Accelerating Discovery Through Structural Insight

FRAGMENT–BASED
HIT GENERATION

SARomics Biostructures at a glance

- Hybrid business model since 2006
  - Independent and co-founder owned
  - CRO generating revenues
  - Internal discovery projects
- Proprietary discovery platform
  - Unique expertise in protein structure determination
  - Hit identification using proprietary WAC™ fragment screening technology and crystallographic fragment screening (XFS)
- Delivered several hundreds of crystal structures to pharma, biotech and academic clients worldwide
  - Protein/small-molecule complexes
  - Antibody/antigen complexes
  - Industrial enzymes
- Experienced and skilled team of 26 (23 PhDs)
- Sales representatives in San Francisco & Japan

Medicon Village, Lund, Sweden

May 2023

New lab 2024

1,000 m^2 at Medicon Village

Joint Fragment-Based Lead Discovery Platform

RG DISCOVERY

50 employees

- Medicinal chemistry
- Custom synthesis
- Peptide chemistry
- Analytical chemistry (NMR & MS)
- *In vitro* ADME & phys chem
- *In vitro* biology

Fragment Screening

- Screening technologies
  - WAC™
  - NMR
  - Thermal shift assay (DSF)
  - *In silico* screening
  - Biochemical screening (HCS)
  - X-ray crystallography
- Fragments
  - SBX/RGD library
  - Client libraries

SARomics

26 employees

- Structure-based drug design
- X-ray crystallography
- Computational chemistry
- NMR screening
- Biophysics
- Protein chemistry

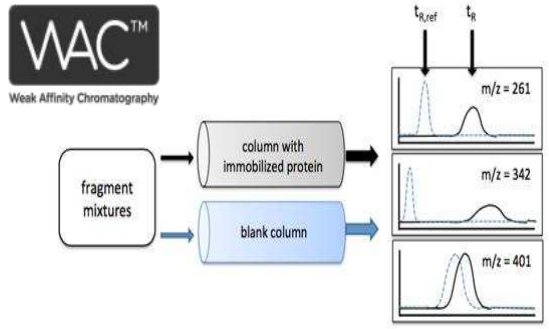
Fragment Screening & Validation of Hits

Suitable combinations of orthogonal techniques designed for each target

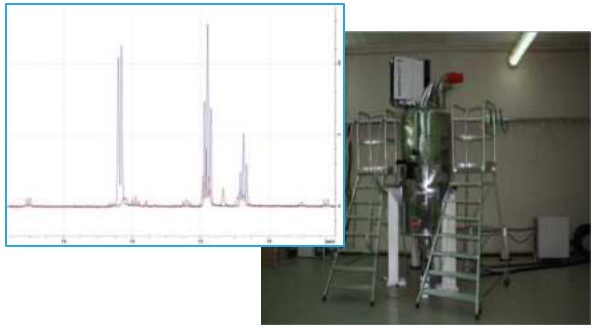
Primary screening technique

Hit validation techniques

Weak Affinity Chromatography

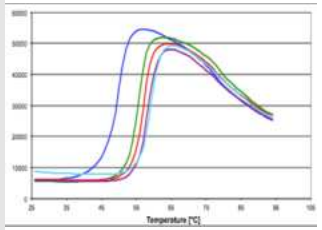


Ligand-observed NMR



Thermal shift assay / DSF

	ΔTm at 62.5 µM
RG200001	7.3
RG200001	6.4
RG200002	10.4
RG200002	10.7
RG200003	-0.48
RG200003	-0.76



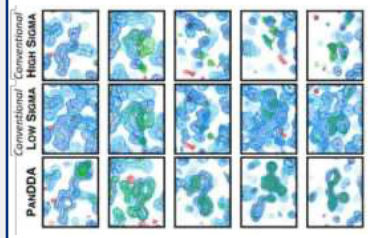
Follow-up techniques

X-ray crystallography

Crystallization robotics

MAX IV

X-ray – Fragment screening

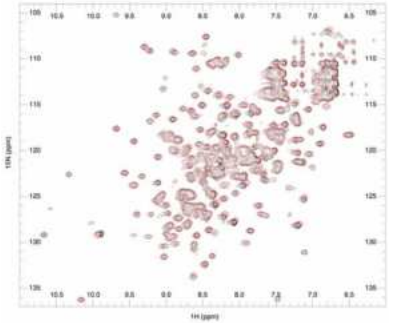


XCHEM Diamond Fragment Screening

FragMAX MAX IV Laboratory

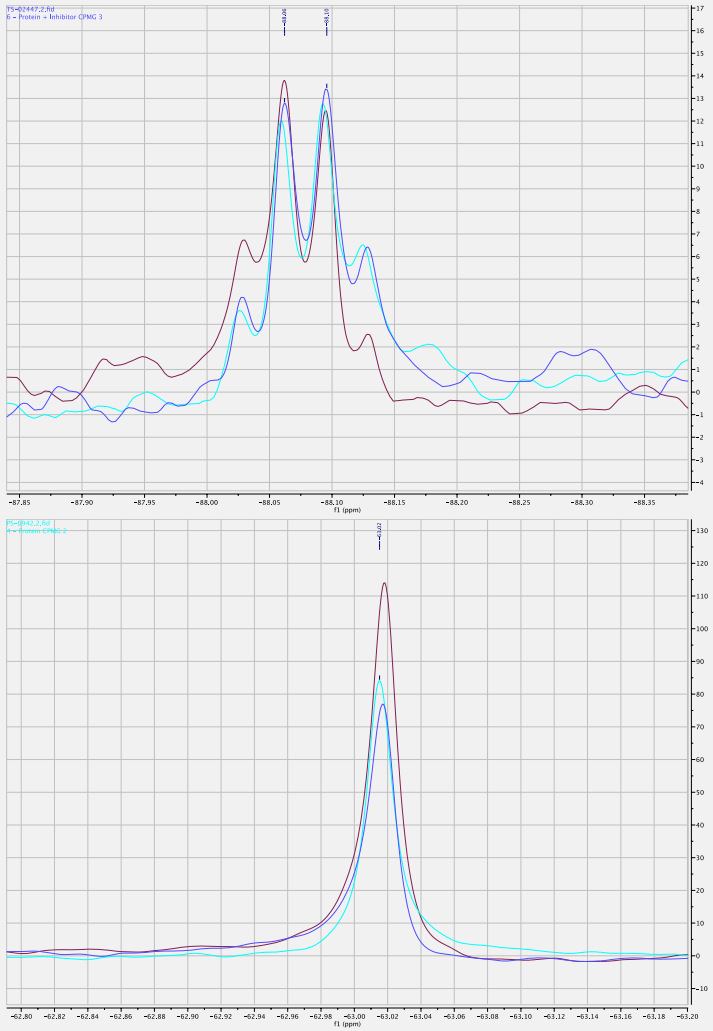
Automatic soaking, data collection and analysis
Suitable for >50 fragments or more

