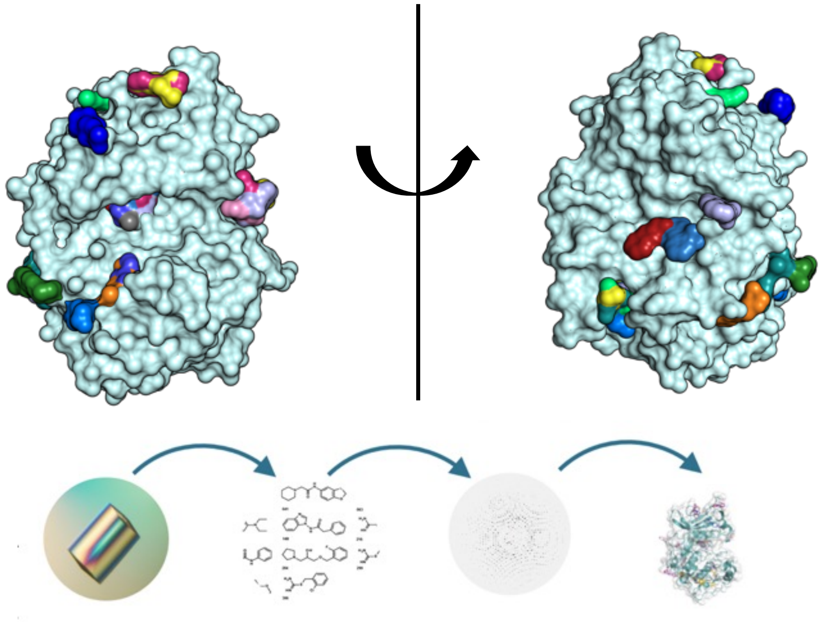
CRYSTALS FIRST

TURNING **INSIGHTS** INTO **MEDICINES**



# Cheaper, faster, smarter lead generation:

## Accelerating discovery of active compounds with protein-fragment complexes

Dr. Serghei Glinca

April 22nd 2021 BioSolveIT DrugSpace Symposium

# WHERE WE COME FROM...

## PROF. GERHARD KLEBE

Philipps University of Marburg


"RED BIBLE OF DRUG DESIGN"

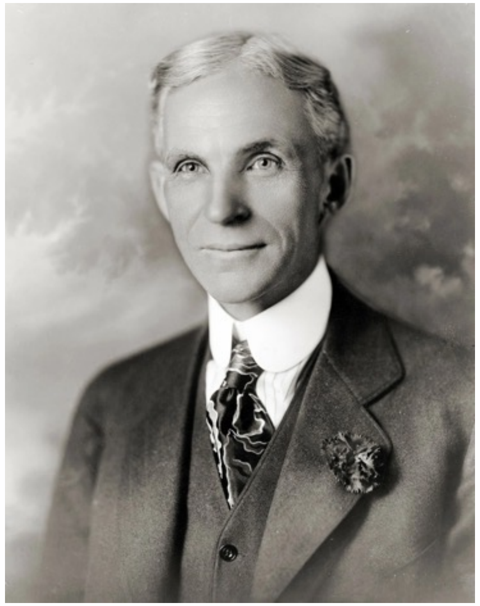
~1% of PDB X-ray data deposited by the Klebe group


Marburg, Germany

“If you always do what you’ve always done,

you’ll always get what you’ve always got.“

- Henry Ford



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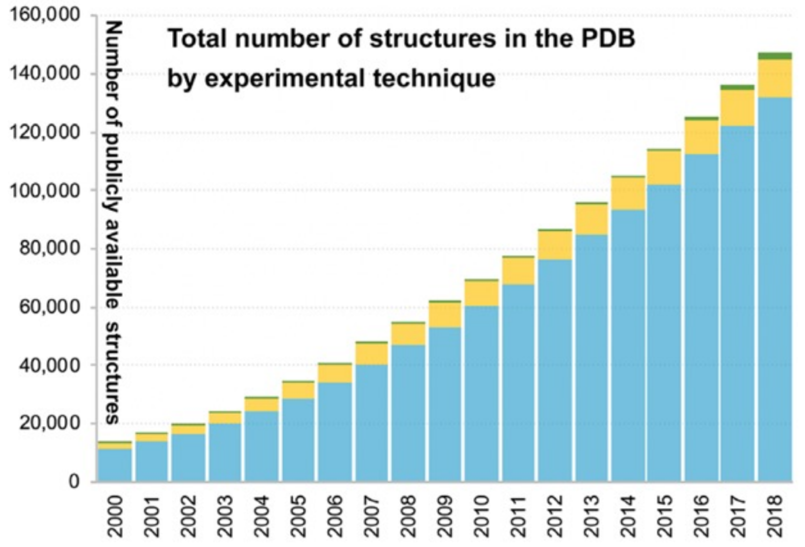
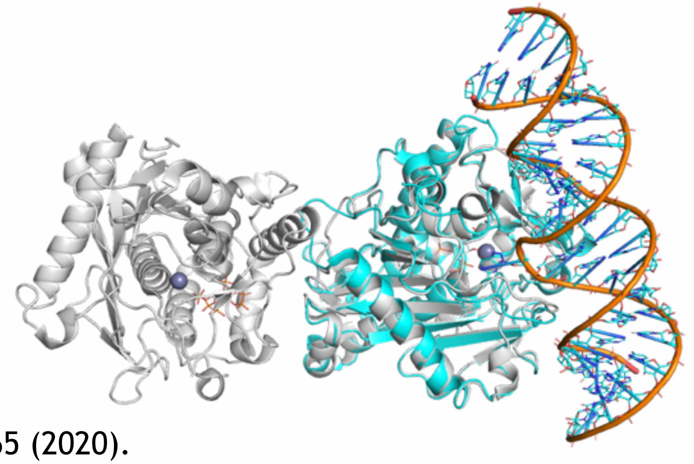
# THE VITAL ROLE OF STRUCTURAL BIOLOGY IN DE-RISKING THERAPEUTICS

Discovery and development of 210 new drugs approved by FDA 2010–2016 were facilitated by 3D structural information.

Structural data helps to overcome challenges inherent to bioactive compounds in terms of safety and efficacy for animals and humans.



1. Goodsell, D. S. et al. RCSB Protein Data Bank: Enabling biomedical research and drug discovery. Protein Sci. 29, 52–65 (2020).
2. Westbrook, J. D. & Burley, S. K. How Structural Biologists and the Protein Data Bank Contributed to Recent FDA New Drug Approvals. Structure 27, 211–217 (2019).

