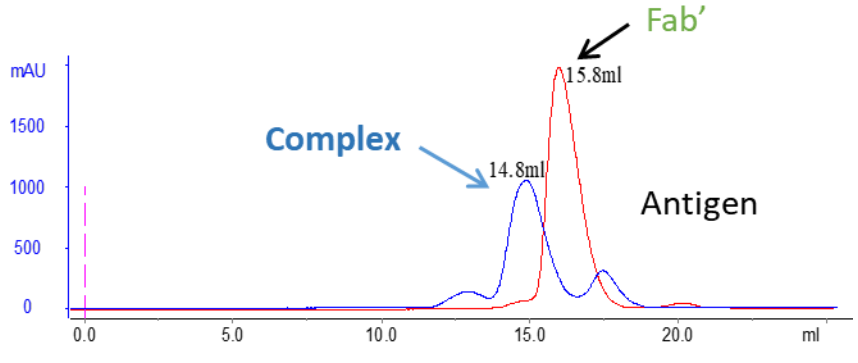
Elucidate binding interface of Ab and Ag via X-ray Crystallography

Pepsin digestion

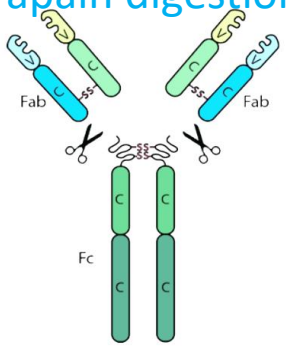


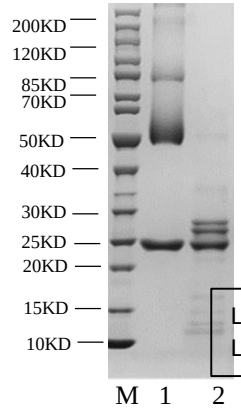


L1: Intact Antibody
L2: Pepsin digestion



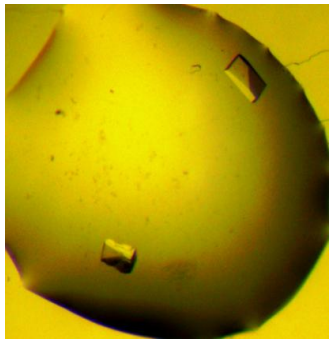
Papain digestion



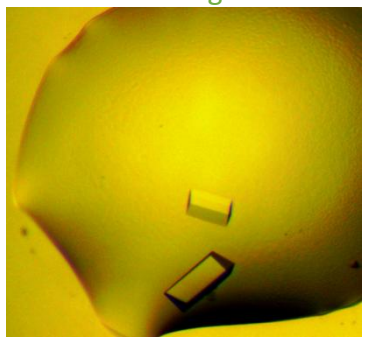


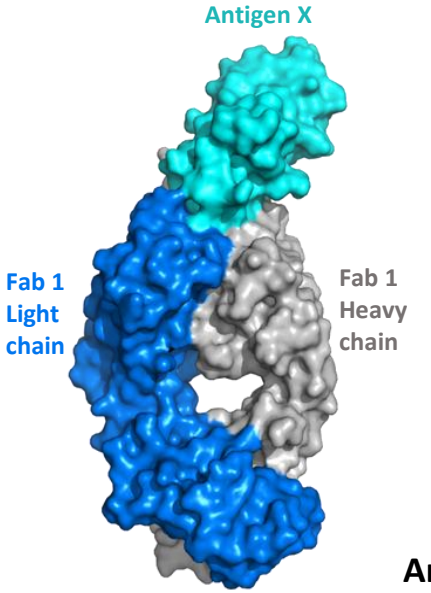
L1: Intact Antibody
L2: Papain digestion