Protein-observed NMR



Fragment libraries for NMR screening

- Internal fragment library
  - Co-owned with Red Glead Discovery
  - ~560 fragments primarily used for WAC fragment screening
  - Experimental confirmed solubility
  - Fragment mixes not formulated for optimal NMR spectra
- Swedish NMR Center fragment libraries
  - Maybridge ^{1}H fragment library with ~850 fragments
  - Key Organics ^{19}F fragment library with ~400 fragments
  - Characterized and mixed for NMR
  - Easy and quick accessibility
- Customer library
  - Needs characterization in terms of:
    - Solubility
    - ^{1}H spectra of each fragment
    - Determine minimal overlap for fragment mixes



T2 spin-echo spectra

Magenta: only fragment

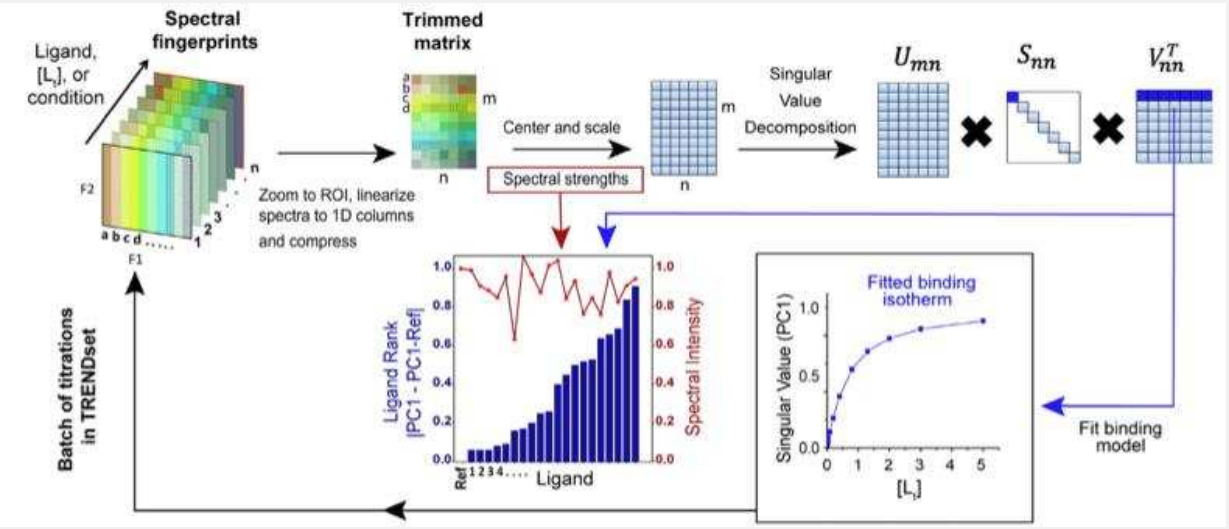
Cyan: fragment + protein (wt)

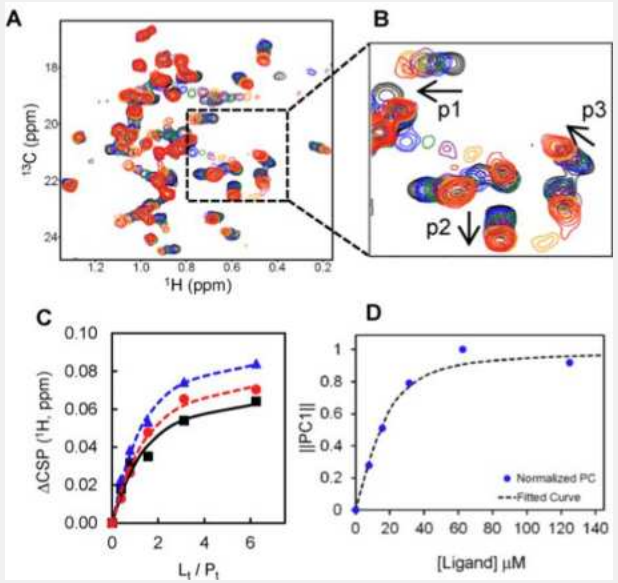
Blue: fragment + protein (mutant)

^{19}F fragment screening

- Requires 19F fragment library
- Ligand detected but simpler spectra
- Increased sensitivity
- 430 ^{19}F fragment library

