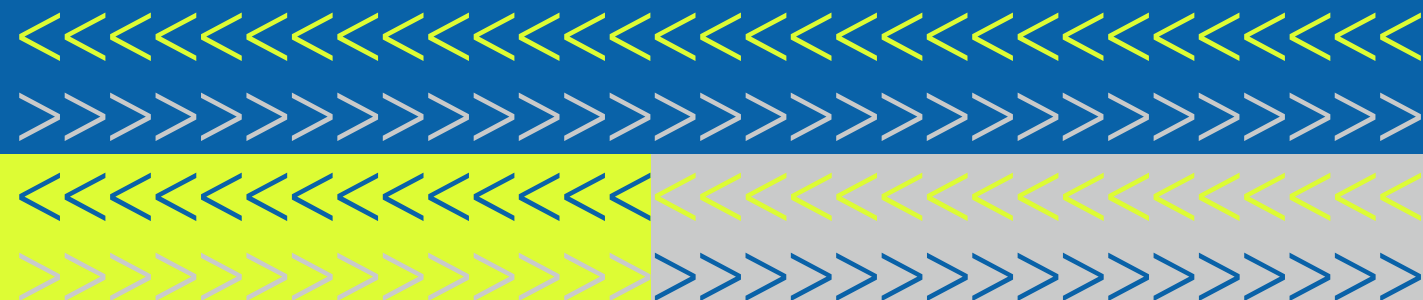


# Hits



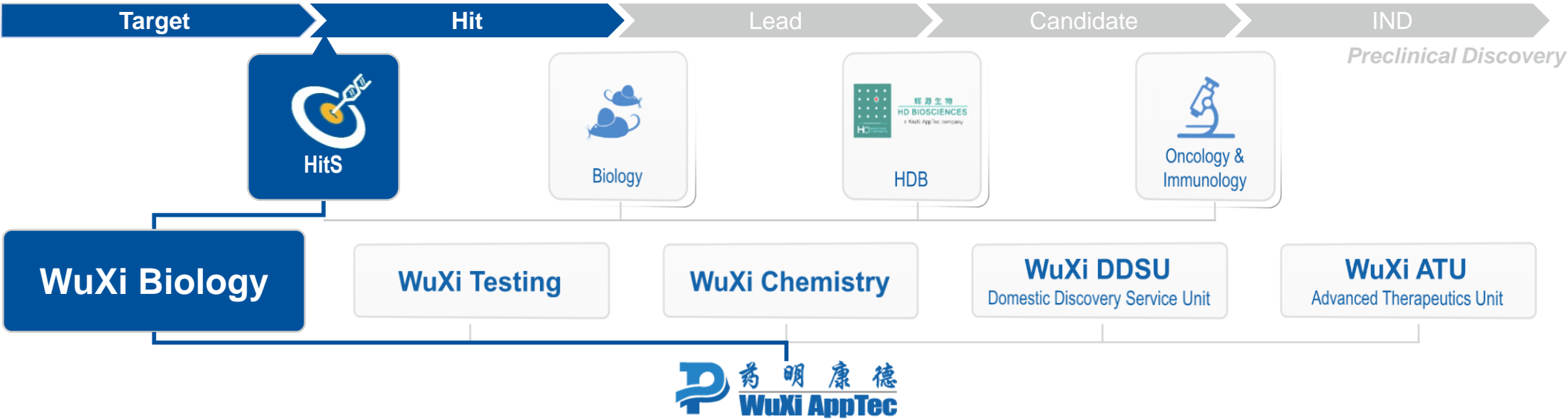
Unleashing the Potential  
of Hit Discovery



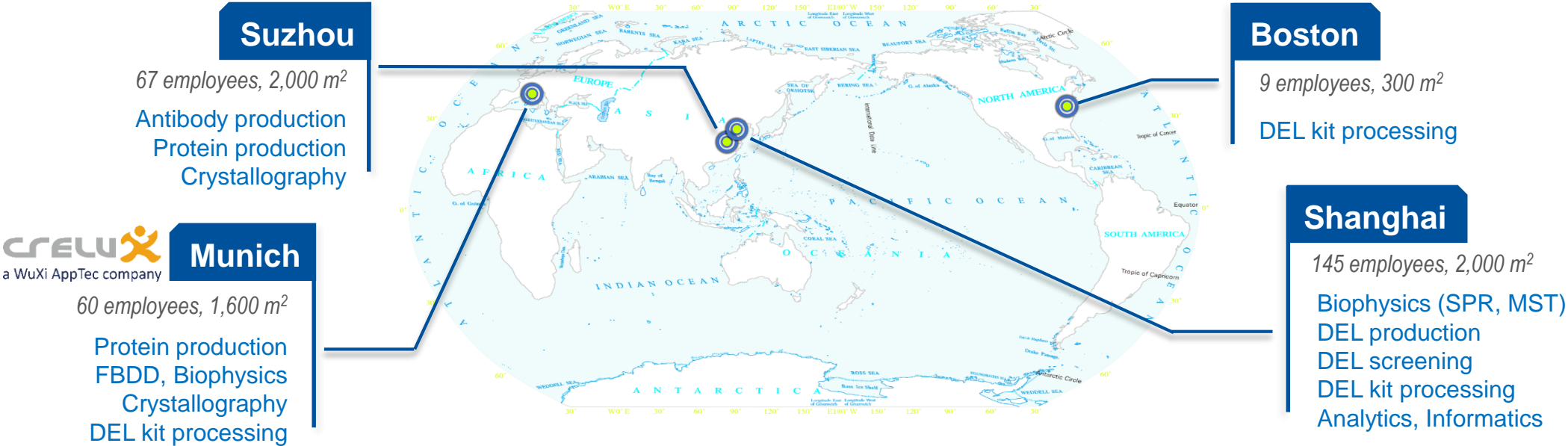
## CONTENT

HitS at WuXi AppTec & Capability Overview .....	3-4
Protein Production .....	5-6
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Hit Validation .....	10-11
Ready-to-Go Services .....	12-13
New Modality .....	14-16
Business Models .....	17-18

# HitS at WuXi AppTec



# HitS Global Presence



## Protein

### Protein Production

- All target classes
- High level protein engineering
- Tailored tagging strategies
- Recombinant protein production
- *E.coli*, yeast, insect cells, mammalian cells
- Customized conjugation (e.g. Biotinylation)
- Isotope labeling (15N, 13C)
- Antibody screening and Nanobody
- Comprehensive protein QC methods

## Screening

### Fragment Based Screening (FBDD)

- WuXi fragment library (~3100 fragments)
- Automated screening (MST/Dianthus, SPR, DSF/nanoDSF)
- Fragment screening via X-ray or NMR