Papain digestion
Fab-Ag

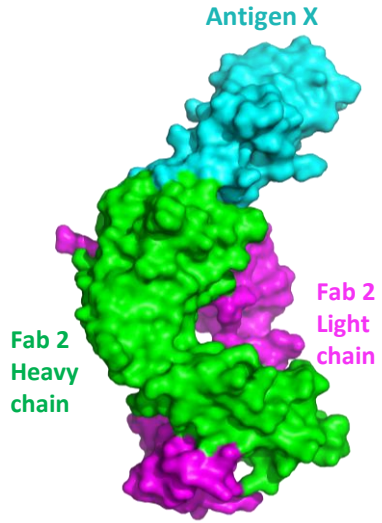


Pepsin digestion
Fab'-Ag





Antigen X at same orientation



Determined point of distinction for Client Fabs for patent data package

Epitope Mapping via Crystallography

BIORTUS

Epitope/Paratope Analysis of Binding Interface

Antigen	Antigen residue	Antibody residue	Distance(Å)
Hydrogen bonds(< 3.5Å)			
Ag (chain A)	E129	H chain N57	3.02
	K154	H chain Y59	2.91
		L chain T97	2.79
Salt bridges(< 4.0Å)			
Ag (chain A)	E129	H chain K54	
	E129	H chain K54	
Stack interaction			
Ag (chain A)	K152, F153	H chain F101	
Ag (chain A)	Y150	H chain F105	
Hydrophobic interaction(< 4.0Å)			
Ag (chain A)	K152, F153, L154	H chain F101 L chain F99	
Other VDW interaction(< 4.0Å)			
Ag (chain A)	E128	H chain S55	3.61
	E129	H chain S55	3.67
	P131	H chain N57	3.46

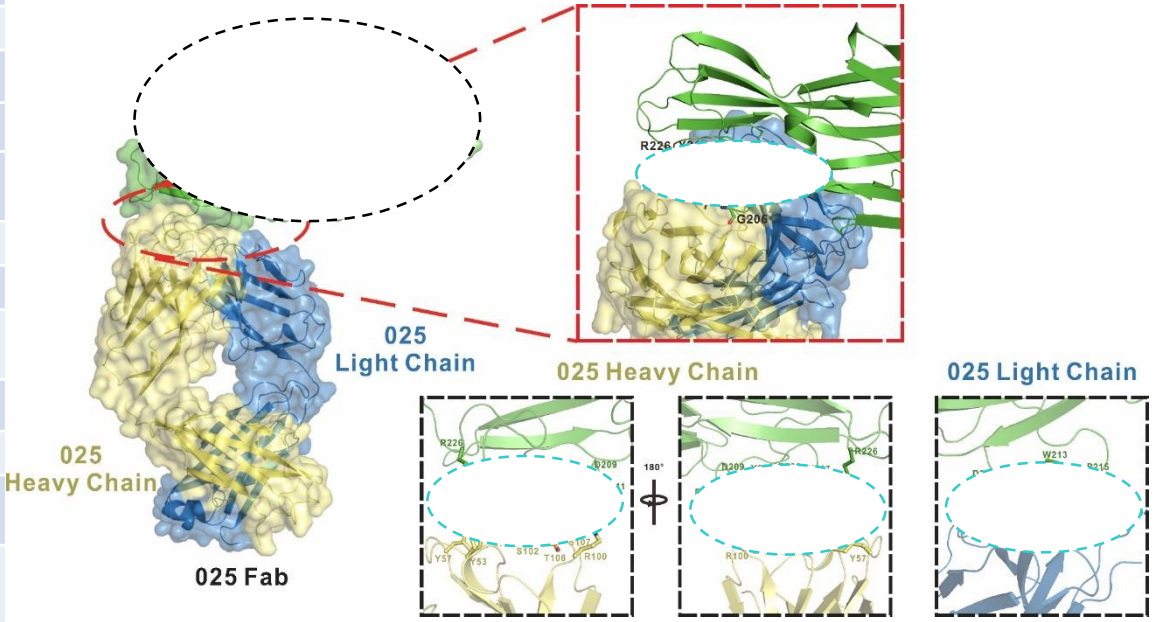
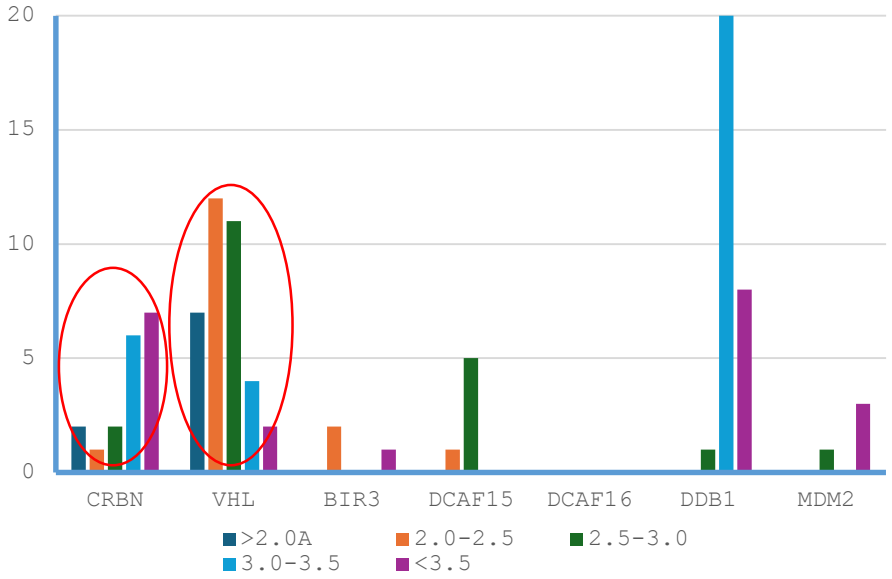