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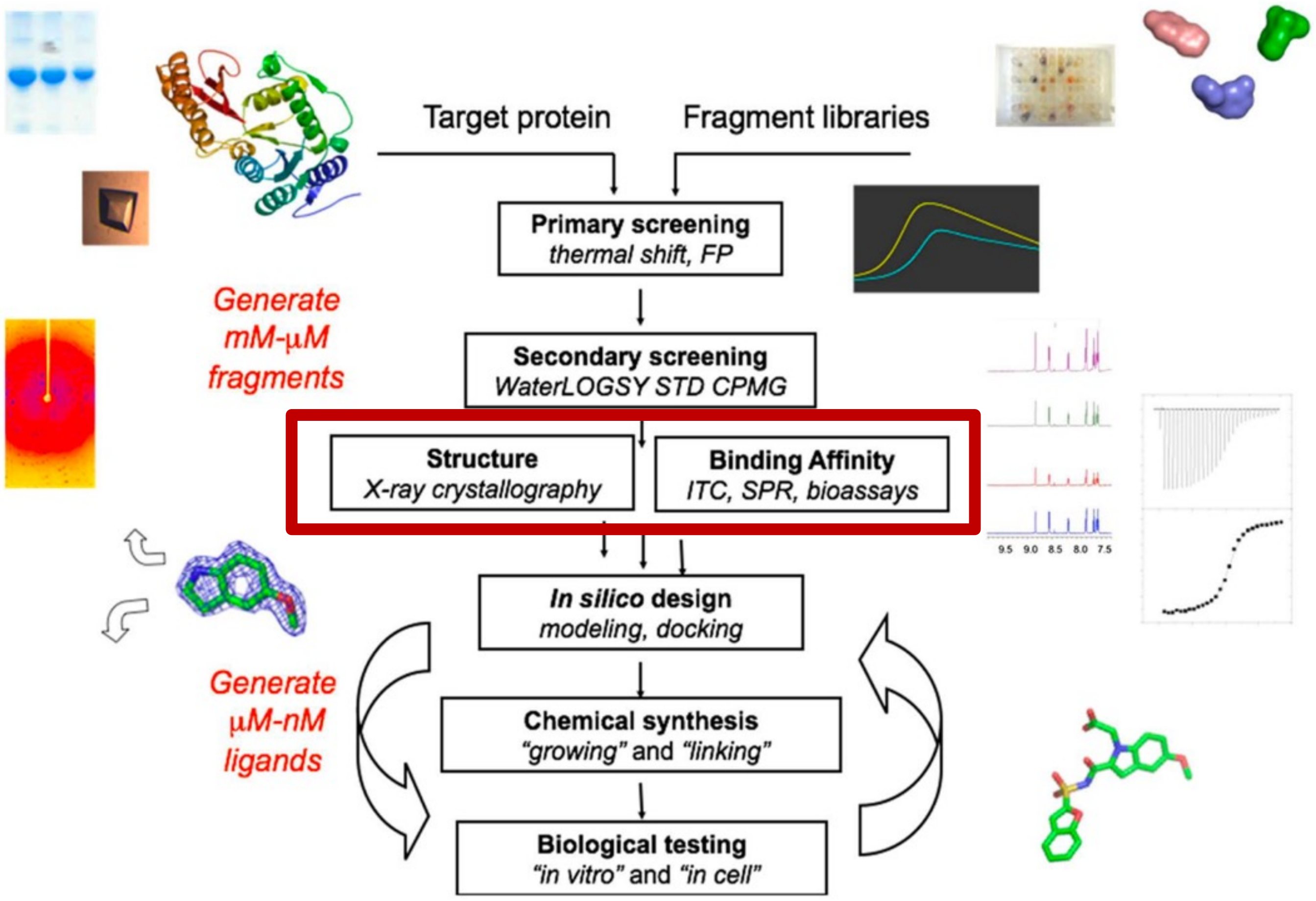
# FRAGMENT SCREENING CASCADE




Abell, C.; Dagostin, C. RSC Drug Discov. Ser. 2015

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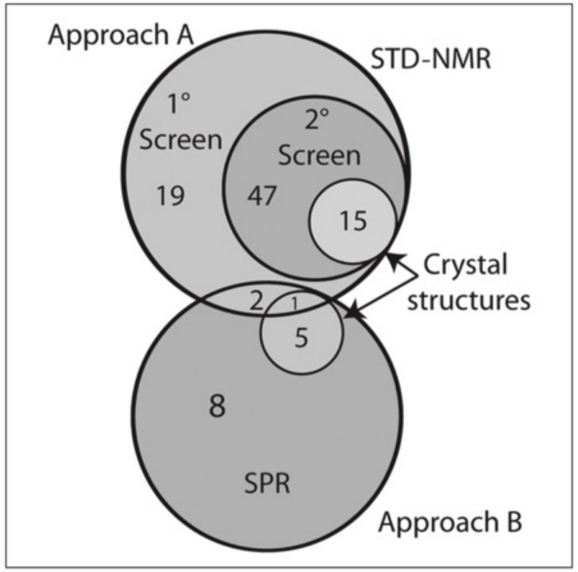
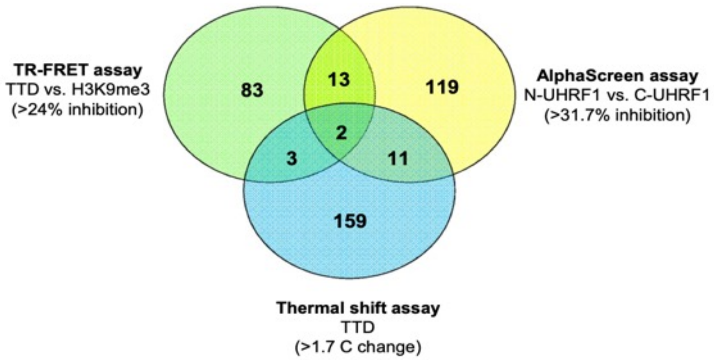
# LOW OVERLAP OF FRAGMENT HITS IN BIOPHYSICAL SCREENINGS