### DNA-Encoded Library (DEL)

- 80+ billion synthetic compounds
- Bioinformatics system to collect, process and analyze data, facilitate involvement of the client to make collective decisions
- DEL + AI

## Hit Validation

### Biophysical Panel

- Over 10 biophysical methods available
- Affinity, thermodynamics, stoichiometry, kinetics, thermal & chemical stability, aggregation

### Biochemical Panel

- Various technologies (e.g. HTRF, AlphaScreen)
- Various luminescent, fluorescent, colorimetric assays

### Structure-based Drug Discovery

- X-Ray crystallography, Cryo-EM, NMR

Protein Production

Screening  
FBDD / DEL

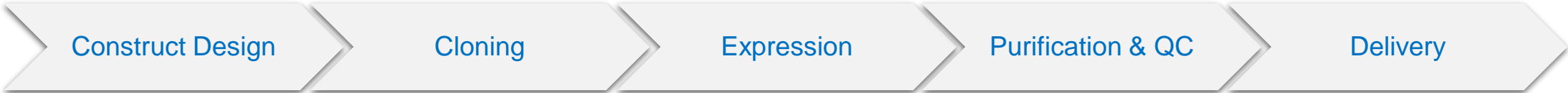
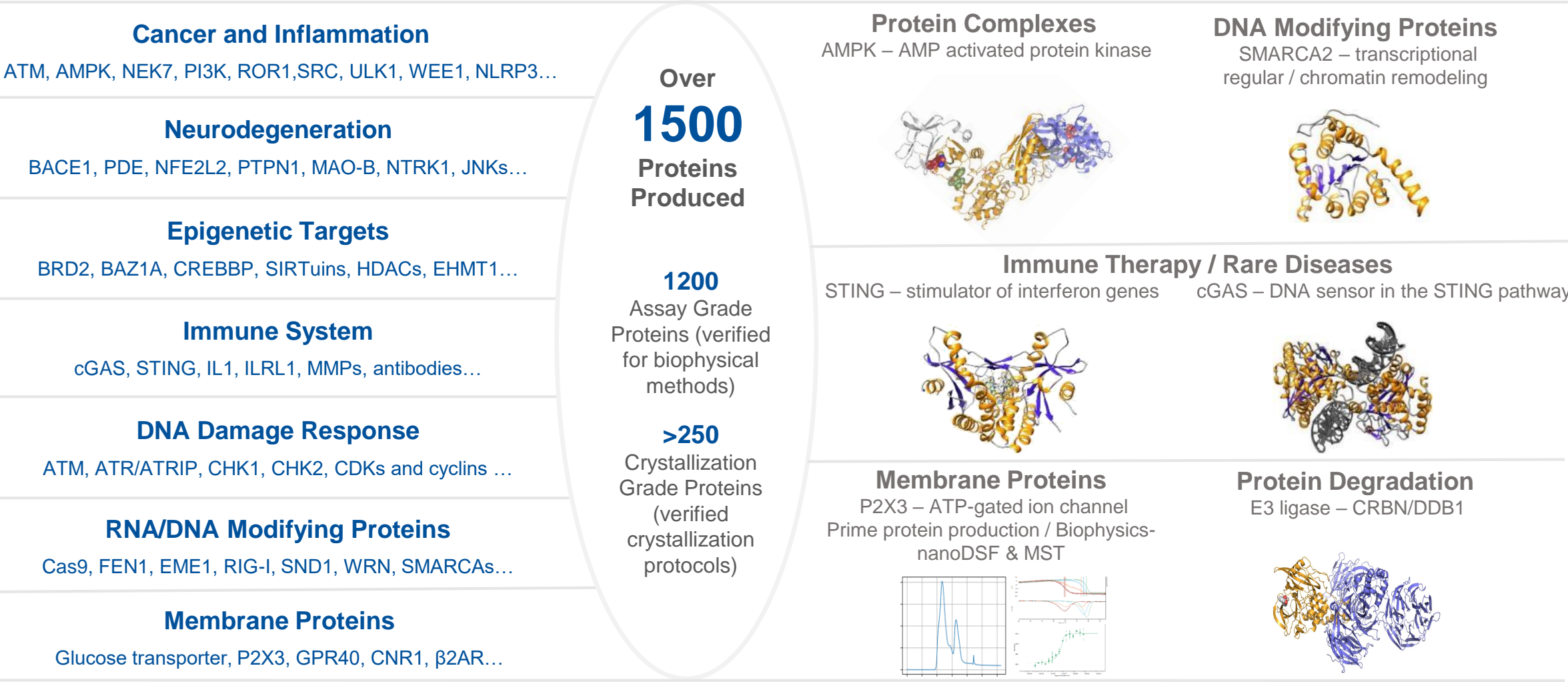
Biophysical &  
Biochemical Assays

Structure  
Determination

Hit-to-lead  
Optimization

# 1.1 Assay Grade Protein Production

--- Covers All Target Classes and Challenging Proteins ---



# 1.2 Protein Expression Solutions

--- Comprehensive Protein Expression Capabilities for Challenging Targets ---

### MultiBac VLP Factory

### Flu VLPs

**Insect cells**

## Co-expression Systems of Protein Complexes

**Mammalian/ Insect cells**

**MultiBac™/ ComplexInc™**

**BVES / BacMAM**

### Mammalian Expression (HEK293 / CHO)

- Transient Expression
- Stable Cell Lines

HEK293 Inducible  
(Protein complexes, toxic proteins, etc.)

HEK293 GnTI-  
(Modified glycosylation profile for crystallization)

Expi293 / ExpiCHO  
(Commercial customized requests or cell lines)