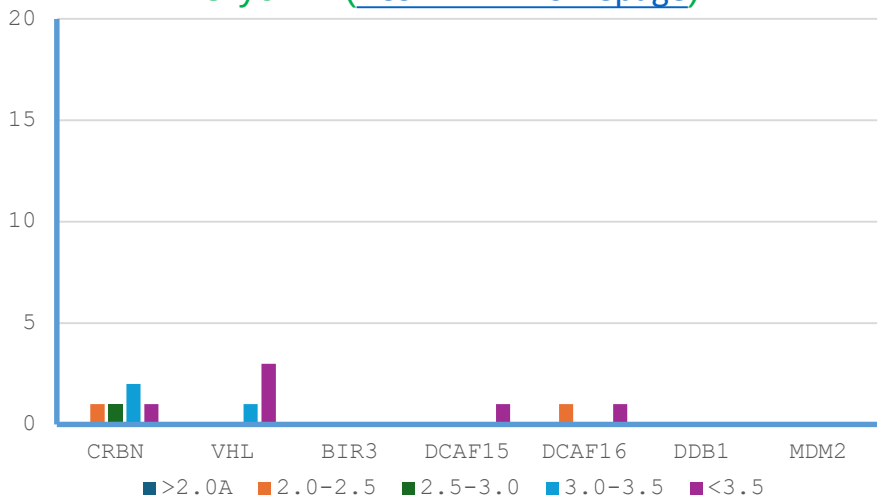
Fig. Detailed Ab-Ag Recognition Interface

PROTAC & Molecular Glue for E3 Based Target Degradation

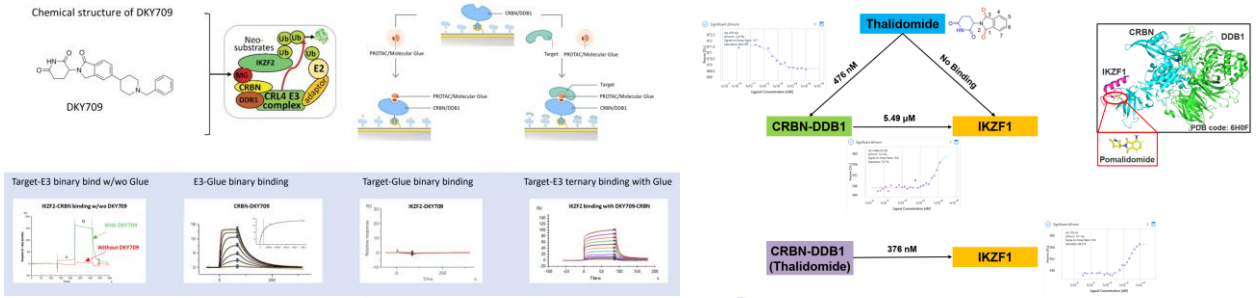
Xray (RCSB PDB: Homepage)

