

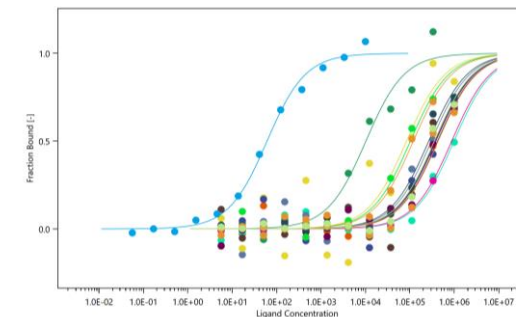
Biophysics at WuXi AppTec's HitS



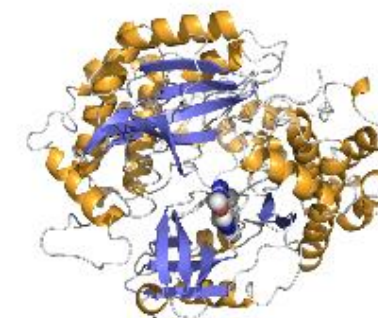
State-of-the-Art Biophysical Methods

The broadest technology suite for hit-finding & confirmation accessible

| | |
|-------------------------|--|
| Binding | MST, SPR, nanoDSF, DSF, ITC, HTRF, TR-FRET, FP, SwitchSense, Mass spectrometry, ^1H and ^{19}F NMR |
| Kinetics | SPR, HTRF, TR-FRET, SwitchSense |
| Thermodynamics | ITC, SPR, MST |
| Stoichiometry | SPR, ITC, MST |
| Protein Quality | nanoDSF, DSF, DLS, Mass spectrometry |
| Kinetics | SPR, FRET, TR-FRET, SwitchSense |
| Structure Determination | X-ray crystallography, solid and solvent state NMR, Cryo-EM |



Complete biophysical characterization of your compound series



Readily Accessible Assays for Many Targets

Various Targets, Various Assays: Some Examples

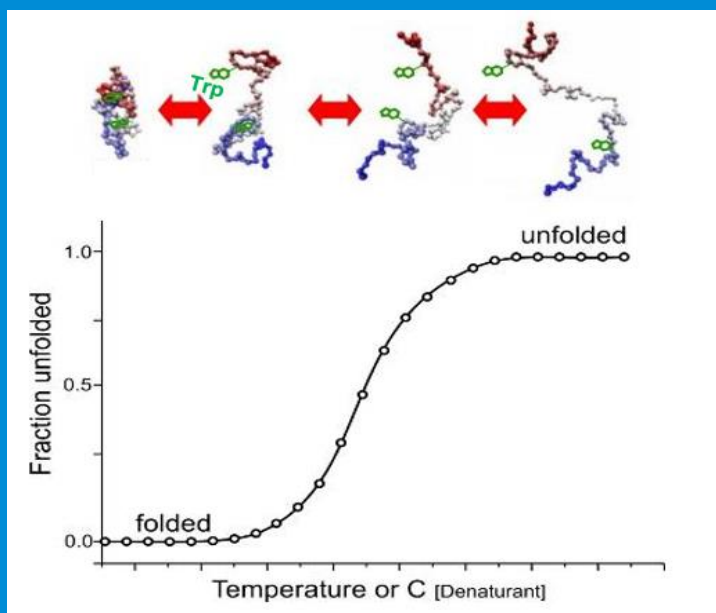
| | | | | | |
|----------------------|--------------|---------------------|--------------------|---------|--------------|
| ACSS1 | Cas9 | CDK9 | MALT1 | Pik-1 | SOS1 |
| ACSS2 | CRBN | IFIH1 (MDA5) | MAP4K1 | PoIQ | SMARCA1 |
| ALAS2 | CREBBP | IL13 | Mb21d1 (mouse) | PRKAA1 | SMARCA2 |
| alphaB-Crystallin | cREL | IL17A / IL17AR | MEK-1 | PRKAA2 | SMARCA6 |
| alphaGal | DDX58 (RIG1) | IL18 / IL18R | MEN1 | QPCTL | SND1 |
| Antibodies | DHODH | IL1R | MER | RAC1 | SNRPC |
| APOBEC3B | Dock5 | IL2 / IL2R | MGP (MUS81) | RET | SOS1 |
| APOE3/E4 | EGFR | IL4 | MLLT1 | SIK3 | STAT6 |
| Aurora A (AURKA) | EXO1 | IL6 / IL6R | MRE11 | SIRT2 | STING |
| AXL | FEN1 | IL7 / IL7R | NLRP3 | SIRT5 | TBK1 |
| AXL1 | FYN | IRAK1 | Norovirus Protease | SKP2 | TNFalpha |
| BCL2 (XIAP, cIAP) | GLI1 | IRAK3 | NR5A2 | SKP2 | TrpR |
| BRD2 | GRB2 | IRAK4 | NRAS | SMARCA1 | ULK1 |
| BRD4 | HMOX1 | Jak-2 | P2RX3 | SMARCA2 | USP7 |
| BRD9 | HPGD | KIT | p38 alpha | SMARCA6 | WDR48 (USP1) |
| | HS2T1 | KRAS | PIK3CA | SND1 | WEE1 |
| | CDC7 | LIG1 (DNA Ligase 1) | PKA | SNRPC | WRN |
| | CDK7 | LIG3 (DNA Ligase 3) | PKR | SOS1 | XRN1 |