## Molecular Biology Strategies for Optimal Expression & Characterization

Test Expression

Production

QC

Real-time Expression Tracking

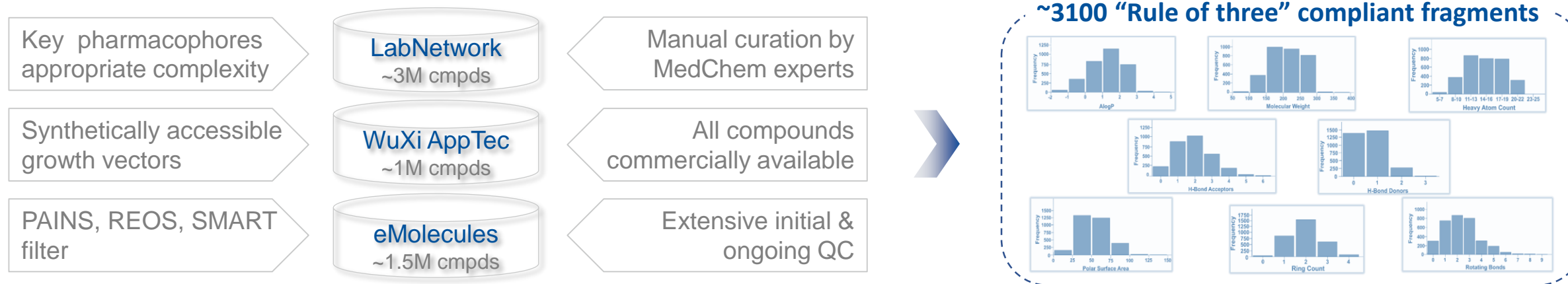
Stable Cell Lines

Targeted Subcellular Expression and Quantification



## 2.1 Fragment Based Screening

### --- HitS Fragment Library ---



### --- High Throughput Biophysical Assays for Fragment Screening ---



#### Biacore 8K: SPR

Affinity range: pM – mM

>1000 cmpds per day

Kinetic characterization of 64 interactions in 4h

8 channels HTS system

Dissociation rates:  $10^{-6}$  to  $0.5 \text{ s}^{-1}$



#### Monolith NT. Automated: MST

$K_D$  range: 10 nM – mM

500-800 cmpds per day

80  $K_D$ s per day

Fully automated MST Screening Unit

Labeled & labelFree MST



#### Dianthus NT.23 PicoDuo: TRIC

$K_D$  range: 10 pM – mM

1500 cmpds per day

> 150 Full  $K_D$ s per day

HTS Screening Unit

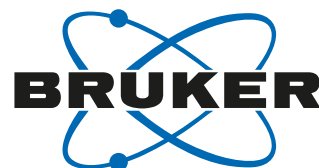
384 Plate format

## 2.1 Fragment Based Screening

### --- X-Ray Crystallography: Solution for Fragment Screening ---



**Bruker METALJET**  
X-ray source in-house



### Capabilities at HitS (Munich & Suzhou)

- >250 different protein crystalized
- >10 *de novo* structures / year
- Complete **XPRESS** Portfolio

click here: <https://www.crelux.com/pdf/Xpress-Portfolio.pdf>

### --- NMR: Solution for Fragment Screening ---

#### Typical NMR Screening Workflow

Target specific experiment design and optimization

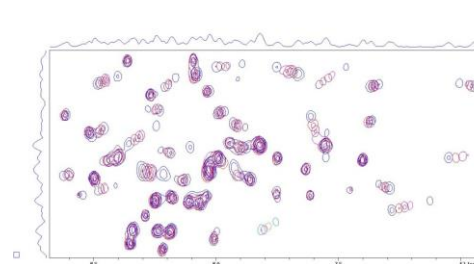
Mixing fragments in cocktails (~ 1500 fragments)

Primary screen: selection of active cocktails

Re-screen of active cocktails (deconvolution)

Data process: primary screen vs. deconvolution

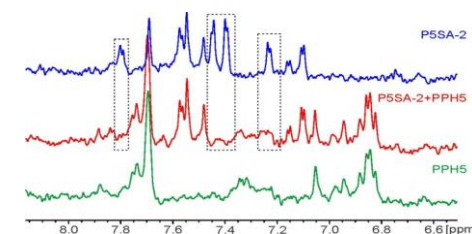
#### Protein-based Method



2D HSQC

Requires isotope labeling  
>100 mg protein required  
Delivers binding site information

#### Ligand-based Method



1D STD

No labeling needed  
10-20 mg protein required  
Detects specific interactions



Bayerisches NMR Zentrum

Partner with



## 2.2 DNA Encoded Library Screening

### WuXi AppTec DNA Encoded Libraries

80+ billion compounds  
 280+ libraries  
 6,000+ proprietary scaffolds & 30,000+ commercial building blocks  
 Covered 70% drug chemical space

### Affinity Screening

Additionally:  
 Cell-based DEL Screening  
 Screening of 'Covalent library'