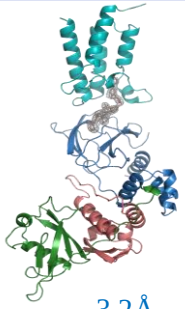


CryoEM (RCSB PDB: Homepage)



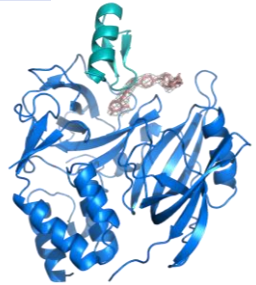
E3 ligase	Degrader	Best resolution	Method
CRBN^{midi}	Molecular glue/PROTAC	1.70	X-tal
CRBN-DDB1	Molecular glue	2.70	X-tal
CRBN-DDB1	Molecular glue/PROTAC	2.70	Cryo-EM
VHL-ElonB-ElonC	PROTAC	2.10	X-tal
VHL-ElonB-ElonC	PROTAC	3.00	Cryo-EM





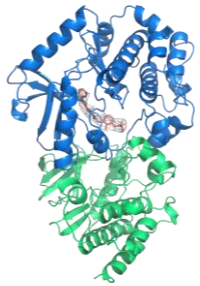
3.2Å

VCB+PROTAC+SMARCA2



3.5Å

CRBN^{midi}+MG+IKZF1



4.0 Å

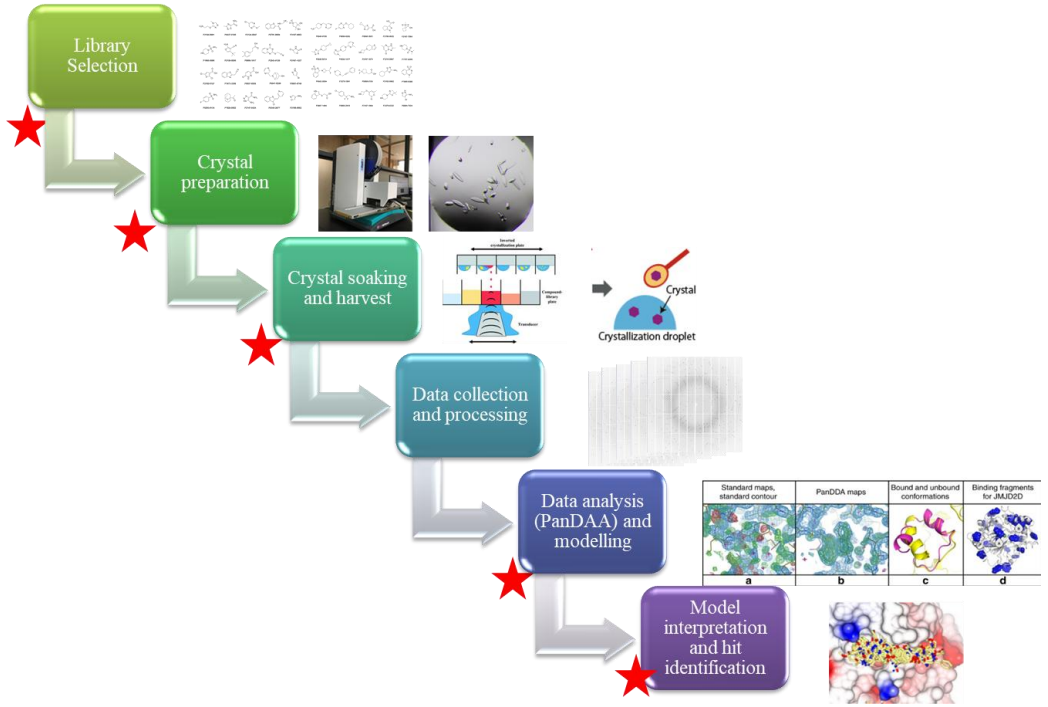