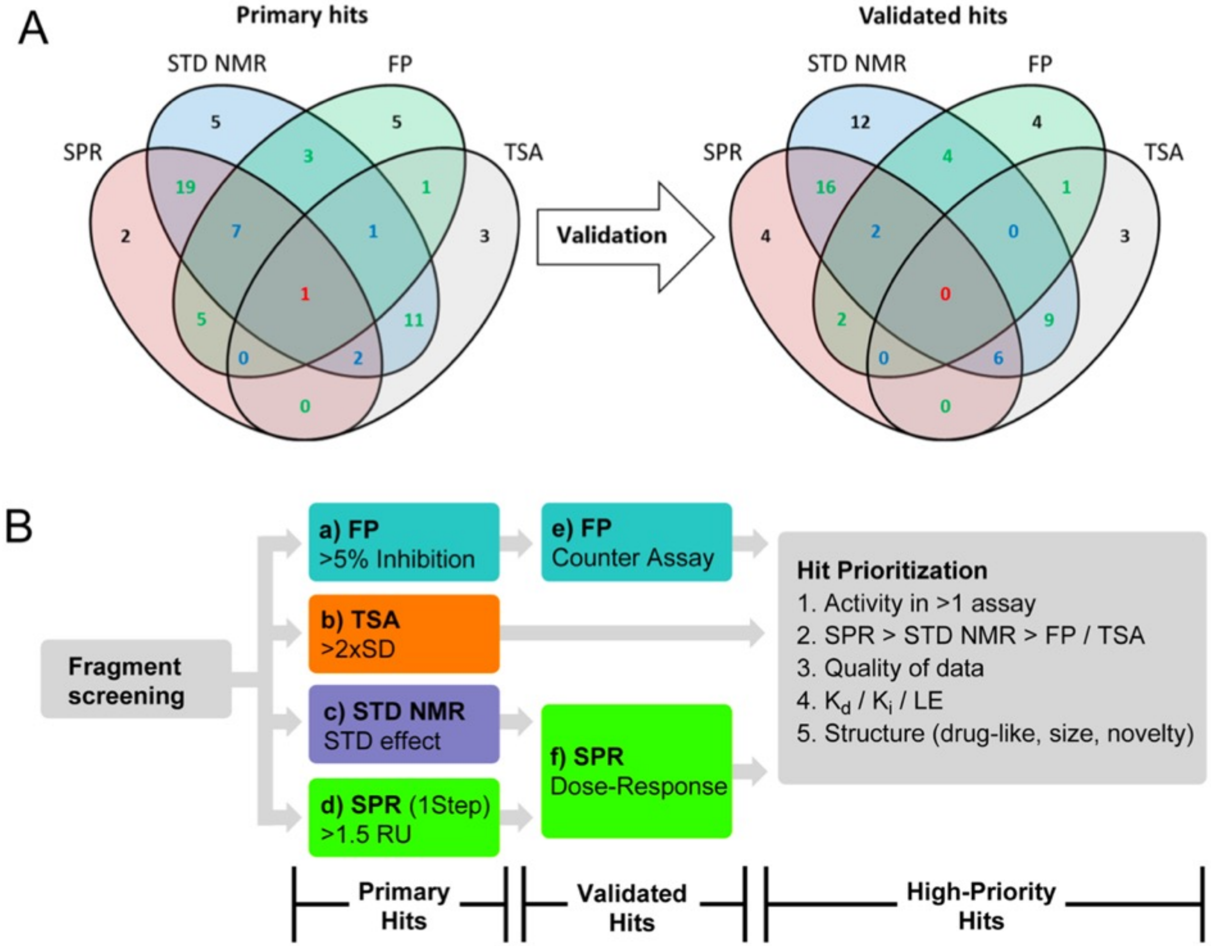

1 integrase core domain

Wielens et al., 2013




Epigenetic factor UHRF1 – 2300 frag lib

Chang et al., 2021




Fragment-based deconstruction–reconstruction for KEAP1 – 77 frags

Pallesen et al., 2021

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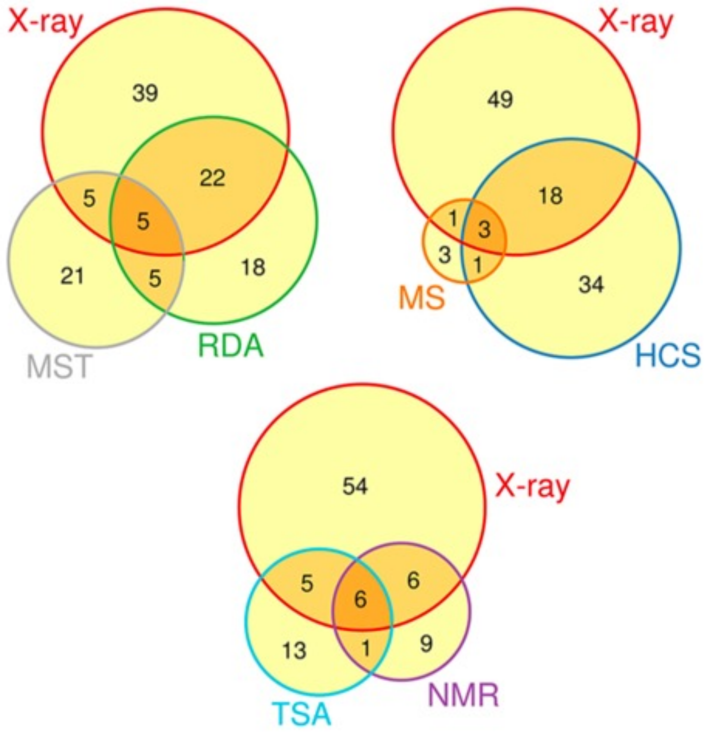
# WHY NOT START WITH CRYSTALLOGRAPHIC SCREENING IN FBDD?

- Fragment library: 361 compounds
- Protein: Endothiapepsin
- Study: 6 biophysical assays + X-ray
- 71 X-ray hits
- 44 % (31) fragments only by X-ray
- Any screening cascade would have retrieved max. 19 X-ray hits
- No hits by all six methods
- Sampling of binding sites:
- 19 hits: 7 pockets vs. 71 hits: 11 pockets




Schiebel, J.; et al., ACS Chem. Biol. 2016

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# WHY NOT START WITH CRYSTALLOGRAPHIC SCREENING IN FBDD?

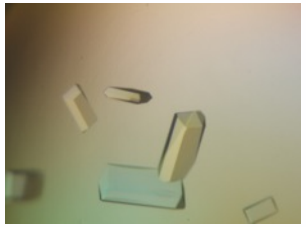
# PROTEIN CRYSTALLOGRAPHY

## DETERMINATION OF PROTEIN-LIGAND COMPLEXES FROM SCATTERED X-RAYS

### COMPOUNDS

### CO-CRYSTALLIZATION

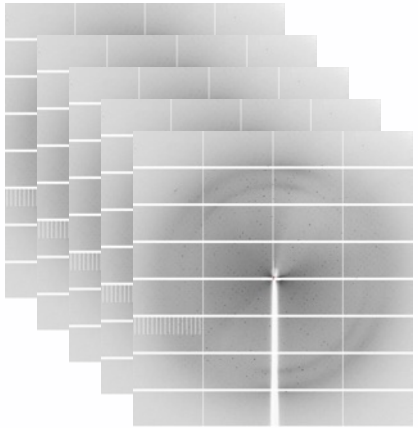
Compound is added to the protein during crystallization setup and the pre-formed protein-ligand complex is crystallized.



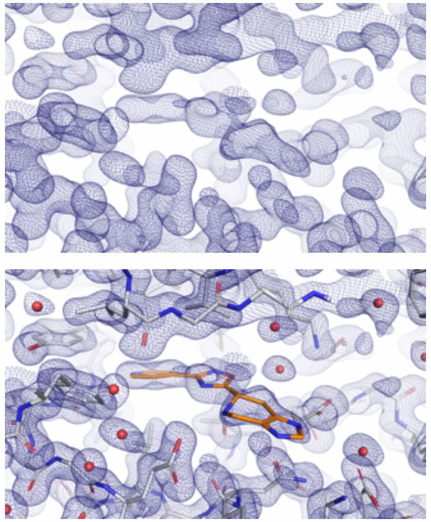
### SOAKING

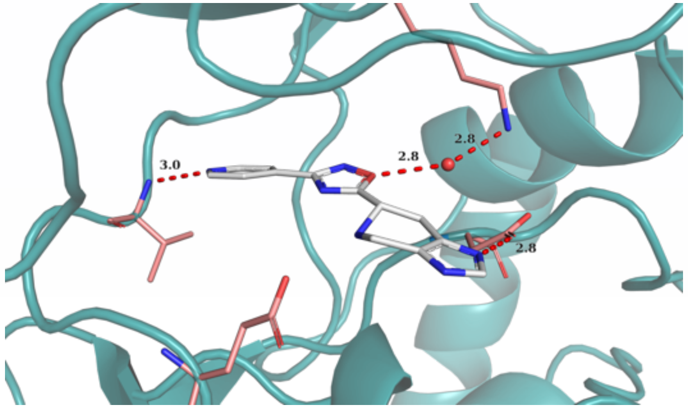
Soaking an apo-crystal in a ligand solution after the crystallisation has taken place.

### DATA COLLECTION



### X-RAY ANALYSIS & REFINEMENT



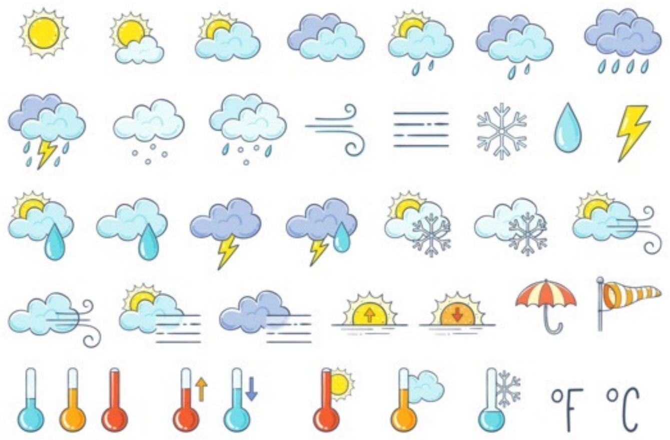


# PROTEIN CRYSTALLOGRAPHY

## CRITICAL FACTORS FOR SUCCESS OF PROTEIN CRYSTALLOGRAPHY

Moon phase

Season




Weather

Voodoo skills

JUST JOKING!

But...there's a grain of truth in every joke.