Fragment ranking and Kd determination using PCA and cluster analysis





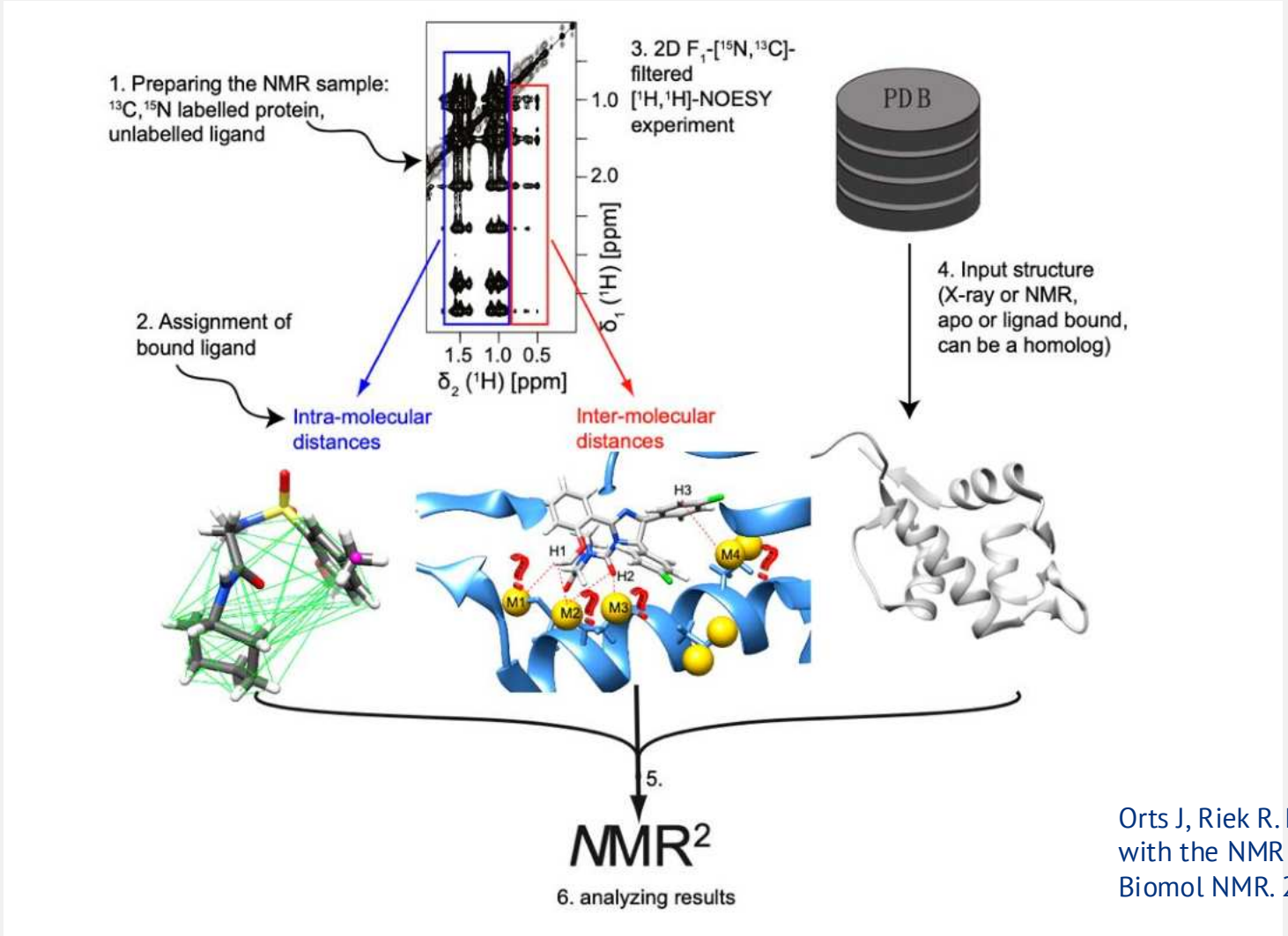
- 2D spectra are linearized and principal component analysis is applied
- No visual analysis required
- Fragments are ranked based on first principal component (PC1)
- Kd can be determined using concentration dependence for PC1

- No assignment is required
- 2D ^{15}N or ^{13}C spectra can be used
- 2D ^{13}C HSQC spectra of non-labelled protein
- Small differences in spectra can be detected
- Large amount of data can easily be processed

Namanja, Andrew T., Jia Xu, Haihong Wu, Qi Sun, Anup K. Upadhyay, Chaohong Sun, Steven R. Van Doren, and Andrew M. Petros. 2019. "NMR-Based Fragment Screening and Lead Discovery Accelerated by Principal Component Analysis." *Journal of Biomolecular NMR* 73 (12): 675–85. https://doi.org/10.1007/s10858-019-00279-9.

NMR² method implemented

Enables fast structure determination of bound ligand



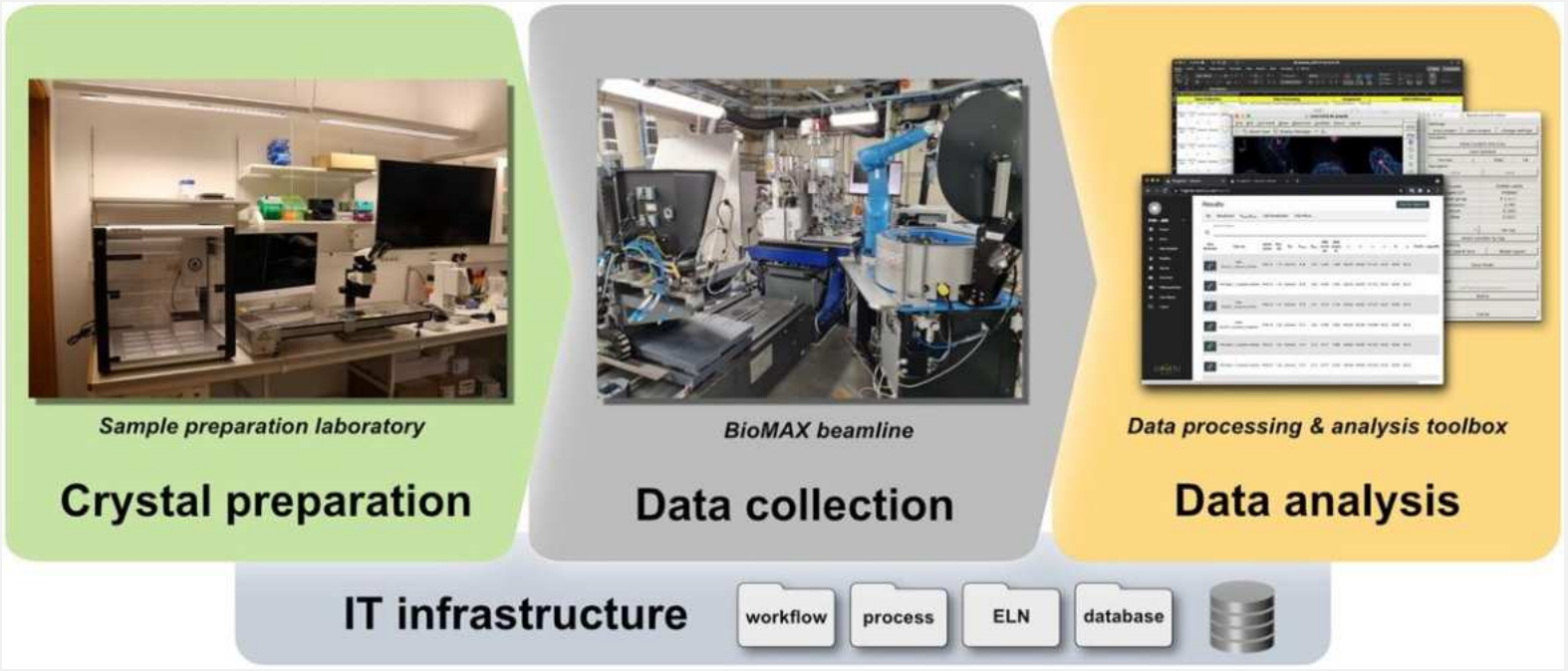
Orts J, Riek R. Protein-ligand structure determination with the NMR molecular replacement tool, NMR². J Biomol NMR. 2020 Nov;74(10-11):633-642.