nanoDSF Example: LabelFree Thermal Shift

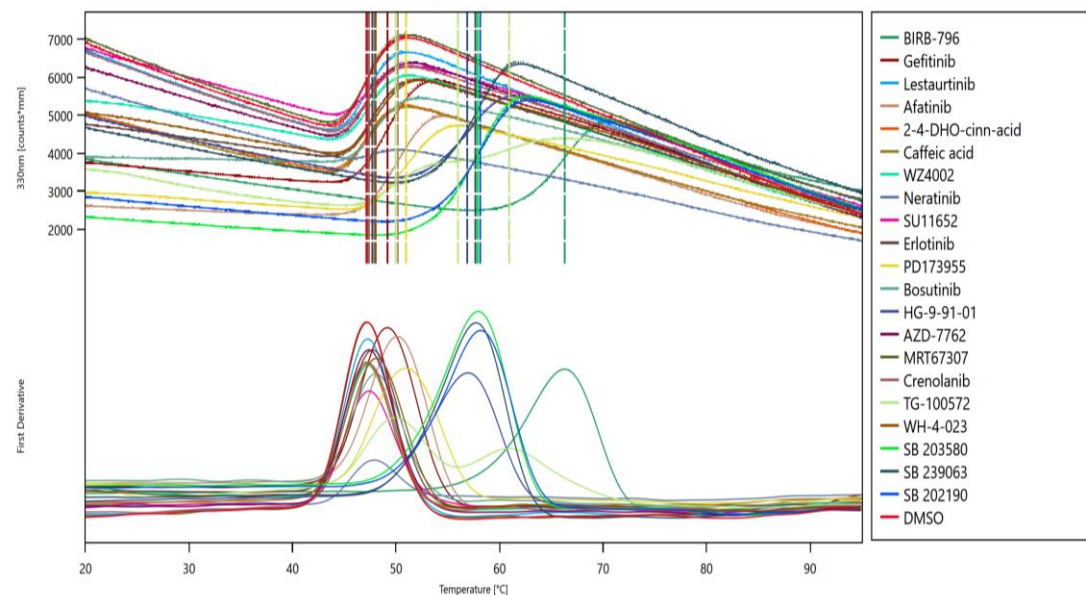
Readout by tracking internal tryptophan fluorescence

Principle of nanoDSF

Determination of thermal stability induced by a ligand



LabelFree
Thermal shift
assay with p38



Microscale Thermophoresis

Determination of binding affinity in free solution



Monolith NT.115

K_d range: 1 pM - mM
 Protein: 60 ng per K_d
 Channels: blue, green, red
 Time: 15 min per K_d



Monolith NT.LabelFree

K_d range: 10 nM - mM
 Protein: 3 μ g per K_d
 Channels: UV
 Time: 15 min per K_d
 Limitation: interfering
 fluorescence of binding partner



Monolith NT.Automated

K_d range: 1 pM - mM
 Protein: 60 ng per K_d
 Channels: blue, green, red, UV
 Time: 50-100 K_d per day