# WHY NOT START WITH CRYSTALLOGRAPHIC SCREENING IN FBDD?

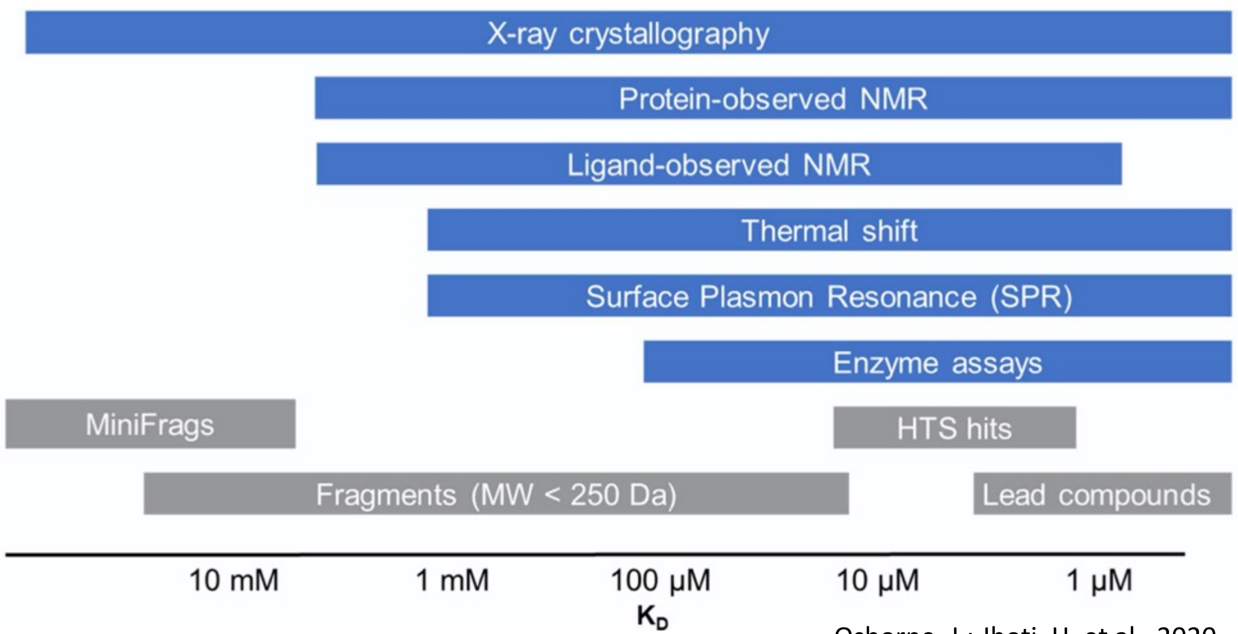
Fragment screening by X-ray crystallography

✓ is the most sensitive screening method delivering binding modalities *ad hoc*.

✓ offers guidelines for further prosecution of identified hits & structurally-enabled lead design.

✓ opens access to novel chemical and IP space.

✓ is an essential tool for SBDD.




Osborne, J.; Jhoti, H. et al., 2020

But many problems of crystal soaking hinder the routine application for fragment screening and co-structure determination.

# MAJOR PROBLEMS OF CRYSTAL SOAKING

## SPEED, RESOLUTION, NO STRUCTURE, "EMPTY" STRUCTURE

"CONVENTIONAL" SOAKING

Protein crystal sensitivity

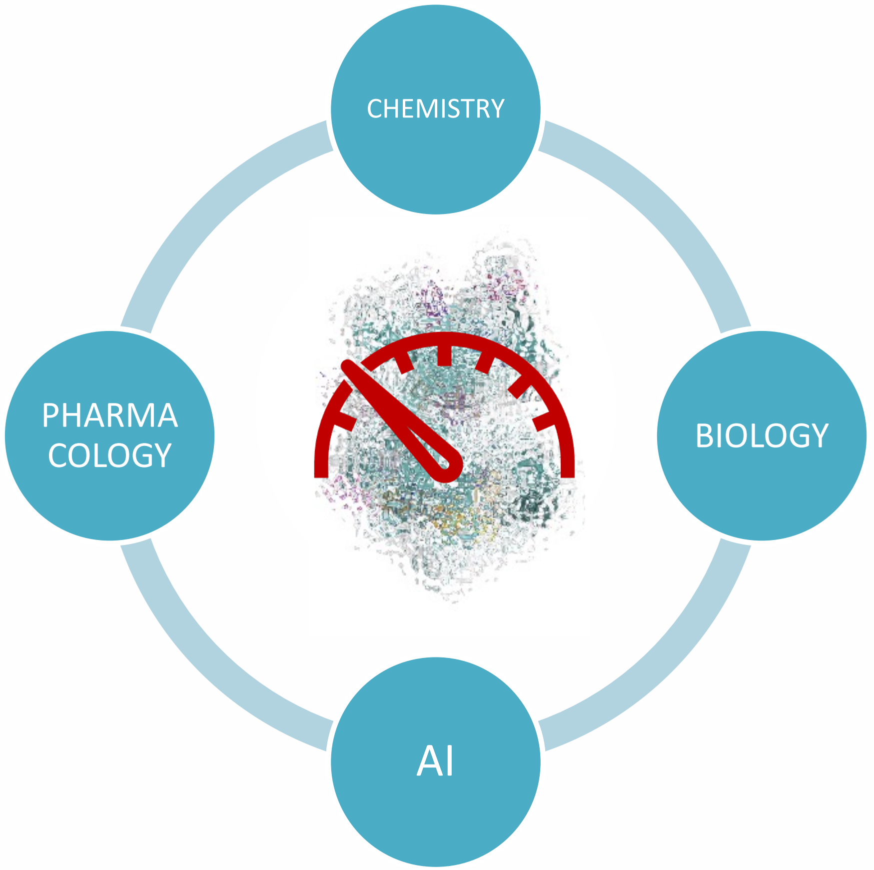
Unpredictable behavior of protein crystals

Reduction of crystal quality

Dissolution of crystals by organic additives and cryoprotectants

Mandatory trial & error optimization of soaking conditions

Solubility issues of compounds at concentrations required for soaking



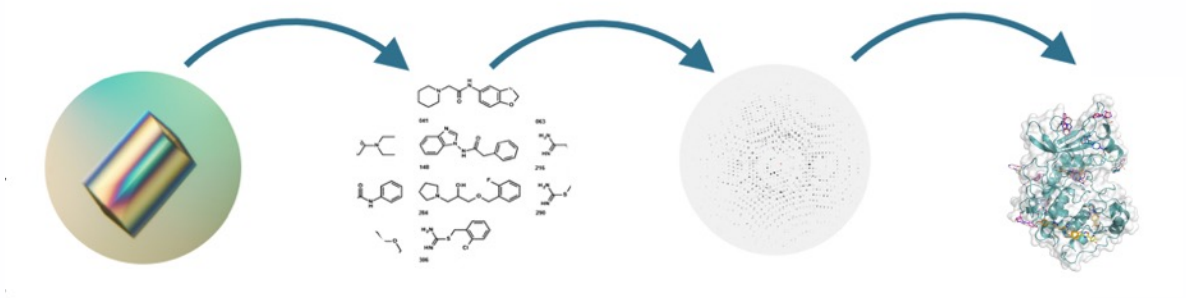

**OVERALL SLOW DOWN OF THE DRUG DISCOVERY PROCESS**

# CRYSTALSFIRST'S DISRUPTIVE SMARTSOAK® TECHNOLOGY

SmartSoak® solves the problems of crystal soaking.

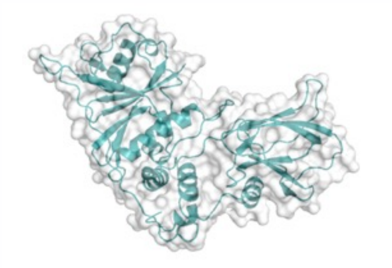
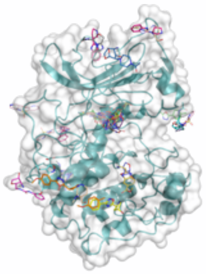
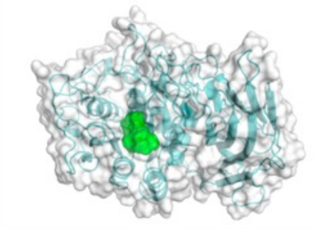
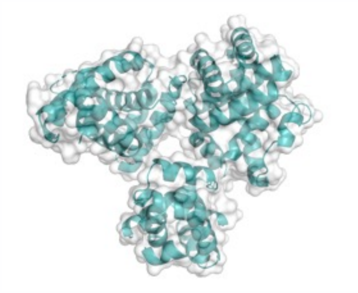
The world's first technology offering an <u>up to 10X accelerated</u> process for soaking of protein crystals.