X-ray crystallographic fragment screening

XFS

FragMAX

A facility at MAX IV for high throughput fragment screening by X-ray Crystallography

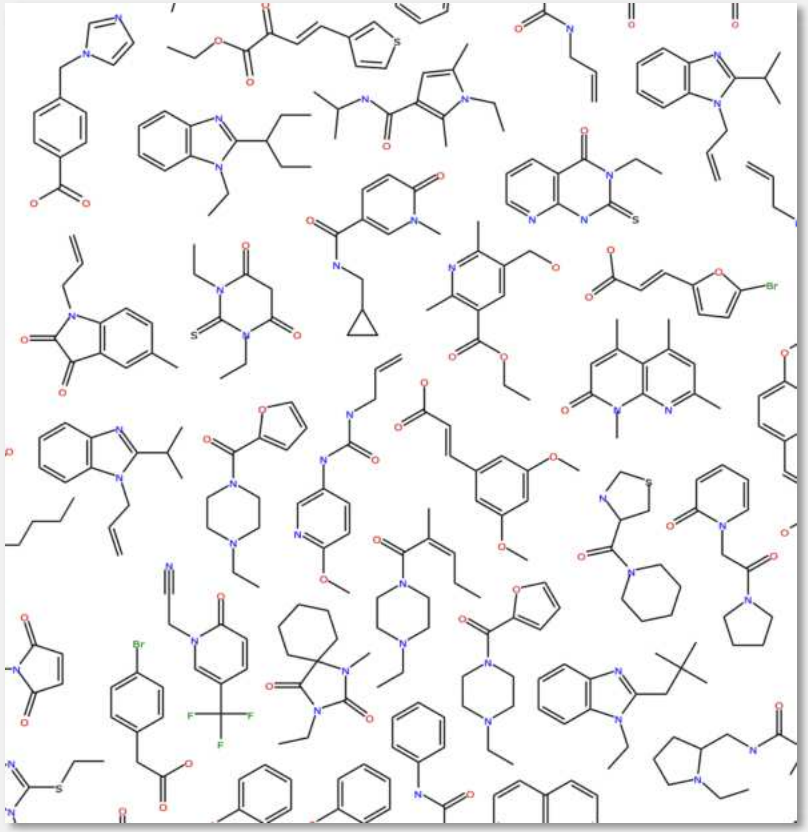


In collaboration with

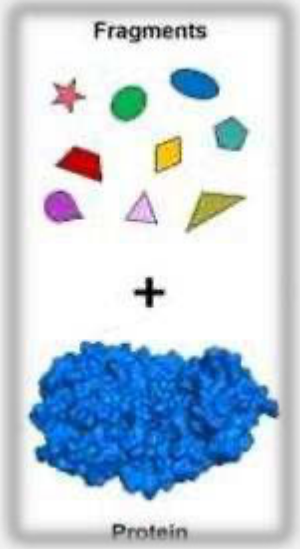
LP3

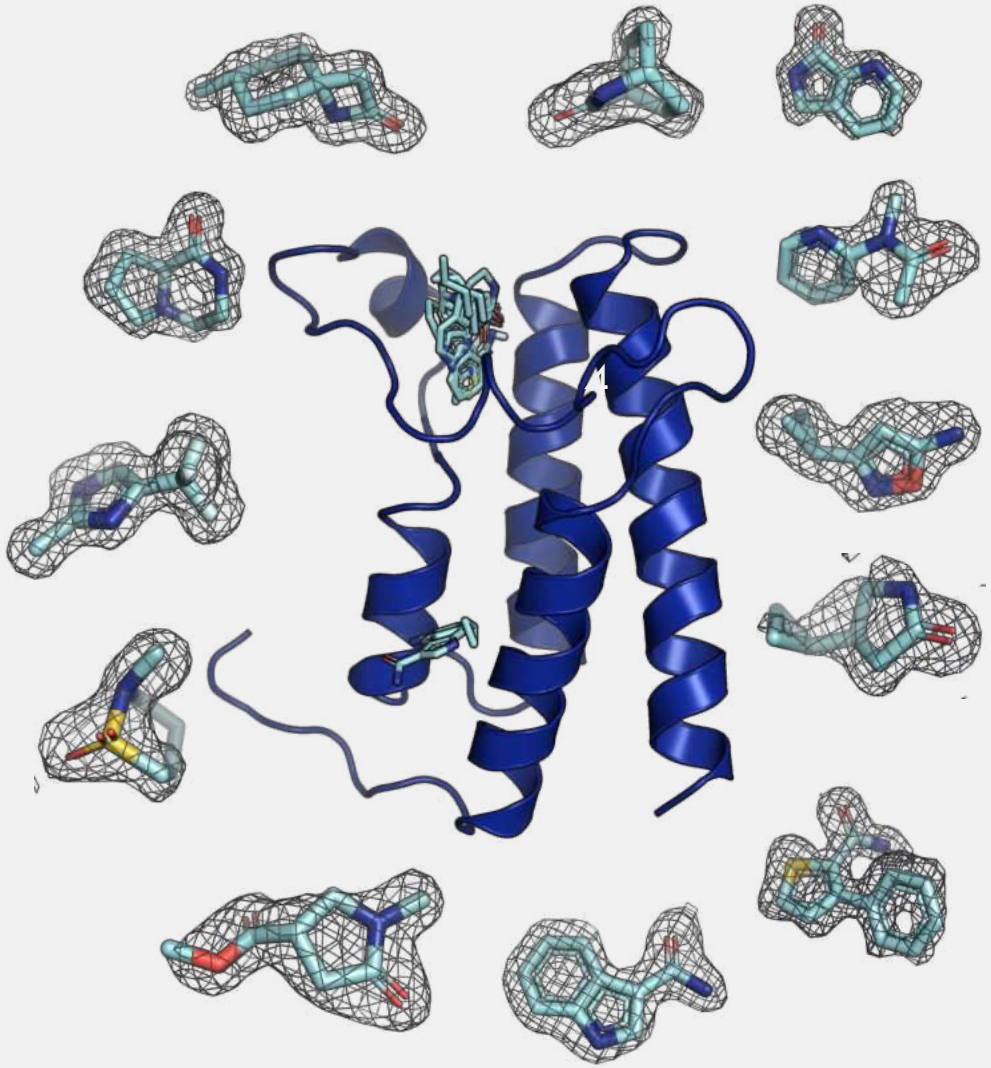
Fragment libraries for XFS

- Proprietary kinase fragment library
  - 300 fragments (1 M in DMSO)
  - Selected from Enamine REAL Space
  - Optimized for number of analogues
- HYPER fragment library
  - Developed in collaboration with Uppsala University