MEK1+MG+BRAF

Supporting of Crystallographic Fragment Screening

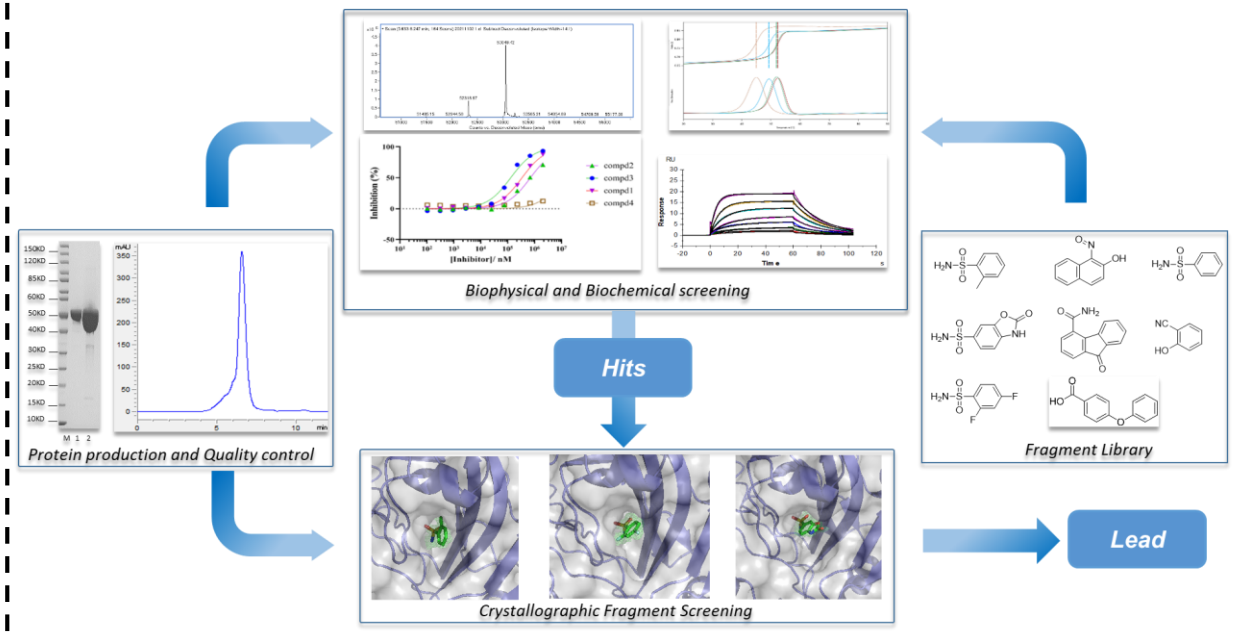
Biortus has **4 commercial fragment libraries: 2 reversible + 2 covalent** (~10,000 fragments).

Coupled with our Assay Screening and Crystallography platforms, can be used for initial candidate discovery

Traditional CFS



Modified CFS

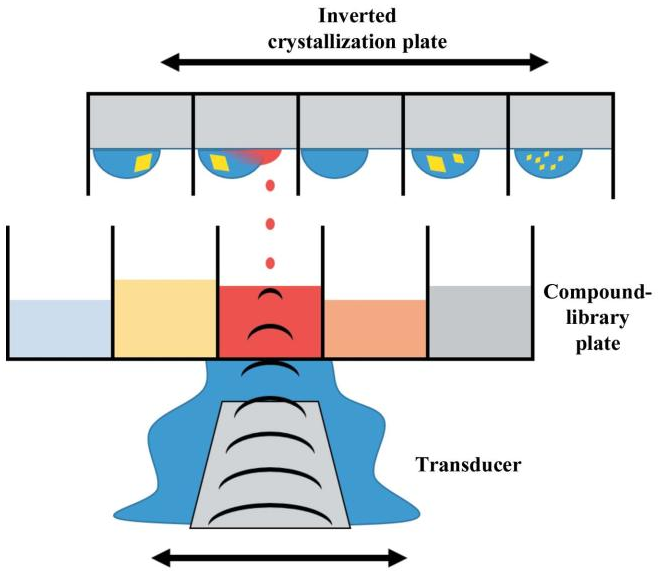


Traditional CFS needs higher cost (synchrotron fee), and it usually give lower hit rate.