CrystalsFirst filed 3 patents for this enabling technology.



# CRYSTALSFIRST'S DISRUPTIVE SMARTSOAK® TECHNOLOGY

- target-agnostic
- successfully applied for over 20 protein targets
- delivering hit rates up to 30 %


<u>Successful applications for</u>

E3 Ligases
Methyltransferases
Helicases
Kinases
Metalloenzyme
Bcl-2 protein
Glycosylase
Cytokine
Hydroxysteroid dehydrogenase

<u>Applicability suited in/for</u>

Transcriptomics
Epitranscriptomics
Phosphatases (allostery)
RAS pathways

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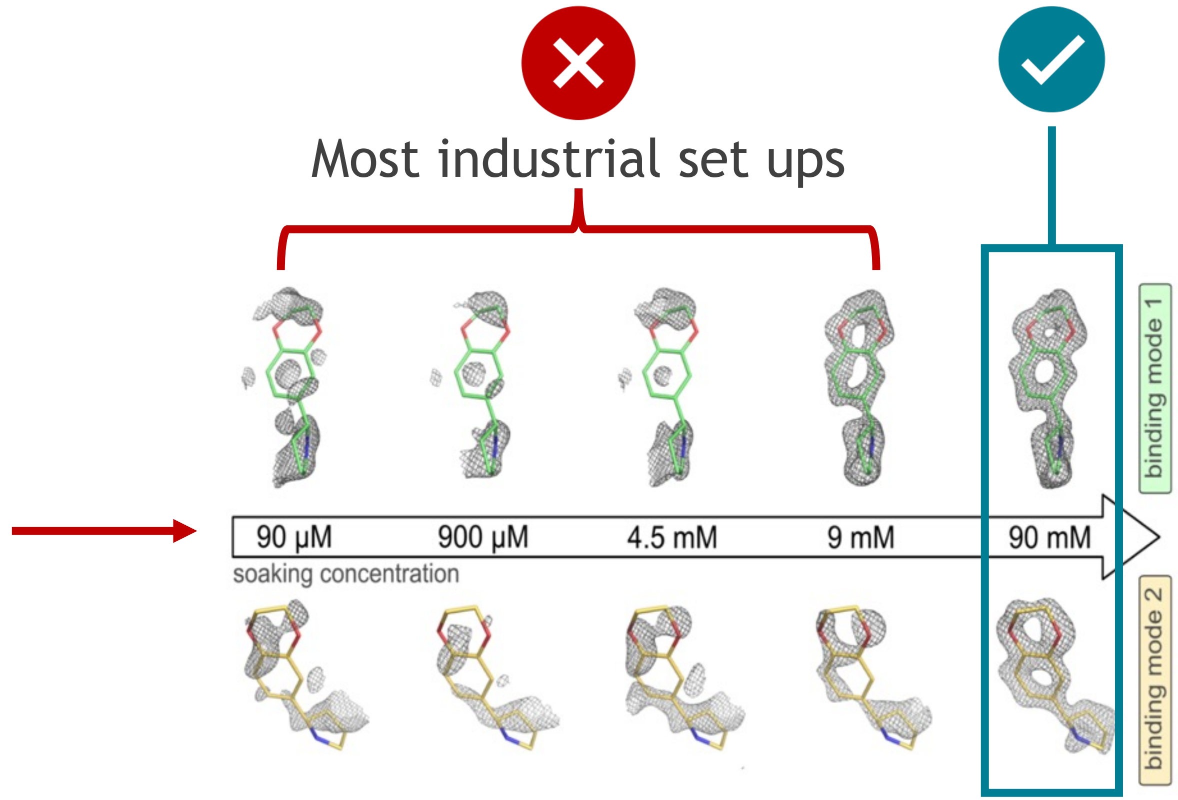
# BETTER STRUCTURES FASTER

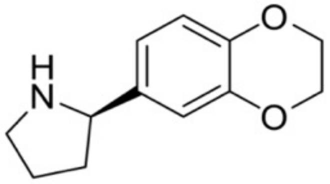
## SMARTSOAK® HIGH PERFORMANCE SOAKING SYSTEMS

- Stabilization of protein crystals
- High concentration soaking using 100mM as a standard setup

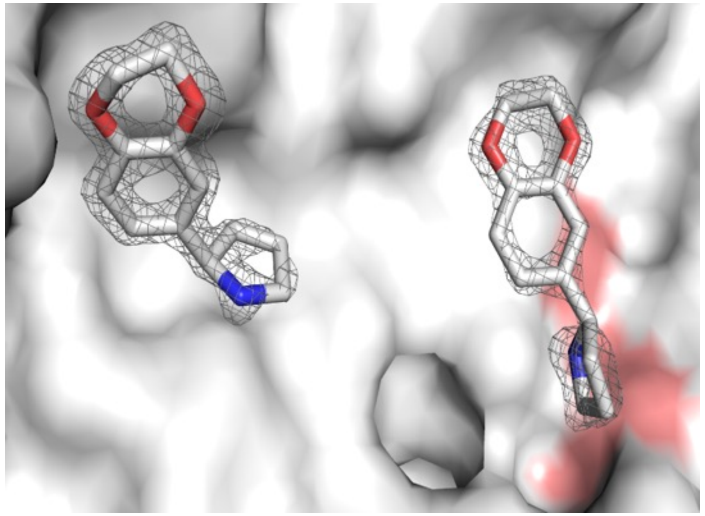
# IMPORTANCE OF SOAKING CONCENTRATION




Soaking Concentration




fragment **112**

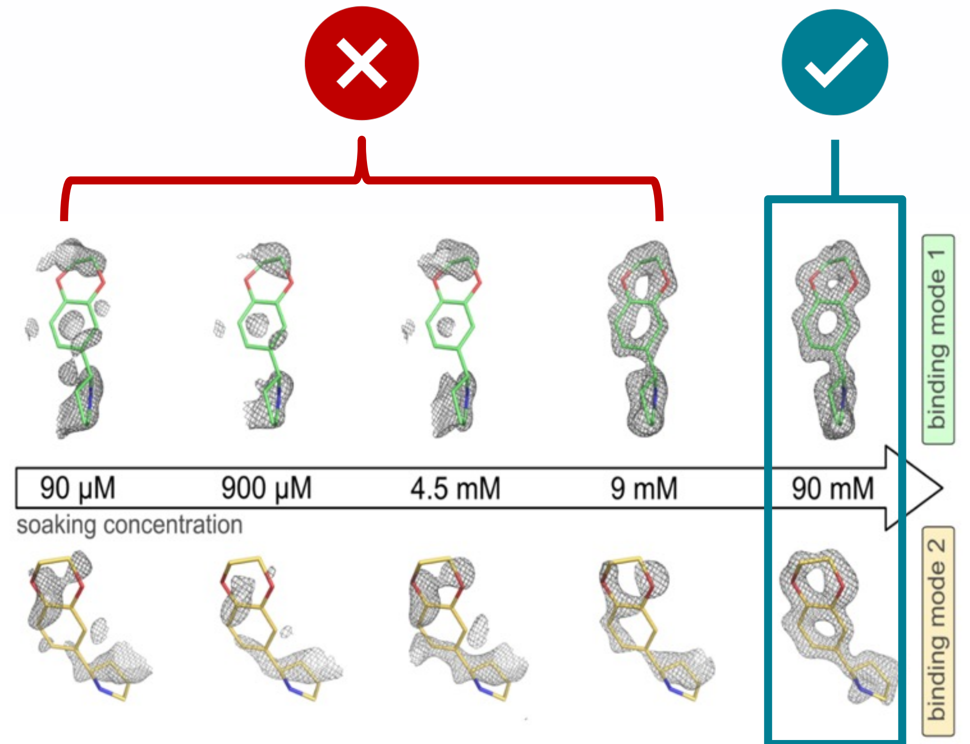


two copies bound

# SMARTSOAK® - HIGH PERFORMANCE SOAKING SYSTEMS

## KEY FEATURES LEADING TO BETTER STRUCTURES FASTER

- Stabilization of protein crystals
- High concentration soaking using <u>100mM as a standard setup</u>
- Long soaking times up to 24h
- Significant increase of data quality & success rates
- Improved ligand solubility