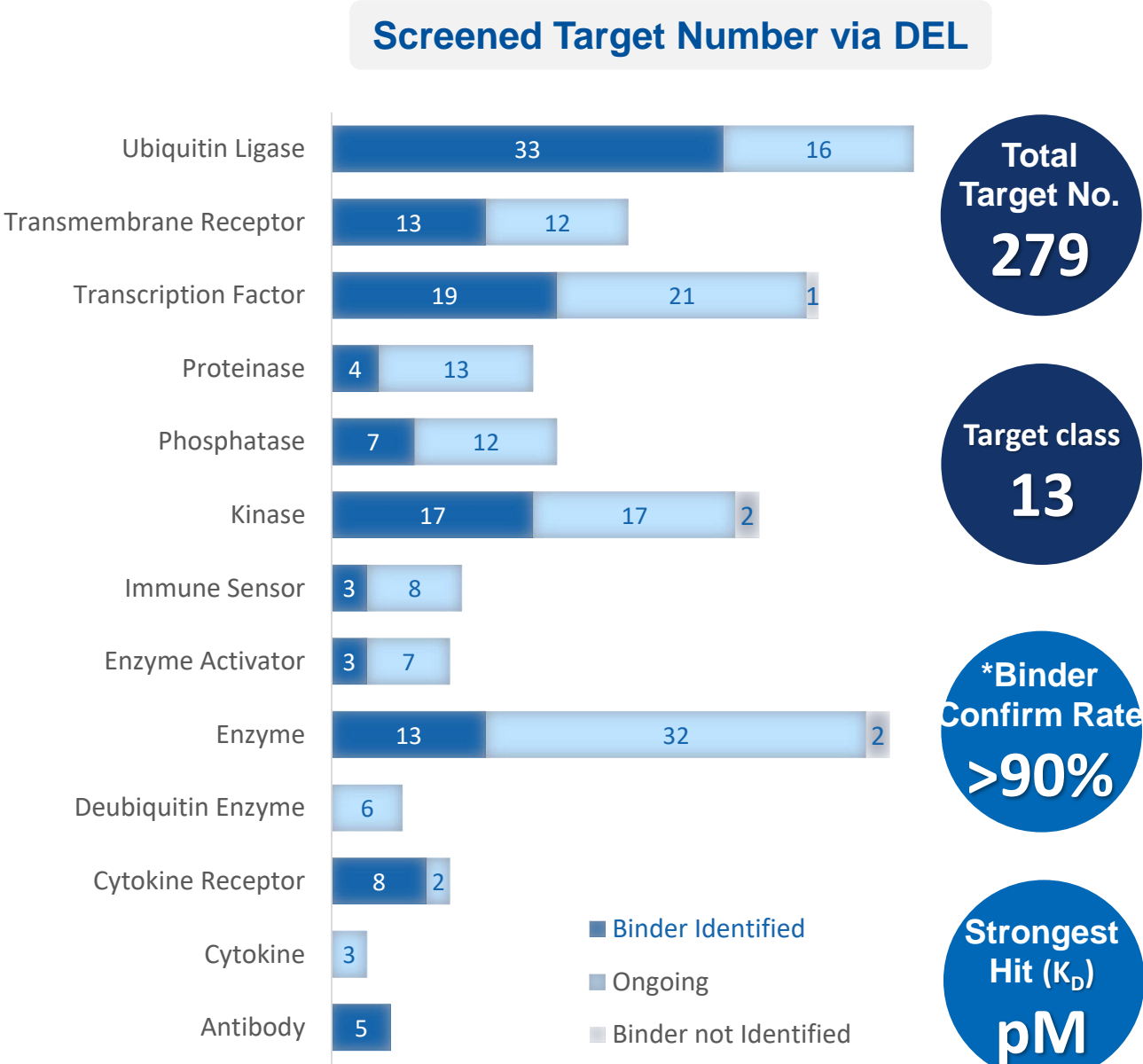
### Sequencing & Decoding

Multi-dimensional & transparent decision-making  
 Cloud-based data analysis system with proprietary algorithm

### Multiple validation methods

On-DNA synthesis & ASMS validation  
 Off-DNA Synthesis & Activity Confirmation

### Tailored business model

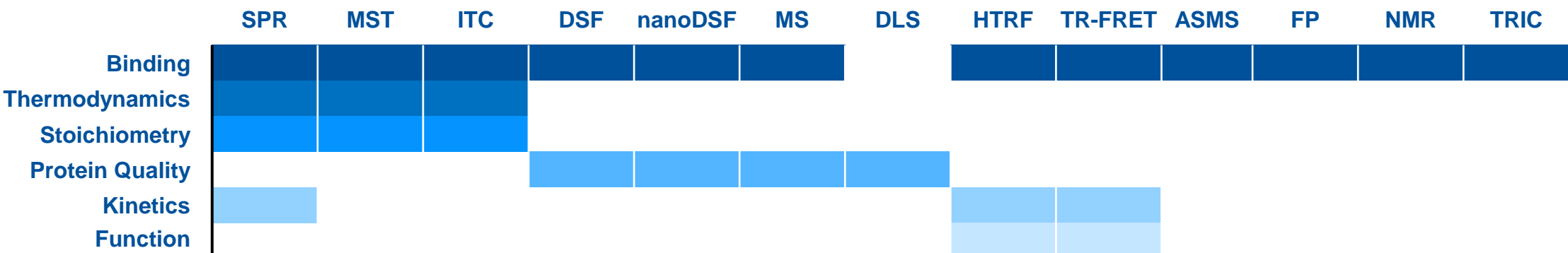


DELpro data until Jun 2021

\*Ongoing project not included

### 3.1 Biophysical and Biochemical Platforms

--- The Broadest Technologies Suite for Hit-finding Globally ---



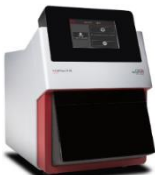
Our assays are validated by following ICH recommendations and inspecting the parameters such accuracy, intra-/inter-assay precision, interference, linearity, stability, specificity, & sensitivity



Surface Plasmon Resonance (SPR)  
Biacore 8000 & 8000+



Microscale Thermophoresis (MST)  
Monolith.115 B/R



Nanodifferential Scanning Fluorimetry (nanoDSF)  
Prometheus NT.48



Bio-Layer Interferometry (BLI)  
Octet RED384



Dynamic Light Scattering (DLS)  
DynaPro plate Reader III



On-DNA Affinity Selection Mass Spectroscopy (ASMS)



Isothermal Titration Calorimetry (ITC)  
MicroCal PEAQ-ITC automated

Critical parameters

$K_d$ : pM – mM  
 $K_{on}$ : up to  $10^9\text{ M}^{-1}\text{s}^{-1}$   
 $K_{off}$ :  $10^{-6}$  –  $0.5\text{ s}^{-1}$

$K_d$ : 1 pM – 1 mM

Thermal Stability:  $T_m$   
 Chemical Stability:  $C_m$   
 Aggregation:  $T_{agg}$

$K_d$ : 10pM –1 mM  
 $k_{on}$ :  $10^1$ –  $10^7\text{ M}^{-1}\text{s}^{-1}$   
 $k_{off}$ :  $10^{-6}$  –  $10^{-1}\text{ s}^{-1}$

Particles size: 0.5 - 1000nm (Radius)  
 Scattering Angle: 90°

Semi-quantitative

$K_d$ :  $10^{-9}\text{ M}$  –  $10^2\text{ M}$   
 $T_{stability}$ :  $\pm 0.00012^\circ\text{C}$

Application

Small molecule drug candidates to high-molecular weight proteins in various sample environments

Proteins, peptides, nucleic acids, vesicles, platelets and whole cells, virus particles and empty capsids

Proteins, peptides, nanoparticles, DNA, RNA, exosomes, liposomes

Proteins, antibodies, peptides, serum containing media, DMSO containing buffers, virus-like particles, untreated cell culture supernatants and crude cell lysates

Proteins, peptides, nanoparticles, liposomes

Hit triaging after DEL selection

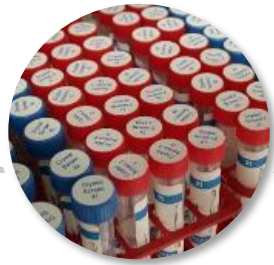
Proteins, antibodies, Peptides, Nucleic acids, nanoparticles, liposomes

## 3.2 Structural Biology Platforms

### --- X-Ray Crystallography ---



Crystal grade protein production



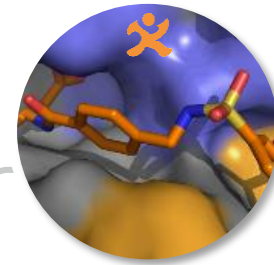
Screening of myriad crystallization conditions



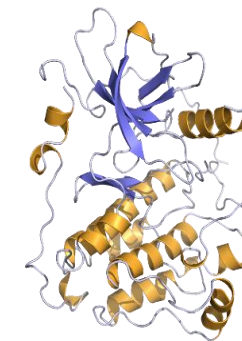
Fine-tuning of crystallization conditions to grow well diffracting crystals



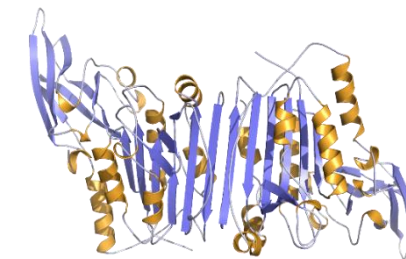
Preparation of crystals for collection of X-ray diffraction data