  - 200 fragments (1 M in DMSO)
  - Selected from Enamine REAL Space
  - Optimized for maximum number of analogues
  - Multi purpose
- FragMAX libraries
  - FragMAXlib (160 fragments, 1 M in DMSO)
  - Enamine DSI-poised (860 fragments, 0.5 M in DMSO)



Case study: XFS – SMARCA4





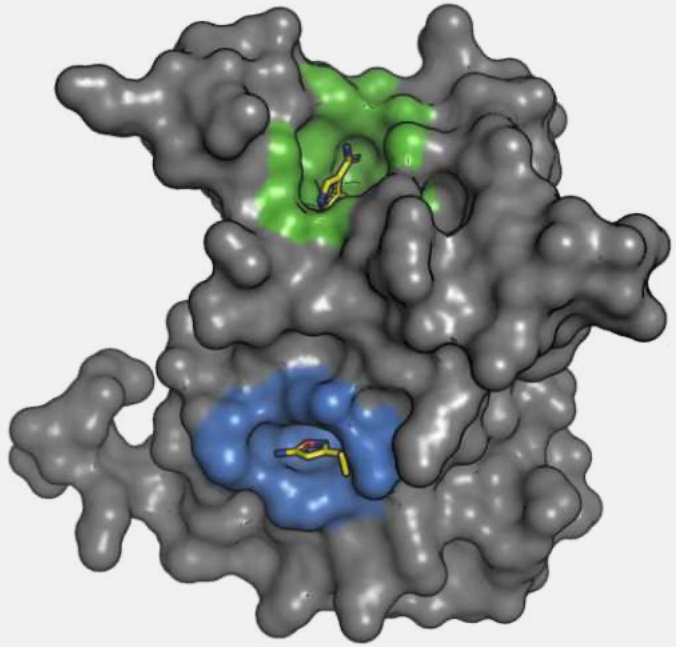
Library of 110 fragments soaked

Primary site (Ac-Lys binding site)

=> 10 fragment hits

Novel site

=> 2 fragment hits

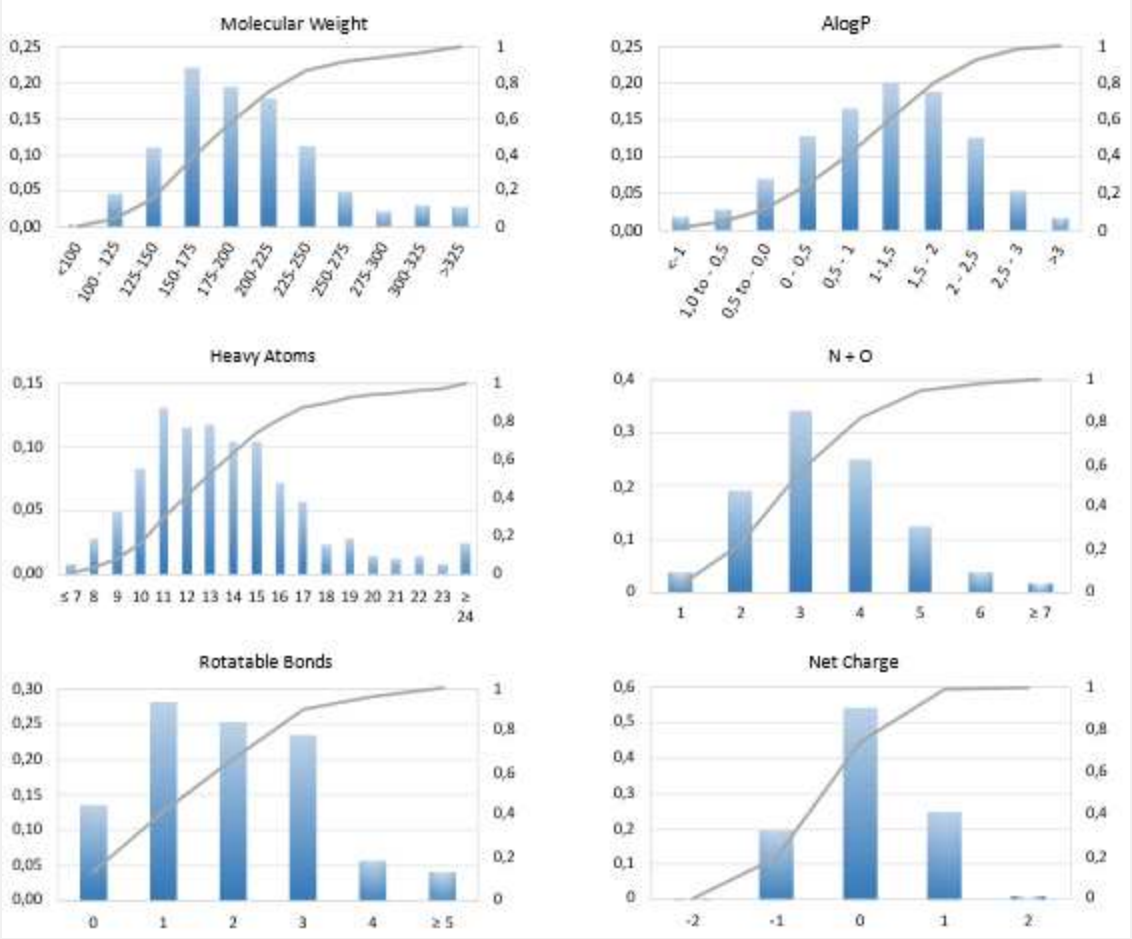


Weak Affinity Chromatography (WAC™)