# SOLVING THE SOAKING PROBLEM USING SMARTSOAK®

| "CONVENTIONAL" SOAKING | SMARTSOAK® |
|---|---|
| Protein crystal sensitivity | Stabilized protein crystals |
| Unpredictable behavior of protein crystals | Predictable behavior |
| Reduction of crystal quality | Increased crystal quality |
| Dissolution of crystals by organic additives and cryoprotectants | Stable despite organic additives and cryoprotectants |
| Mandatory trial & error optimization of soaking conditions | No trial & error optimization of soaking conditions |
| Solubility issues of compounds at concentrations required for soaking | High standard soaking concentrations |

# SMARTSOAK®-ENABLED FBDD

## 1

**Risk analysis
&
project plan**

**1 week**

## 2

**Design of a high-quality crystallization system
&
SmartSoak®-stabilization**

**4-8 weeks***

## 3

**SmartSoak®-enabled soaking
&
SmartRefine data evaluation**

**1-2 week**

## 4

**Structure refinement
&
Rapid fragment evolution**

**1-2 weeks**

# DOES THE INDUSTRY BEGIN TO ADAPT?

## BOEHRINGER INGELHEIM PUTS X-RAY AND FRAGMENT-BASED APPROACH FIRST

### Drugging all RAS isoforms with one pocket

Dirk Kessler*[,1], Andreas Bergner[1], Jark Böttcher[1], Gerhard Fischer[1], Sandra Döbel[1], Melanie Hinkel[1], Barbara Müllauer[1], Alexander Weiss-Puxbaum[1] & Darryl B McConnell**[,1]

called 'x-ray first' approach where we crystallized every newly synthesized compound in the active $KRAS^{G12D}$ form before proceeding toward biophysical or biochemical affinity testing. Based on the binding mode we selected the interesting molecules for further measurements to neglect the typical affinity biased optimization strategies that often lead to wrong conclusions with respect to binding interactions.

Our company's DNA and systematic approach has been established 4 years ago.

The industry begins to adapt but the industry's standard is still trial-and-error.

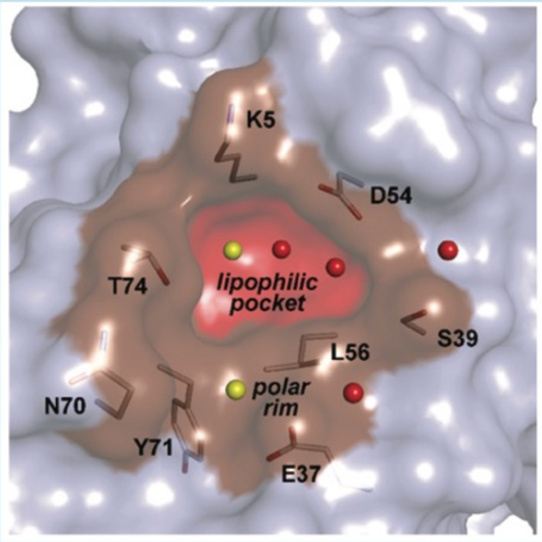
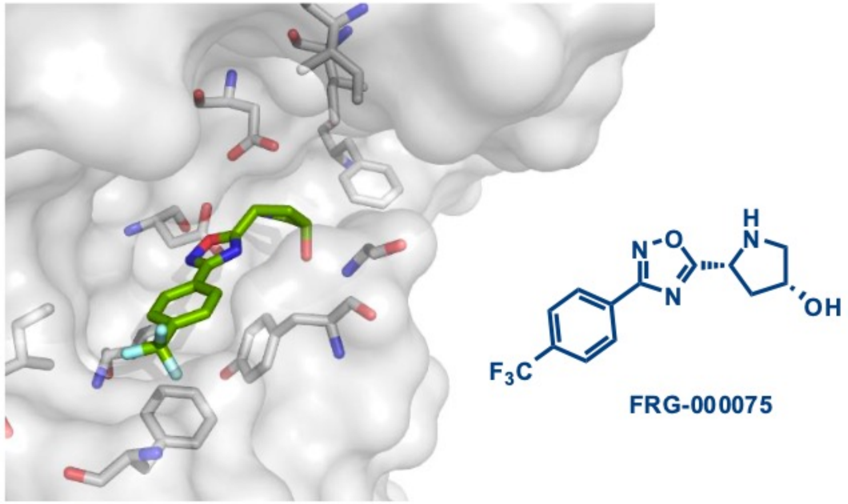


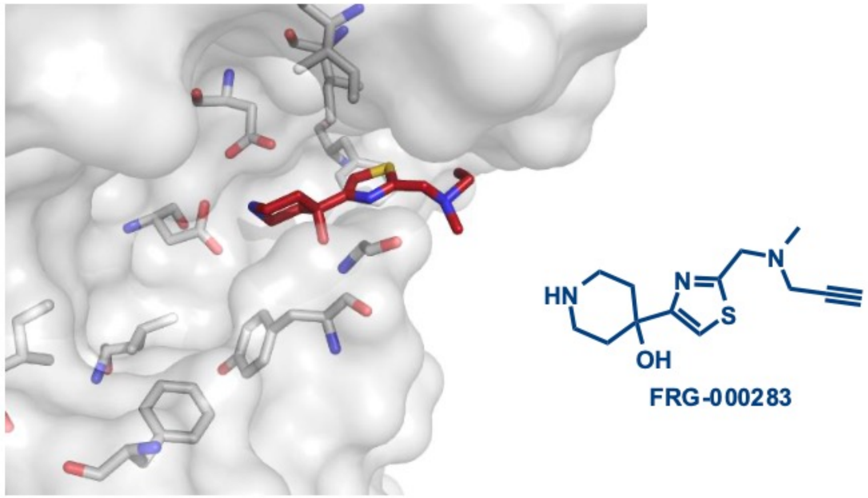

**Figure 3. The lipophilic hot spot of switch I/II.** SI/II-pocket with the relevant crystallographic water molecules and amino acids in the small lipophilic pocket and the shallow polar rim surrounding the small cavity.