Data processing, structure solution and refinement



EGFR, 1.7 Å



MALT1, 2.0 Å

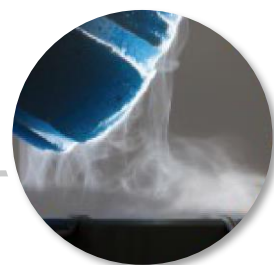
### --- Cryo-EM: A Comprehensive Solution for Challenging Targets ---



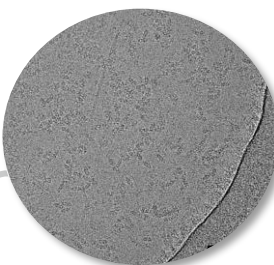
Cryo-EM grade protein production



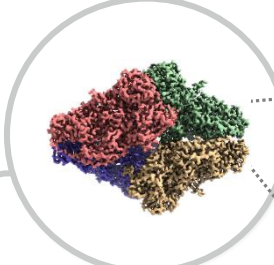
EM feasibility analysis (negative staining EM)



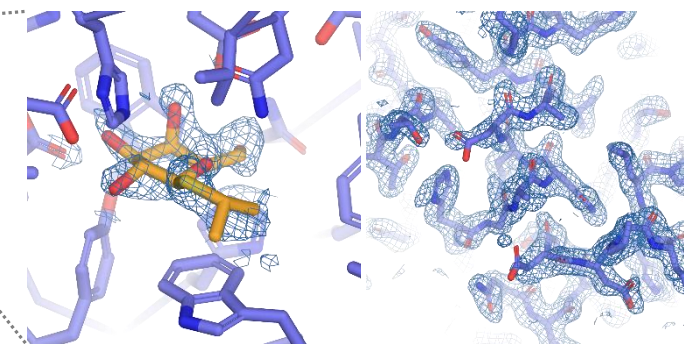
Cryo-EM conditions optimization



Cryo-EM imaging



Data processing structure solution



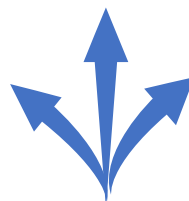
$\beta$ -Galactosidase, 2.1 Å

--- Fast Track to Characterize Small Molecule Target Interaction ---



### Established Assays

*Optimized assay conditions*



### Flexible

*Single/multiple assays in parallel*



### Cost-effective

*High quality data*



### Fast Turnaround Time

*Less than 2 weeks*

--- nanoDSF • MST • SPR • ADP-Glo • Nucleotide-exchange ---

### Protein Supply

Established protein constructs in house

- Tailored high quality assay grade proteins
- Protein QC: SDS-PAGE, aSEC, MS

Complete protein supply included

### Assay Initiation & Measurements

Established assay conditions

- Established labeling/coupling method
- Published tool compounds

Up to 3  $T_m$ s /  $K_d$ s /  $IC_{50}$ s included

### Results & Report

A tabulated report included

Extended report available including measured curves, assay conditions, analysis and scientific summary in pdf-format

# 4.1 Ready-To-Go Assay Launched!

--- Covers a Broad Range of Drug Targets! ---

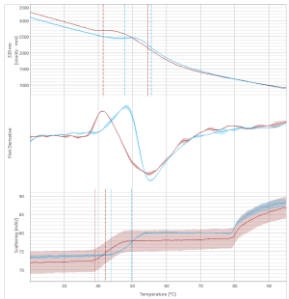
Target	UniProt ID	PP	MST	nanoDSF	SPR	Biochemical Assay
CDK7	<a href="#">P50613</a>	✓	✓	✓	✓	–
CRBN/DDB1	<a href="#">Q96SW2/Q16531</a>	✓	✓	✓	developing	–
cREL	<a href="#">Q96HD1</a>	✓	✓	✓	–	–
DOCK5	<a href="#">Q9H7D0</a>	✓	✓	✓	✓	Nucleotide-exchange
GLUT1	<a href="#">P11166</a>	✓	✓	✓	–	–
GRB2	<a href="#">P62993</a>	✓	✓	✓	–	–
KRAS	<a href="#">P01116</a>	✓	✓	✓	✓	Nucleotide-exchange
MALT1	<a href="#">Q9UDY8</a>	✓	✓	✓	✓	–
MAP4K1	<a href="#">Q92918</a>	✓	✓	✓	developing	ADP-Glo
NLRP3	<a href="#">Q96P20</a>	✓	✓	✓	–	ADP-Glo
NRAS	<a href="#">P01111</a>	✓	✓	✓	✓	Nucleotide-exchange
P2RX3	<a href="#">P56373</a>	✓	✓	✓	–	–
p38	<a href="#">Q15759</a>	✓	✓	✓	–	–
PolQ	<a href="#">O75417</a>	✓	✓	✓	–	–
SHP2	<a href="#">Q06124</a>	✓	✓	✓	–	–
SMARCA2	<a href="#">P51531</a>	✓	✓	✓	developing	ADP-Glo
SOS1	<a href="#">Q07889</a>	✓	✓	✓	–	Nucleotide-exchange
STAT3	<a href="#">P40763</a>	✓	–	✓	✓	–
STAT4	<a href="#">Q14765</a>	✓	–	–	✓	–
STAT6	<a href="#">P42226</a>	✓	✓	✓	✓	–
STING	<a href="#">Q86WV6</a>	✓	✓	✓	✓	–
USP7	<a href="#">Q93009</a>	✓	✓	✓	✓	–
WRN	<a href="#">Q14191</a>	✓	✓	✓	✓	–

PP: Protein Production. About details (e.g., DEMO Report, Price, etc), please enquire!