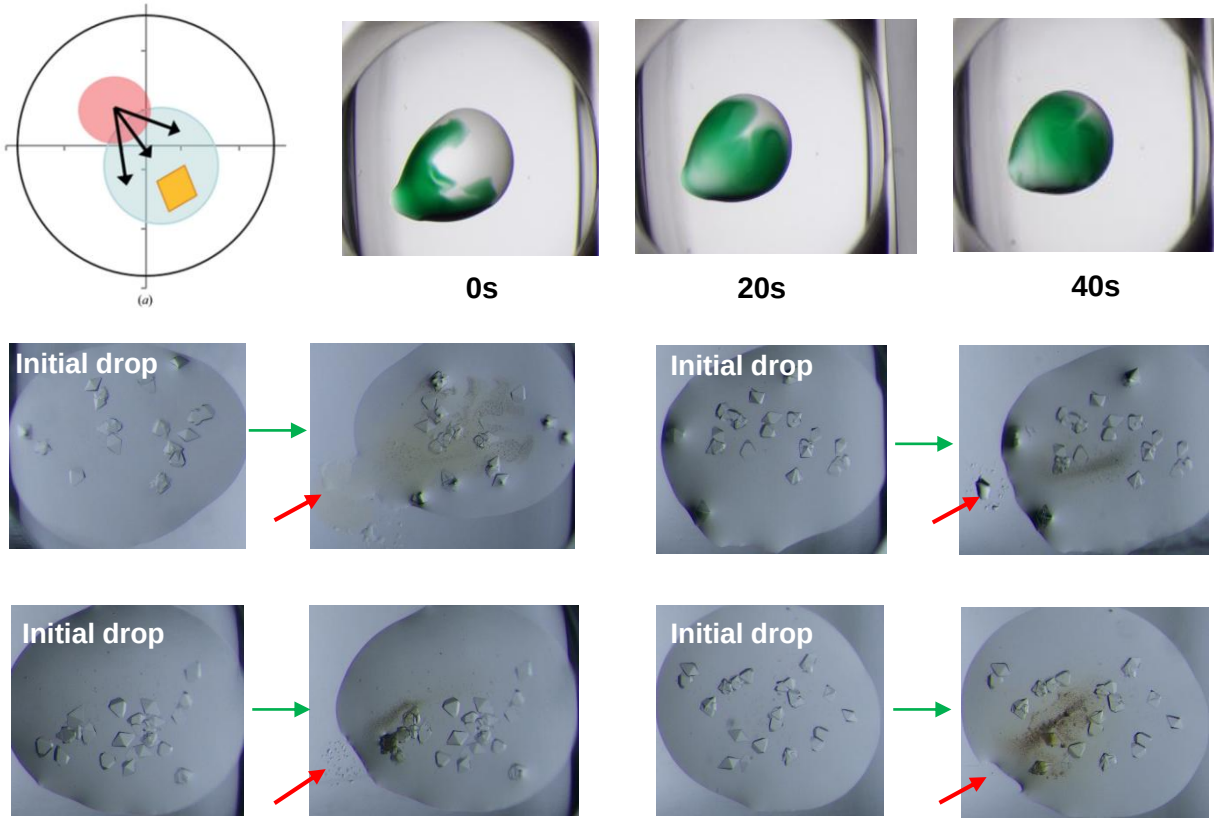
Coupled with assay screening, the cost could be lower and hit rate will be higher.