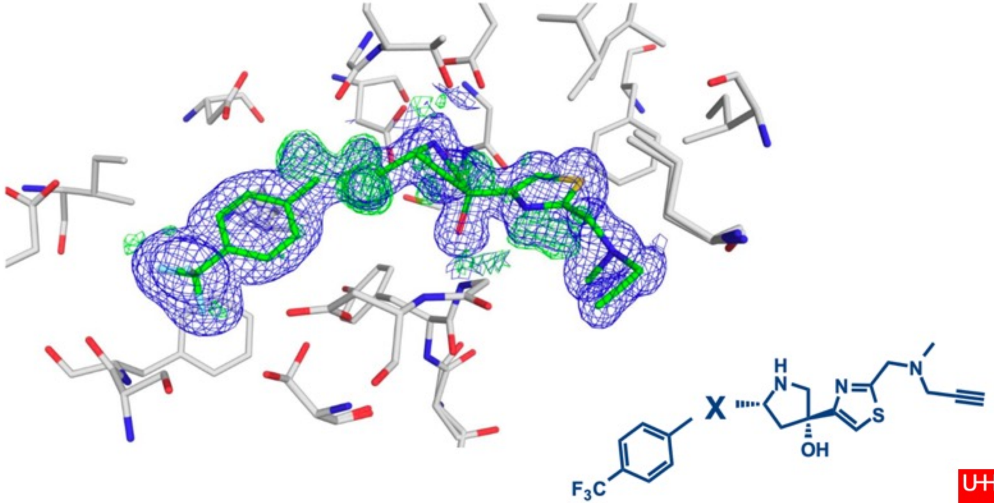
x-ray crystal structures elucidating in detail how ligands bind to the SI/II-pocket in KRAS, NRAS and HRAS in both the on and off states. The establishment of robust cocrystallization systems [26] and high throughput soaking systems [29] has allowed us to generate a high coverage of relevant RAS crystal structures and thus gain insights into designing more potent and specific SI/II-pocket inhibitors or proteolysis targeting chimeras (PROTACs) for the three RAS family of proteins. The high throughput crystallization system also allowed us to develop our so called 'x-ray first' approach where we crystallized every newly synthesized compound in the active $KRAS^{G12D}$ form before proceeding toward biophysical or biochemical affinity testing. Based on the binding mode we selected the interesting molecules for further measurements to neglect the typical affinity biased optimization strategies that often lead to wrong conclusions with respect to binding interactions.

# USE CASE I: MERGING

## CRYSTALLOGRAPHIC FRAGMENT SCREENING: ENDOTHIAPEPSIN



- High Resolution < 1.0 Å
- Conserved binding mode of fragments and merged compound.
- ITC:

  $K_D$ Fragments: Single digit mM

  $K_D$ Merged Cpd: 2.7 μM

Universität Hamburg

Philipps Universität Marburg

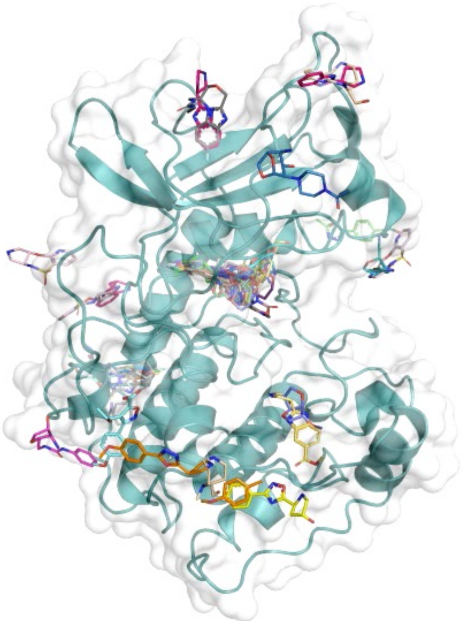
AnalytiCon discovery

CRYSTALS FIRST

# USE CASEII: UNTAPPED CHEMICAL SPACE

## CRYSTALLOGRAPHIC FRAGMENT SCREENING: KINASE

Crystallization of PKA → SmartSoak stabilization of crystals → NP-like fragments: fsp3 58% → SmartSoak screening: 87 fragments → SmartRefine



- 87 refined structures
- 1.3 -1.8 Å resolution
- 27 complexes with fragment bound to the ATP-binding pocket
- **Hit rate** over **30%**
- **mean Fsp$^3$** of hits **47%**

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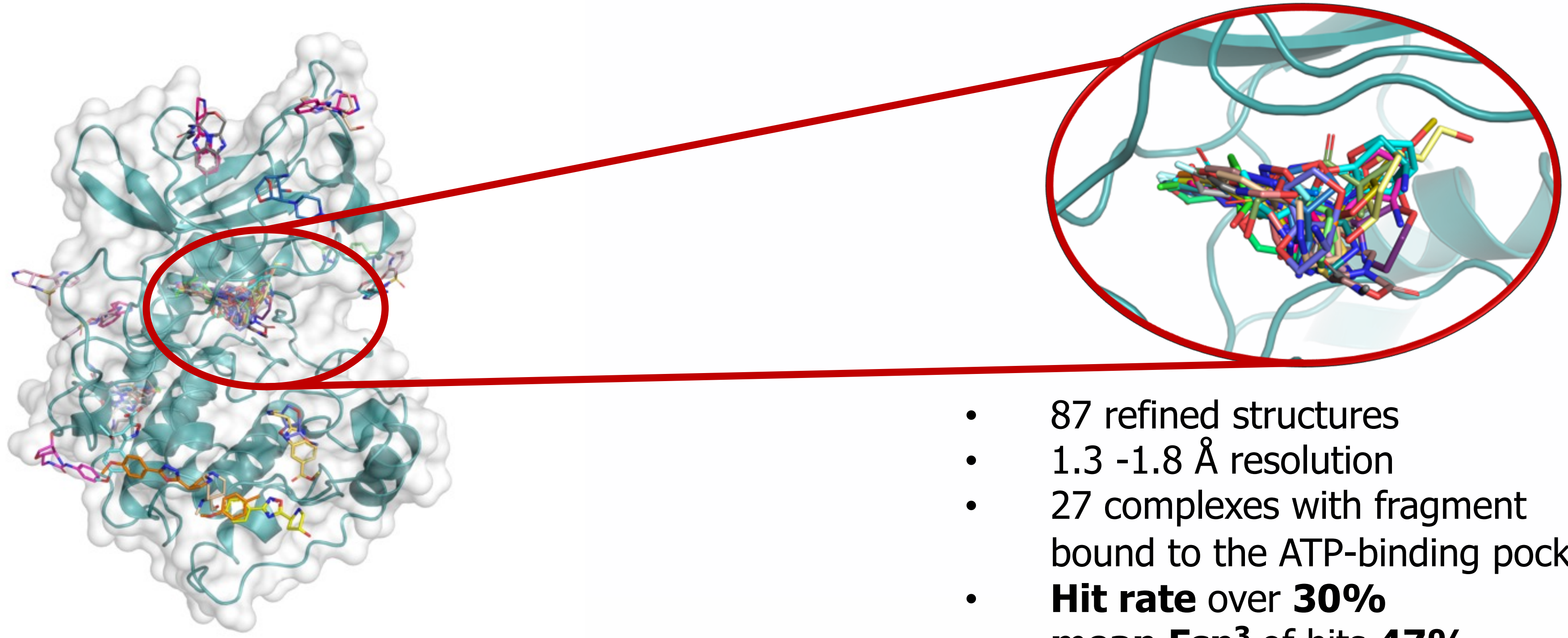