## Show case:

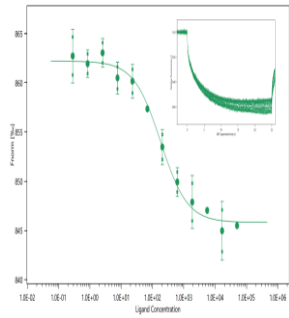
### Activity-Validated Assays of NLRP3 vs. MCC-950

- High quality assay grade protein produced in house
- Protein QC: SDS-PAGE, aSEC, MS



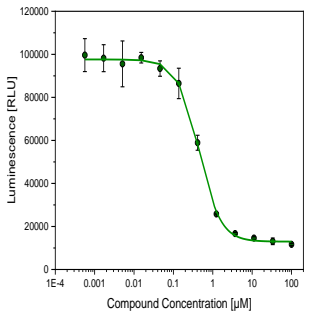
**LabelFree TSA  
(nanoDSF)**  
**Impact of MCC-950 on  
NLRP3 thermal stability**

Control: DMSO  
MCC-950: 1  $\mu$ M



**TRIC/Dianthus**  
**Binding affinity of  
MCC-950 to NLRP3**

**Labeled MST assay**  
 $K_D$ : 180 nM



**ADP-Glo assay**  
**Impact of MCC-950  
on NLRP3 activity**

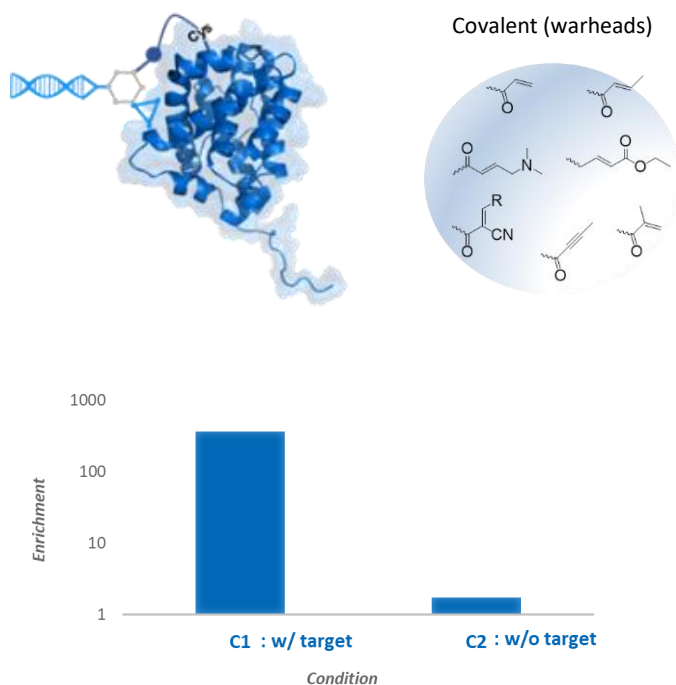
$IC_{50}$ : 450 nM



## 4.2 New Modality – DEL Related

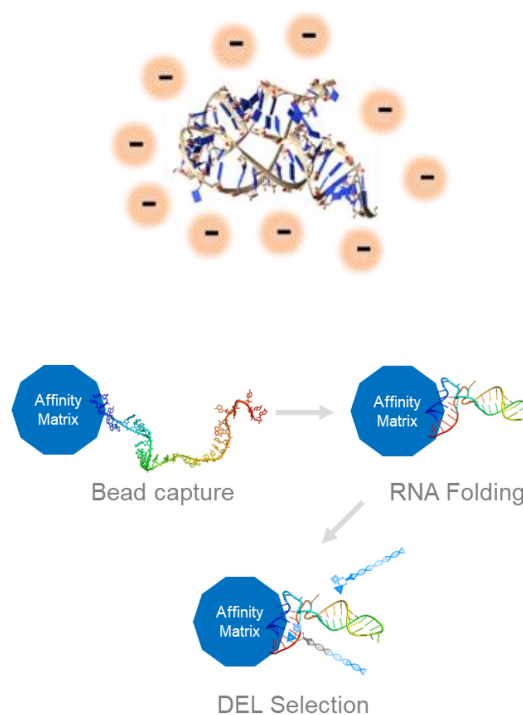
### Irreversible Covalent Screening

- Specially designed library collection with **electrophilic warheads**
- Optimized selection method to enhance the **signal-to-noise** ratio



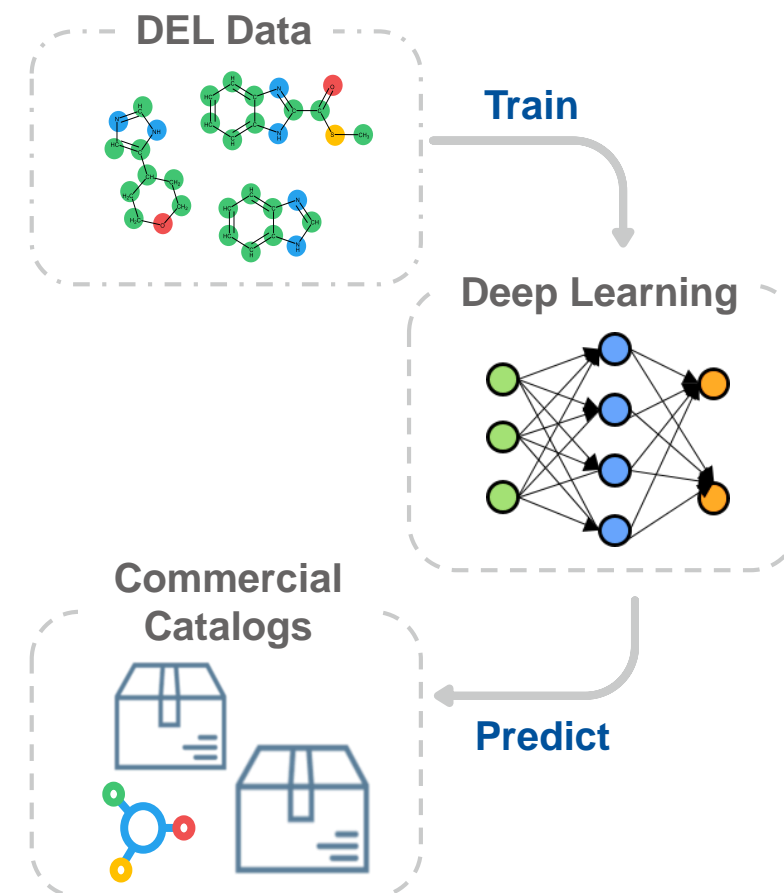
### RNA Target

- Optimized selection procedure to counter **charge, RNA/DNA interaction and structural integrity** of RNA during DEL screen



### DEL+AI

- Ligand-based Virtual Screening
- Lead-like Chemical Space
- Cost-effective Compound Acquisition