Scalable

Microscale Thermophoresis

Determination of binding affinity in free solution



Monolith NT.115

K_d range: 1 pM - mM

Protein: 60 ng per K_d

Channels: blue, green, red

Format: Single capillaries

Time: 15 min per K_d



Monolith NT.LabelFree

K_d range: 10 nM - mM

Protein: 3 μ g per K_d

Channels: UV

Format: Single capillaries

Time: 15 min per K_d



Monolith NT.Automated

K_d range: 1 pM - mM

Protein: 60 ng per K_d

Channels: blue, green, red, UV

Format: Capillary chip

Time: 50-100 K_d per day



Dianthus NT.23PicoDuo

K_d range: 1 pM - mM

Protein: 60 ng per K_d

Channels: red

Format: 384 well plate

Time: up to 1500 K_d per day

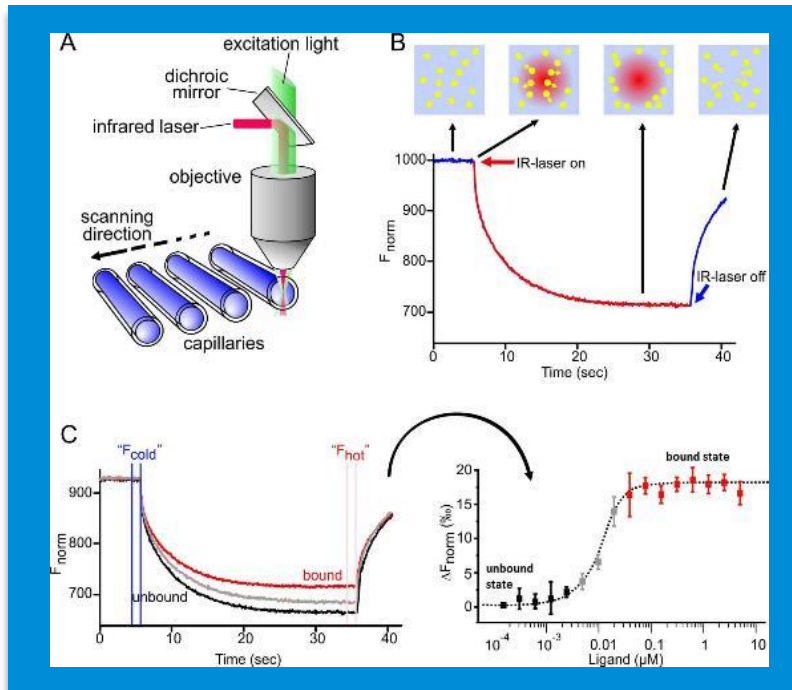
Scalable

MST Example: Binding Assay for Kinases

Quantification of protein – ligand interaction in free solution

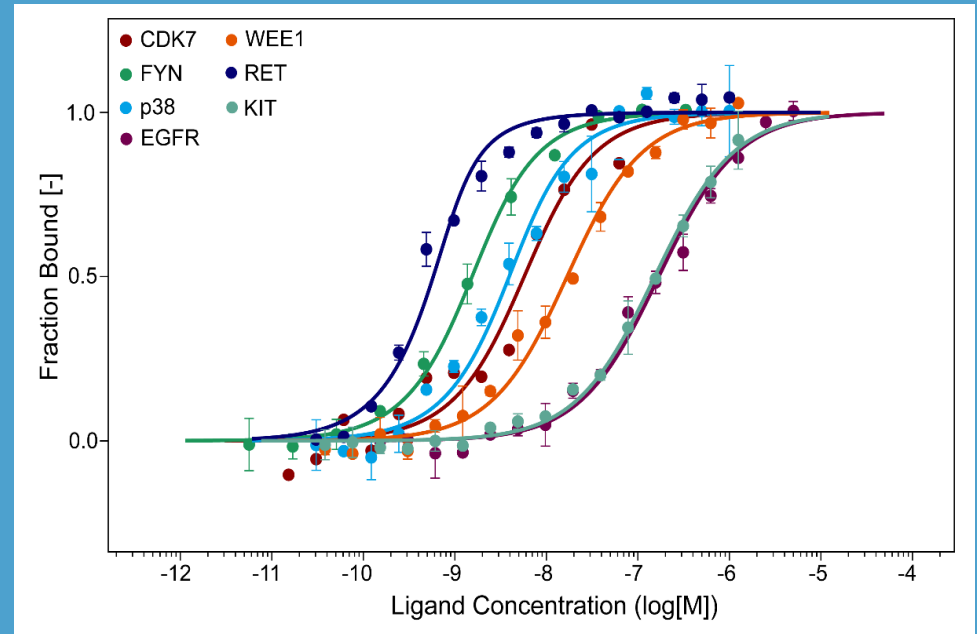
Principle of MST

Determination of binding affinity



Staurosporine binding to selected kinases (Kd):