Echo for CFS (Soaking)

Echo 650
at Biortus



Acoustic offset targeting test with Dye and Compound at Biortus

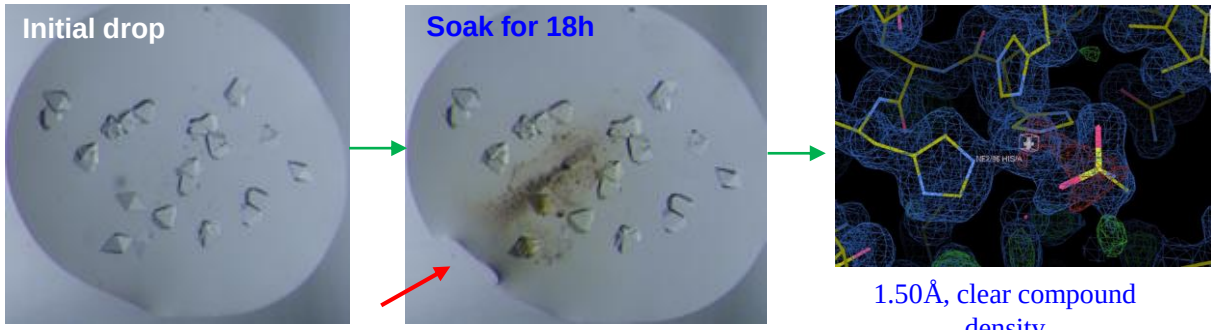


Compound gradual diffusion

- **Advantage**
  - **Wide applicability;**
  - **High throughput;**
  - **Cost-effective (compound/solvent): >20nL**
  - **Accuracy and Repeatability (non-contact transfer)**

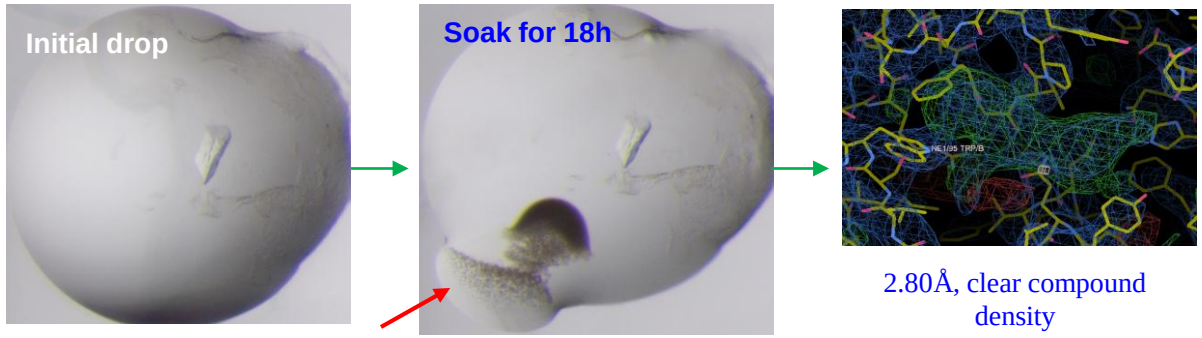
Success rate: **Almost 90% drops could be successfully offset targeting.**