## 4.2 New Modality – MST Related

### --- Development of Bifunctional Small Molecules ---

#### Selected List of RING E3 Ligases

##### Protein Production:

KEAP1

KLHL40

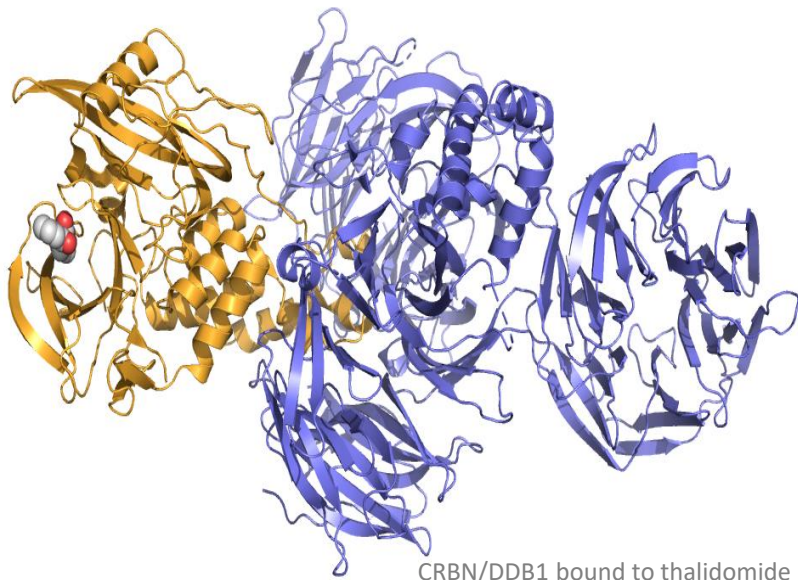
ITCH

VBC

UBR5

KCTD5

(more in evaluation,  
please inquire)



CRBN/DDB1 bound to thalidomide

#### Selected Complexes

##### Structures Resolved for:

CRBN / DDB1

SKP1 / SKP2 / Cks1

VHL / Elongin B / Elongin C

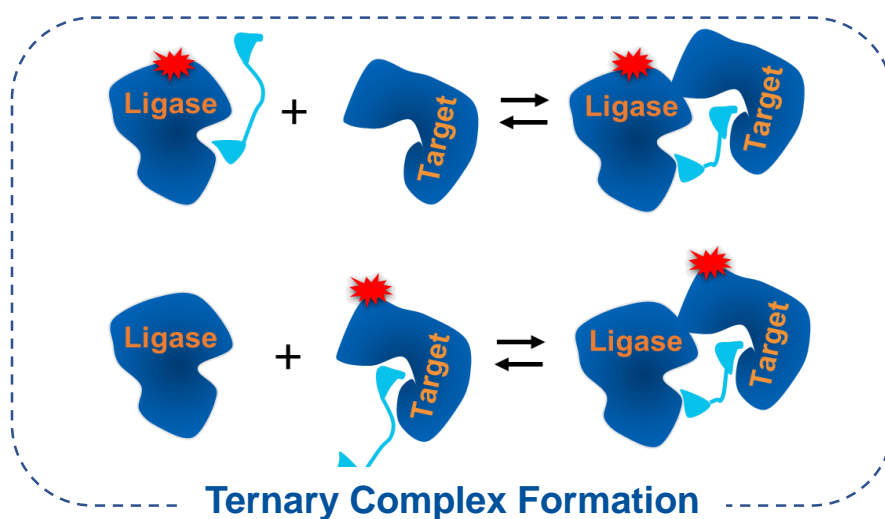
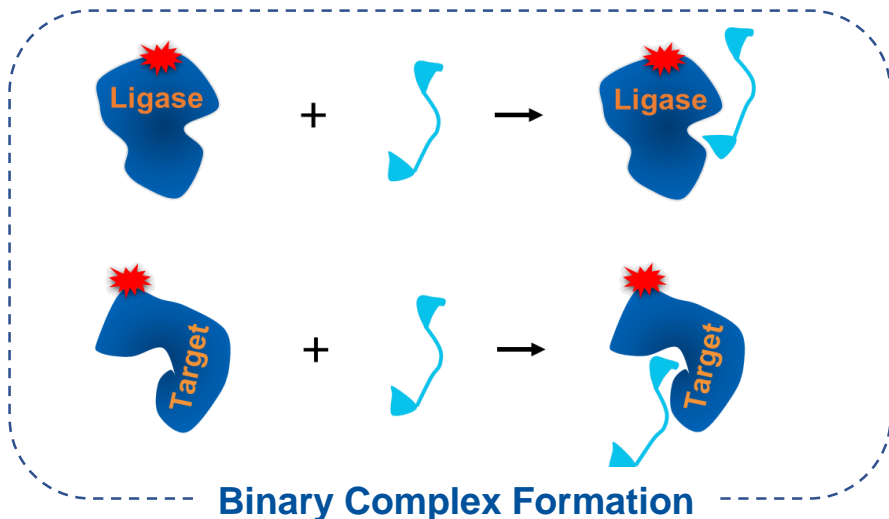
CUL4A

ITCH

MDM2

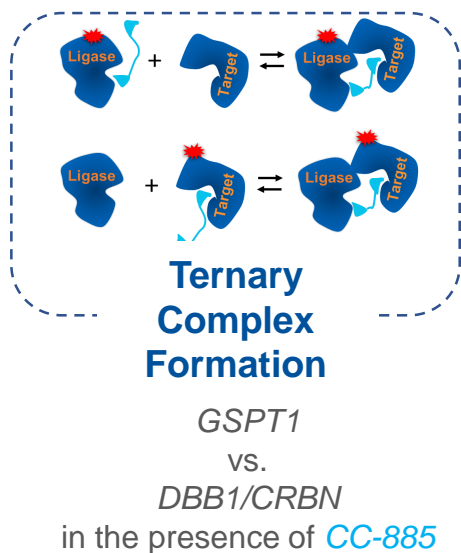
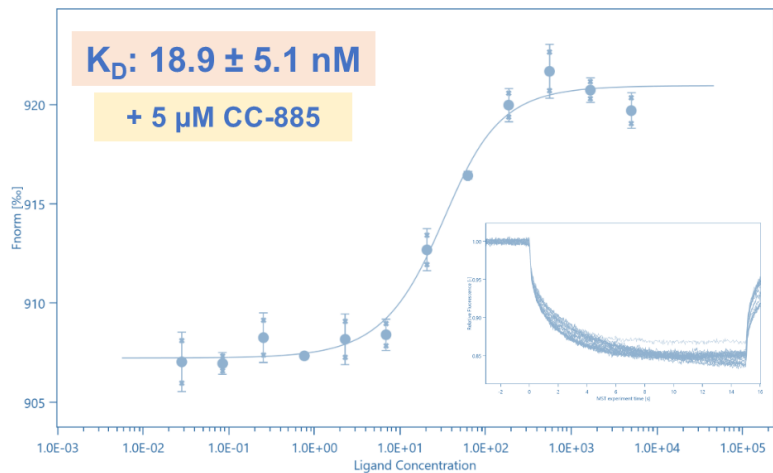
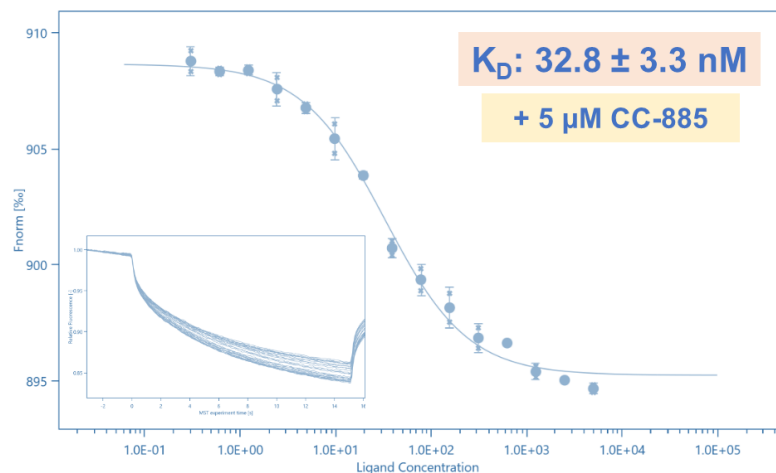
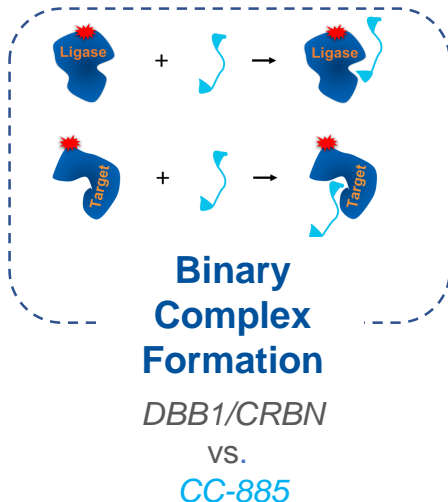
### --- Characterization Of Bifunctional Small Molecules Using MST Platform ---