WAC™

SBX/RGD Fragment Library

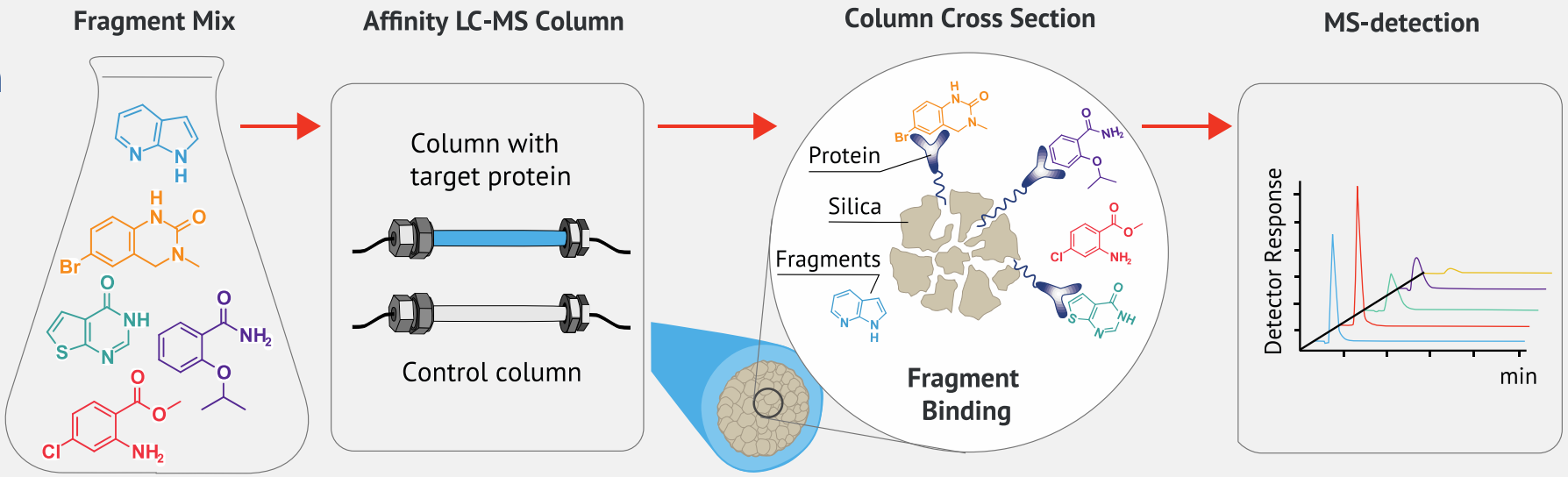
- Internal library
  - Collection of ~560 fragments (Essential & Privileged)
  - General purpose, (not target-directed) covering diverse chemical space
  - Focus on low-molecular weight fragments (<240)
  - Experimental solubility (DMSO, water) on >45% of fragments
  - Analytical data (LCMS) on all fragments
  - MedChem friendly
- Privileged Library
  - Diverse set of 250 fragments all with experimental verified solubility (>0.5 mM)
- >90% commercially available -> rapid SAR-by-catalogue follow-up



Principles of WAC™

Weak Affinity Chromatography

- Immobilized target protein
- High throughput
- **mM hits by screening at 1-5 µM**
- Made in Sweden



Affinity LC/UV/MS Platform

Characteristics of WAC™

Affinity

- Immediate K_D ranking from ~0.5 mM - 0.1 µM with isocratic elution
- Fragments are screened at low concentration (1-5 µM)
- Excess of protein sites enable detection of multiple binding fragments

Quality

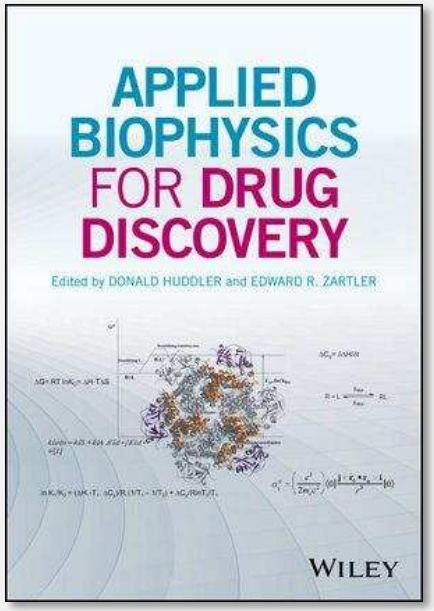
- High precision and robustness
- Automatic quality control (MS or MS/MS detection)
- Low sensitivity to impurities