Showcase at Biortus

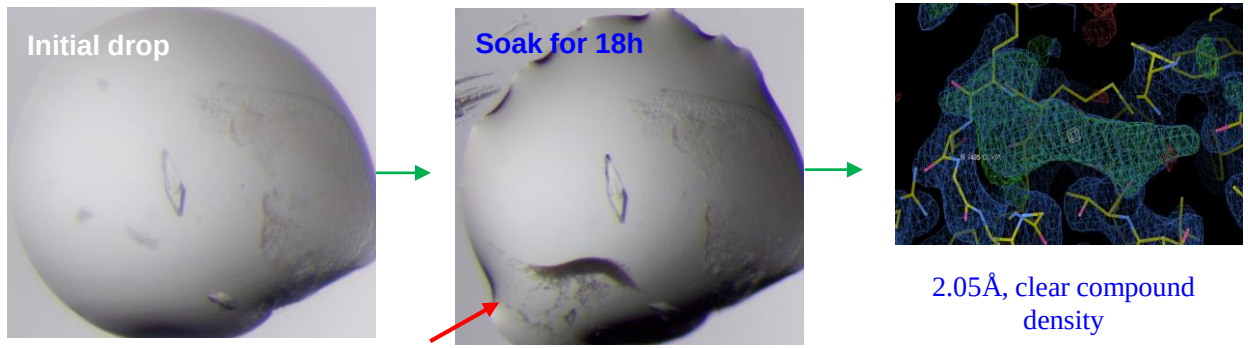


1.50Å, clear compound density

2.85Å, clear compound density



2.80Å, clear compound density



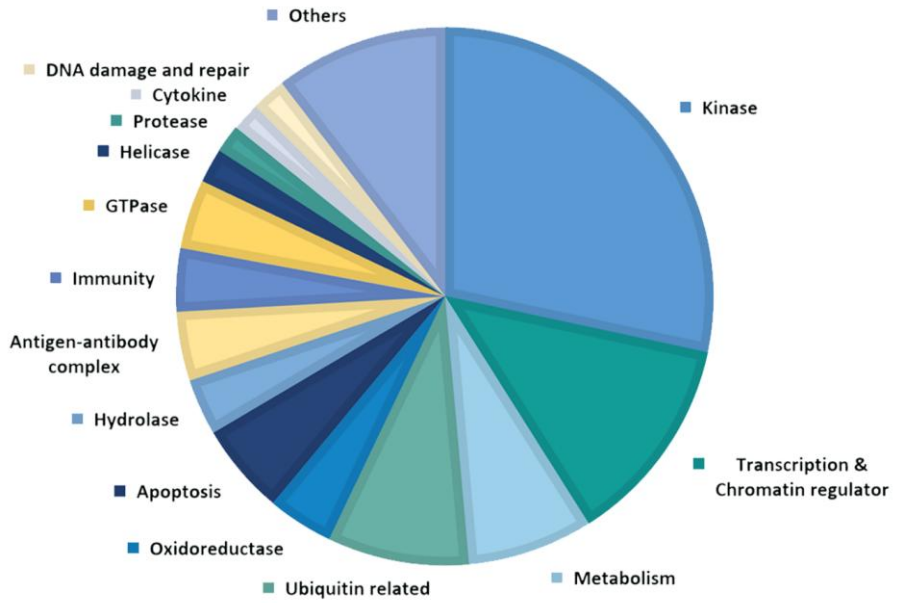
2.05Å, clear compound density

- **Crystals soaked by Echo and send to synchrotron, get good dataset.**

Gallery Structures Ready for Use

- Biortus has compiled a gallery of internal projects
  - Client confidentiality and exclusivity is of highest priority to Biortus.
  - All constructs are internally generated
- Proteins from the gallery can be generated and have a structure ready mostly in < **2** months

Unique Crystal Structures in Biortus



Sampling of Structure Gallery (>200 structures)

Target	Uniprot Number	PDB
Bcl-XL	Q07817	7CA4
JMJD2A	O75164	7D4A
MYST1	Q9H7Z6	7CMR
JNK2	P45984	7CML
USP7	Q93009	7CM2
LDHA	P00338	6ZZR
PHGDH	O43175	7CVP
DJ-1	Q99497	7C62
MTH1	P36639	7ESF
PARP14	Q460N5	7D2C
BRPF1	P55201	7C4I
DHFR	P00374	7ESE

*Deposited coordinates for recognition at PDB server

Target	Uniprot Number	Res. (Å)
PPARg	P37231	2.80
PDK1	O15530	2.35
IRAK4	Q9NWZ3	2.00
ACVR1	Q04771	2.20
ERK3	Q16659	2.30
JNK3	P53779	1.85
RSK1	Q15418	2.60
RSK2	P51812	2.65
PGK1	P00558	2.50
PLK1	P53350	1.95
PLK2	Q9NYY3	2.00
PLK4	O00444	2.70

*Long on-shelf structure list can be provided if interested

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THANKS

Biortus Biosciences Co., Ltd.
https://en.biortus.bio/