1. CDC7 (44.7 ± 18.6 nM)
2. FYN (10.4 ± 1.2 nM)
3. EGFR (1671 ± 227 nM)
4. WEE1 (150.1 ± 21.1 nM)
5. RET (1.18 ± 0.60 nM)
6. KIT (1527 ± 138 nM)
7. p38 (SB203580, 24.2 ± 5.9 nM)



Surface Plasmon Resonance

Determination of binding affinity and kinetics after surface immobilization



Biacore X100

Dissociation rates: $10^{-5} - 0.1 \text{ s}^{-1}$

Affinity range: pM - mM

Assay setup and optimization

Small scale interaction analysis



Biacore 8K

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

Affinity range: pM - mM

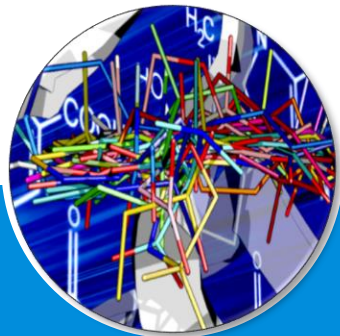
Channels: 8 channels HTS system

Screening capacity: < 2300 compounds per day

Scalable

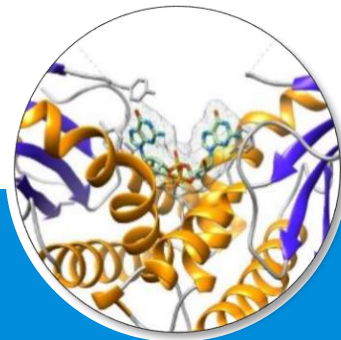
Screenings

Hit finding with the state-of-the-art technologies



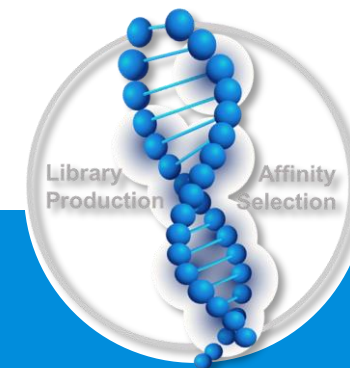
Fragment Screening by:

MST/TRIC and/or SPR
NMR
X-ray Crystallography



Compounds Library Screening by:

MST and/or SPR
LabelFree Thermal Shift
NMR
Crystallography

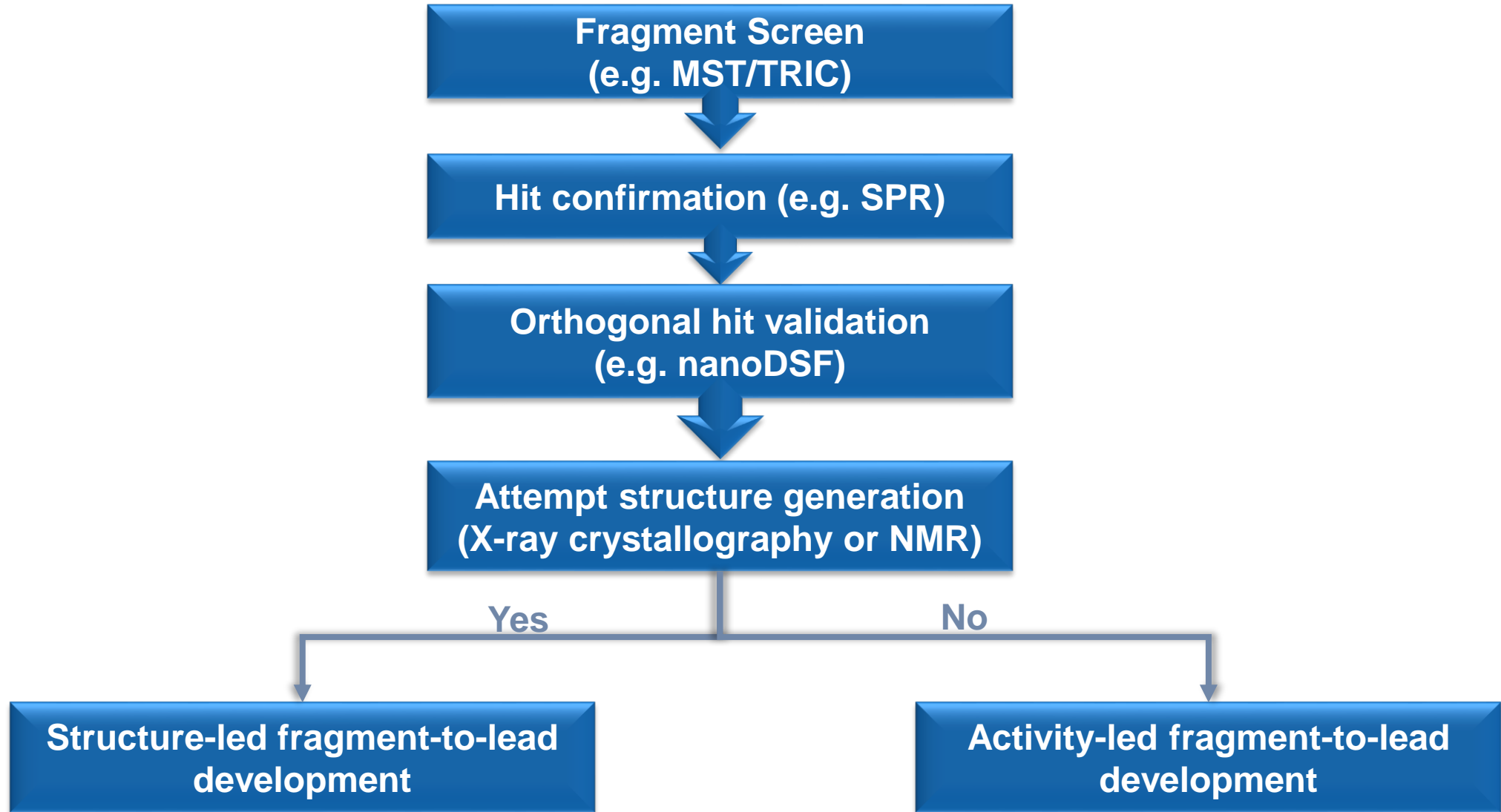


WuXi DEL Technology Service

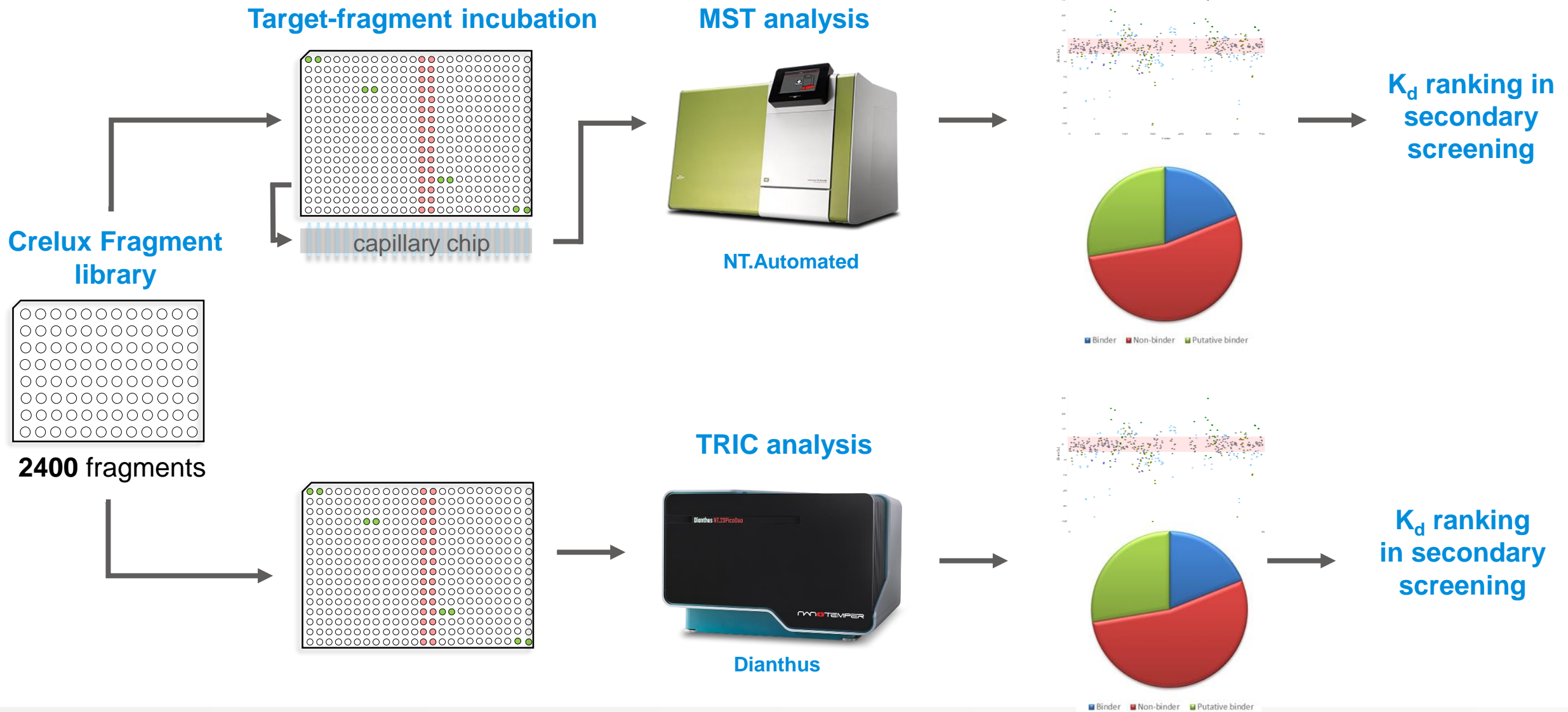
DEL platform (~90B compounds)
<http://rsd.wuxiapptec.com/dna-encoded-library-technology-services>

From fragment or small molecule to IND with WuXi Platform

Typical Fragment Screening Workflow

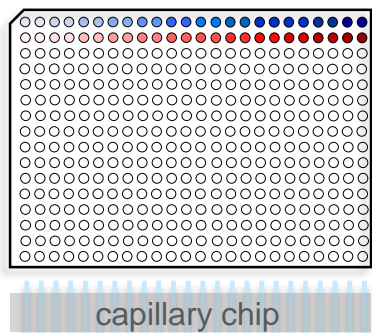