Bifunctional  
compound

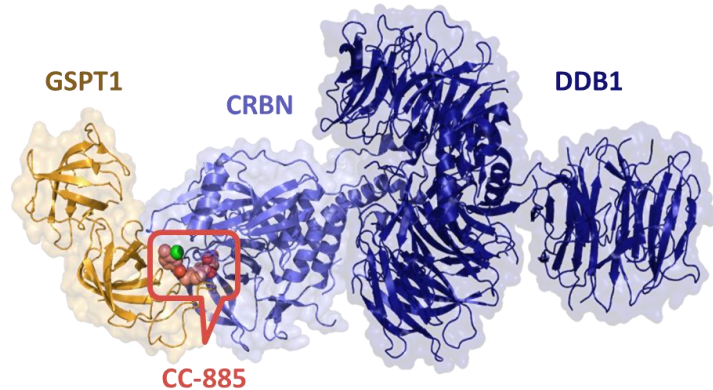
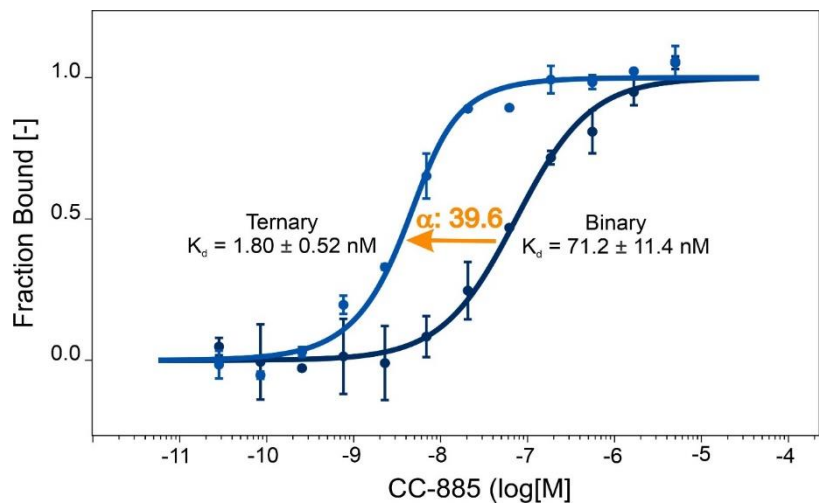
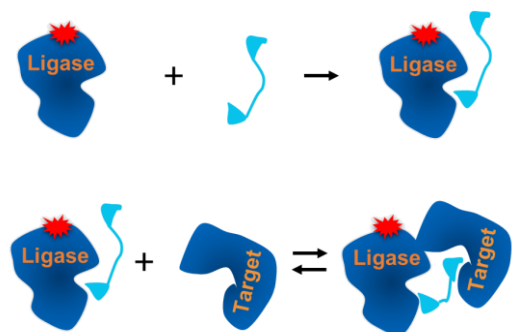


## 4.2 New Modality – MST Related

### --- Development of Bifunctional Small Molecules ---



### --- Binary / Ternary Complex Formation Measured by MST ---



DBB1/CRBN vs. CC-885 in the presence & absence of GSPT1 cooperativity

# HitS Business Models

- Experienced employees with multidisciplinary background
- Cutting-edge equipment and IT infrastructure to facilitate the experiment and management
- Integrated project management

- Tailored hit discovery
- Full professional service
- Standard workflow
- Customized study design
- Integrated project management

Full-Time Equivalent

Fee for Service

## Flexible Business Models

Ready-to-Go Assays

Products & Services

- Protein on stock
- Established assay conditions
- Cost-effective
- Fast turnaround time
- nanoDSF • MST • SPR • ADP-Glo
- Nucleotide-exchange

- Choice of DEL business models



- One-stop target-to-hit solutions
- Protein production for all target classes
- Broadest biophysical/biochemical methods
- Fragment based screening
- Structure-based drug discovery:  
*X-Ray crystallography, Cryo-EM, NMR*



## Quality

Highest standards of Professionalism and confidentiality

Proven track record of success

Recognized by world-class bio-tech / pharmaceutical companies

> 70% employees are Ph.D level



## Capabilities

One-stop target-to-hit solutions

Tailored approach to client's molecules

Broadest technologies suit for hit-finding globally

Latest target-to-hit technologies



## Customer Relations & Communication

Customer orientation

Effective communication  
Commonly customer relations team supplies  
feedback in 24 hours

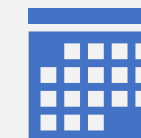
Customized scientific evaluation directly from the senior scientists responsible in the related departments



## Project Experience & Expertise

Expertise in all target classes and challenging proteins

Professional project management with designated project manager, including fixed bi-weekly progress meetings ensuring the highest level of effective communication and project continuity



## Timeline

Fast turnaround time

Accelerate target-to-hit finding processes

Strictly follow planned timelines

*Hits, Hit Success*



# *Improving Health. Making a Difference.*

HitS web



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