Throughput

- The use of fragment mixtures (< 100) gives high throughput

Consumption of Target

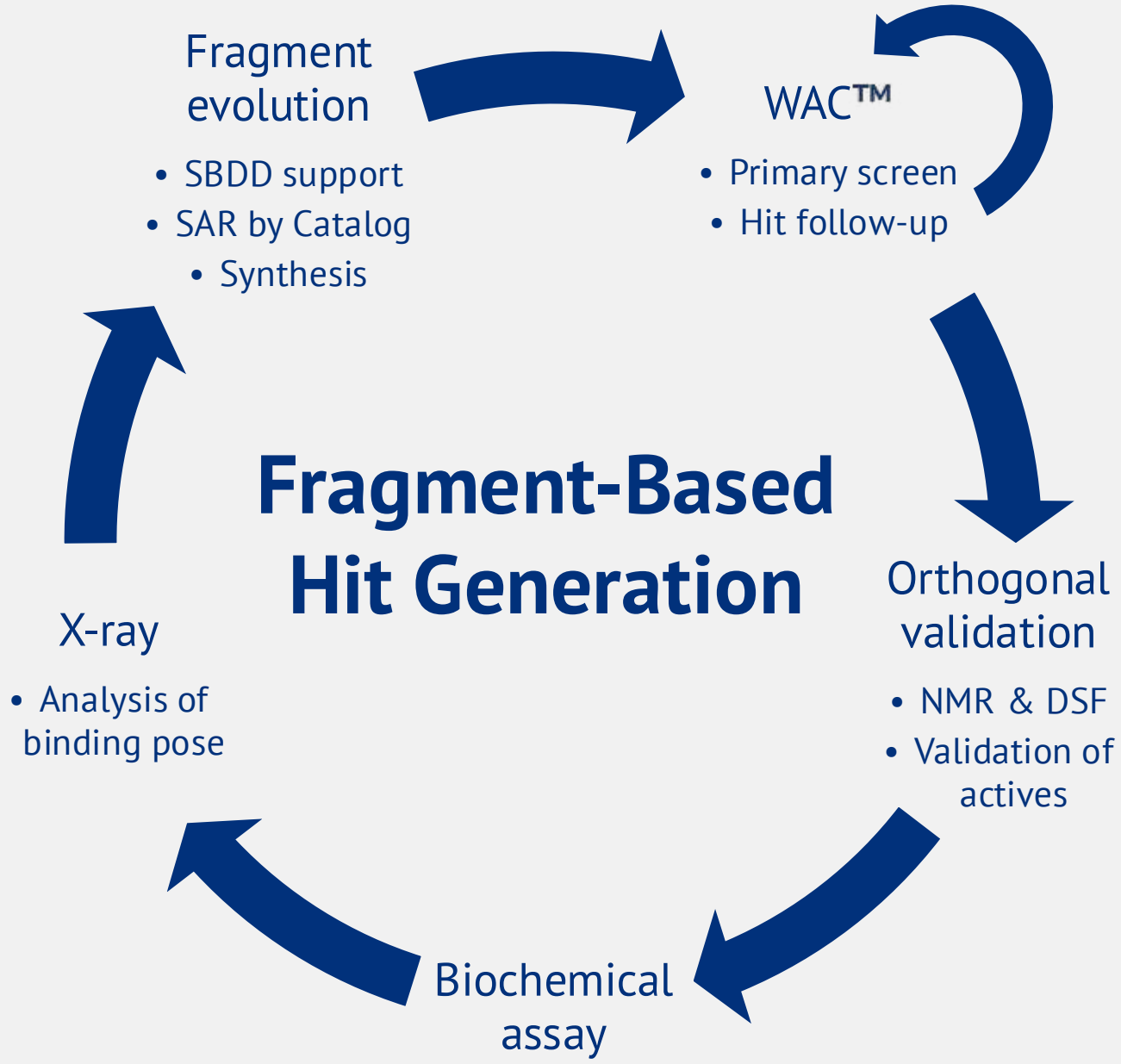
- Consumption of target is low (normal column: 5-7 mg; micro column: 2-5 mg)
- Protein immobilization using exposed lysines or AVI-tag for NeutrAvidin binding
- Long WAC column lifetime



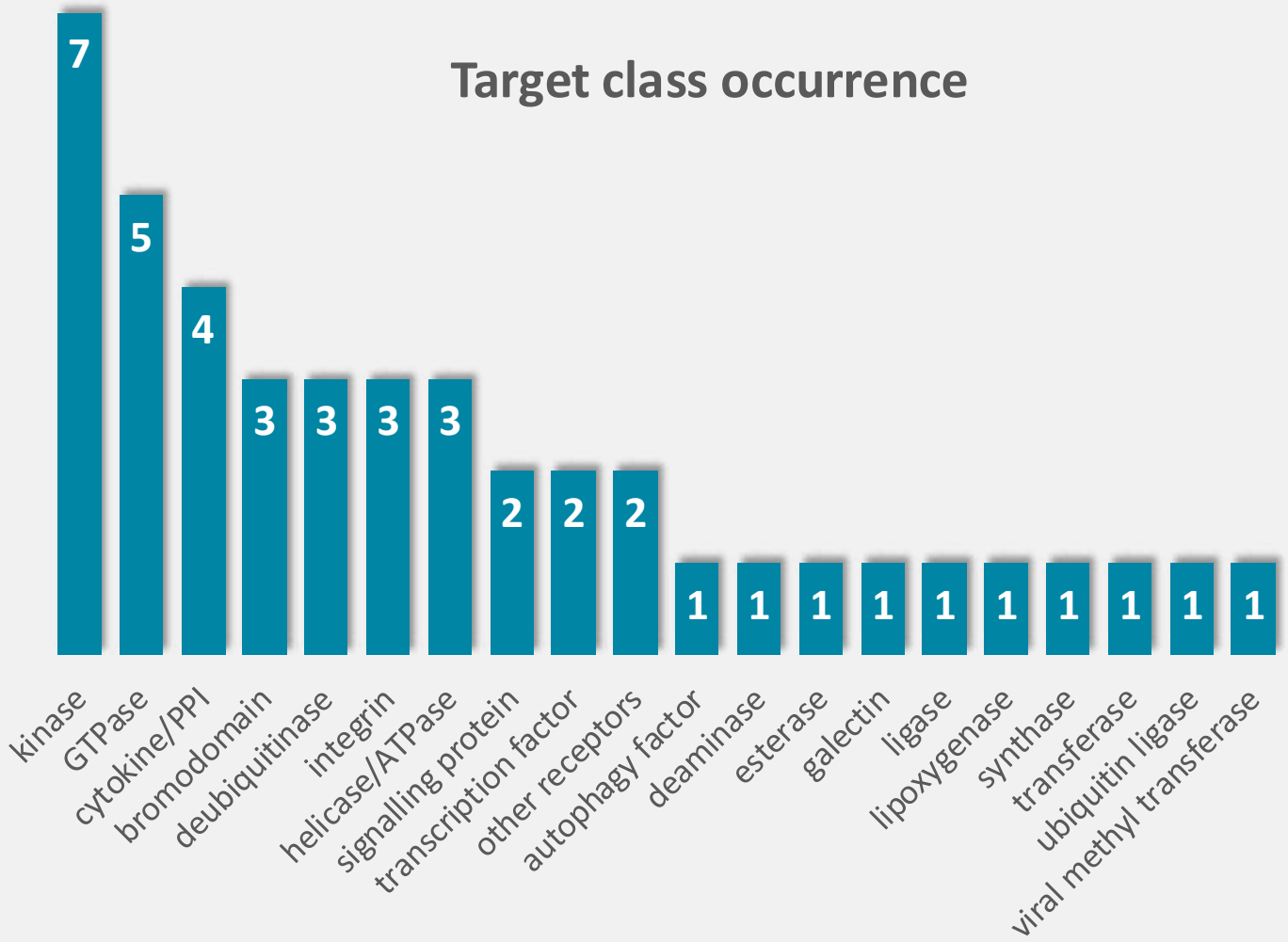
Applied Biophysics for Drug Discovery, 2017
Eds. Donald Huddler, Edward R. Zartler