Primary screening – single dose response



Primary screening – K_d ranking

Target-fragment incubation

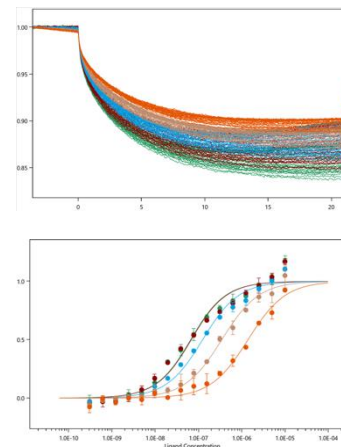


MST analysis



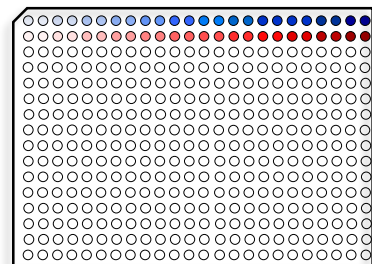
NT.Automated

K_d ranking

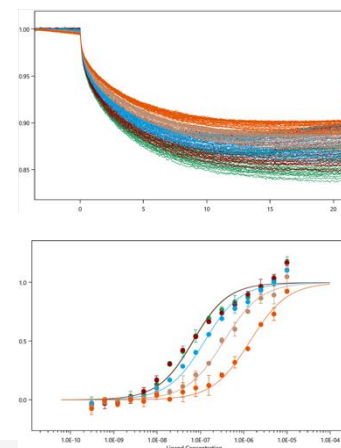


orthogonal
hit
validation

TRIC analysis



Dianthus



orthogonal
hit
validation

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