Chapter 7
Sten Ohlson and Minh-Dao Duong-Thi
Weak Affinity Chromatography (WAC)

Chapter 3
Björn Walse, Andrew P. Turnbull and Susan M. Boyd
Tailoring Hit Identification and Qualification Methods for Targeting Protein–Protein Interactions


Fragment evolution
• SBDD support
• SAR by Catalog
• Synthesis
WAC™
• Primary screen
• Hit follow-up
Orthogonal validation
• NMR & DSF
• Validation of actives
Biochemical assay
X-ray
• Analysis of binding pose
Fragment-Based Hit Generation

Working with WAC™ for 7 years



Facts & learnings

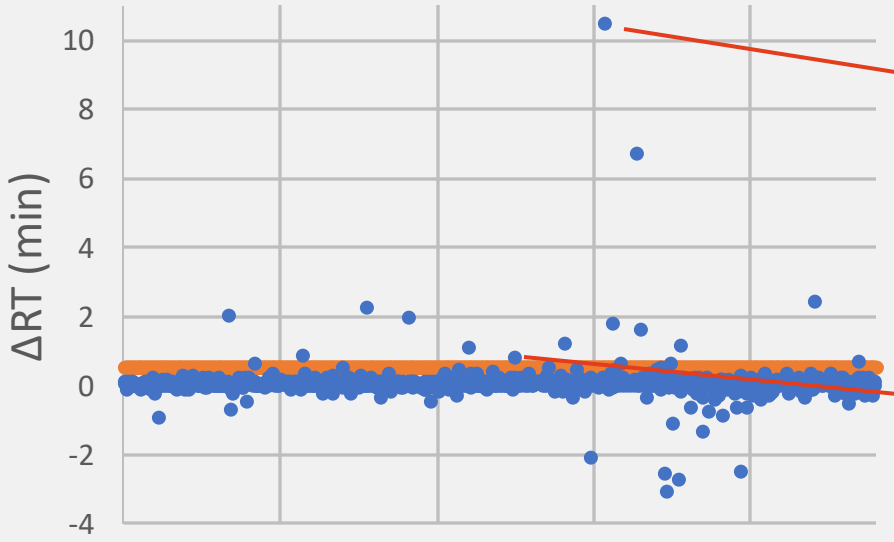
- >50 FBLD projects over 7 years
- 20 distinct target classes
- WAC hit rates from 1% to 20% (avg 6%)
- Library quality and size matters
- Best follow-up: NMR, SPR, X-ray

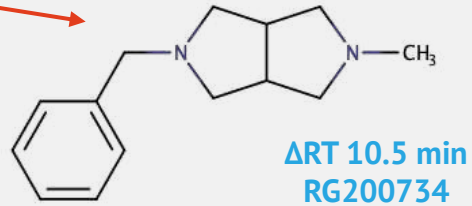
Quirks & drawbacks

- Buffer with low ionic strength, less charge shielding
- Ranking order can be disrupted by ionic interactions
- Non-volatile additives not tolerated by MS

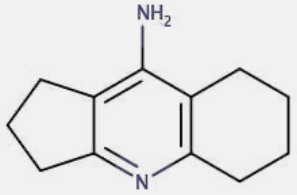
Example of WAC™ Hits

- Challenging PPI target (IL-23)
- Crystal structures of two hits from WAC™ screen
- No structure of this target with a small-molecule ligands has previously been reported

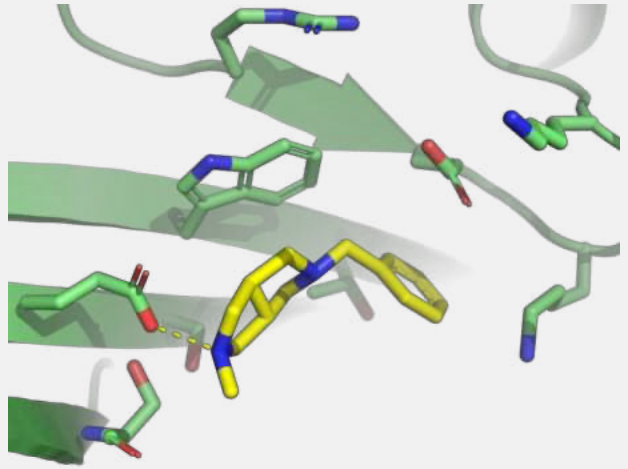


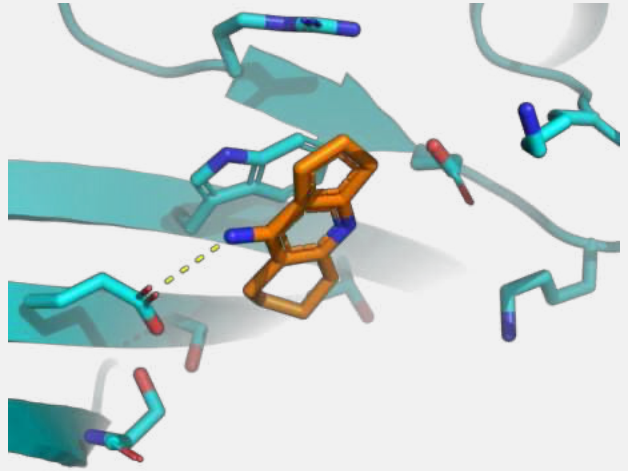


ΔRT 10.5 min
RG200734



ΔRT 0.8 min
RG200616





WAC™ offers

WAC™ Full screen	WAC™ Full screen + analogue	WAC™ screen
• 500 fragments from SBX/RGD library • Characterized library • 18 000 USD*	• 500 fragments from SBX/RGD library • Includes one round of analogue search, selection and testing • 22 000 USD*	• Client library screen • Startup: 12 000 USD* • 5 000 USD* per 1000 fragments

Follow-up studies

- NMR verification
- Co-crystallization
- Fragment evolution
- And more …

All results are owned by the client

*New 2025 prices

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