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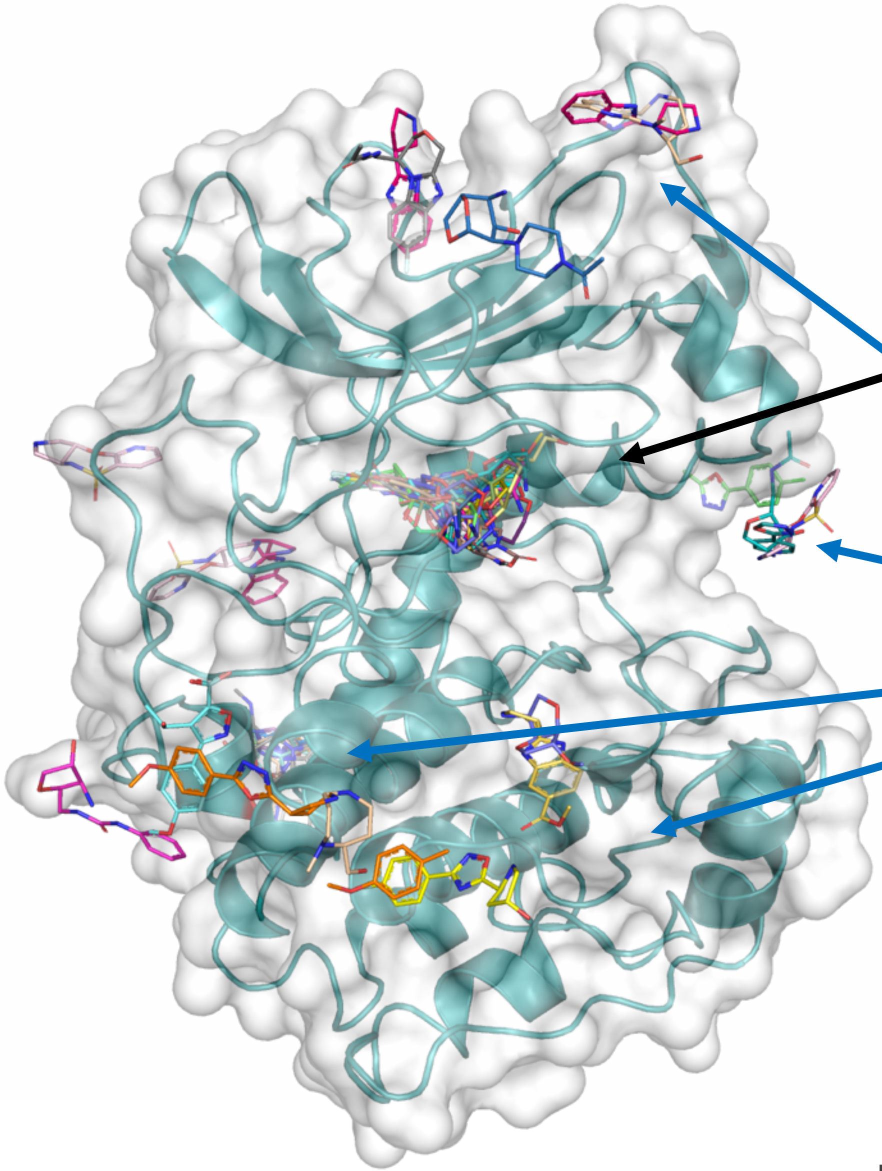
# USE CASEII: UNTAPPED CHEMICAL SPACE



| Categorization of binding events | |
|---|---|
| **ATP pocket binders** | **27** |
| hinge, direct | 18 |
| hinge, via water | 1 |
| DFG, direct | 14 |
| DFG, via water | 12 |
| **Secondary binding events** | **28** |

**Multitude of diverse starting points offering tailor-made inhibition modes for type I, (II), III or IV inhibitors**

Liu Y. & N. Gray, 2006, Nature Chemical Biology, 358–364

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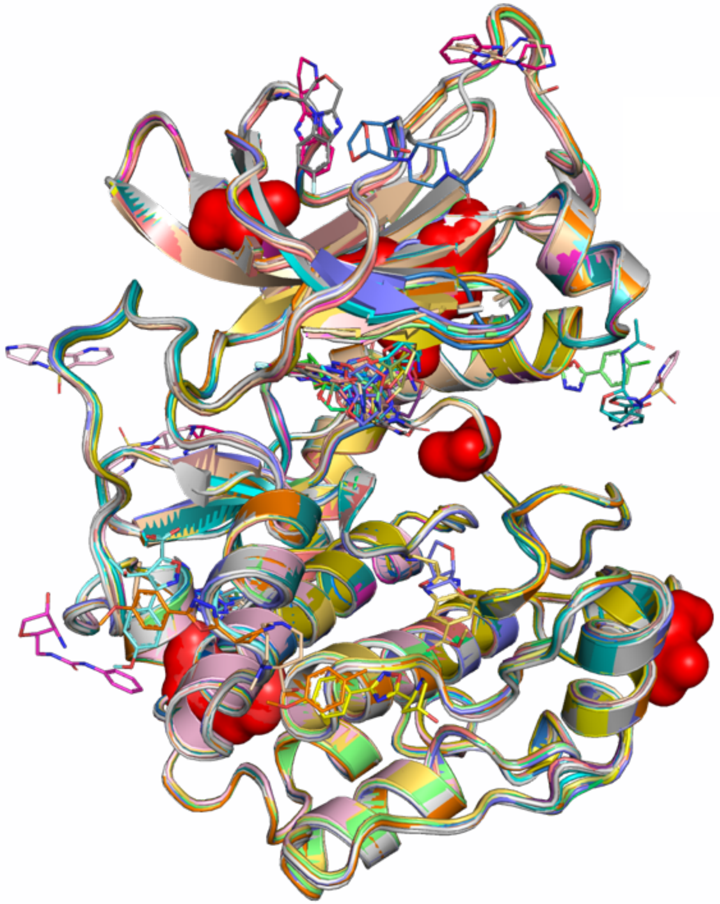
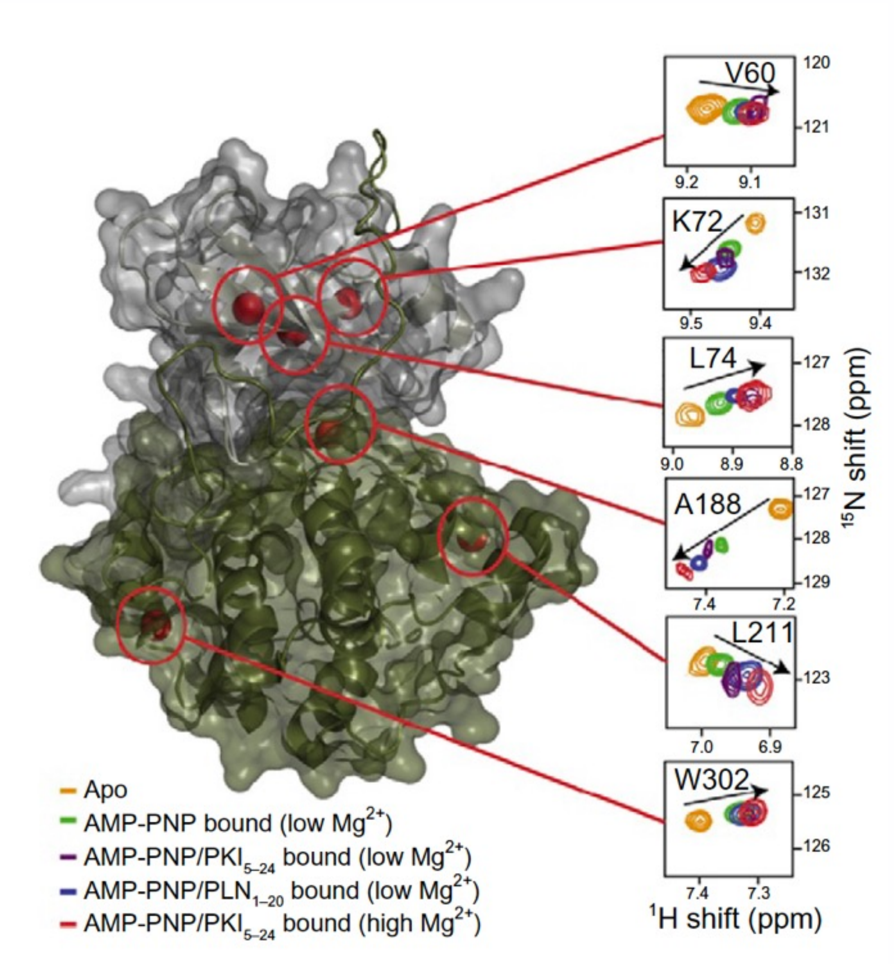
# USE CASEII: UNTAPPED CHEMICAL SPACE



Natural-product like fragments cluster at 4 out of 5 allosteric and cooperative sites (red surface) of PKA revealed by NMR spectroscopy by Masterson et al.




Masterson et al.,. *Adv. Protein Chem. Struct. Biol.* 2012

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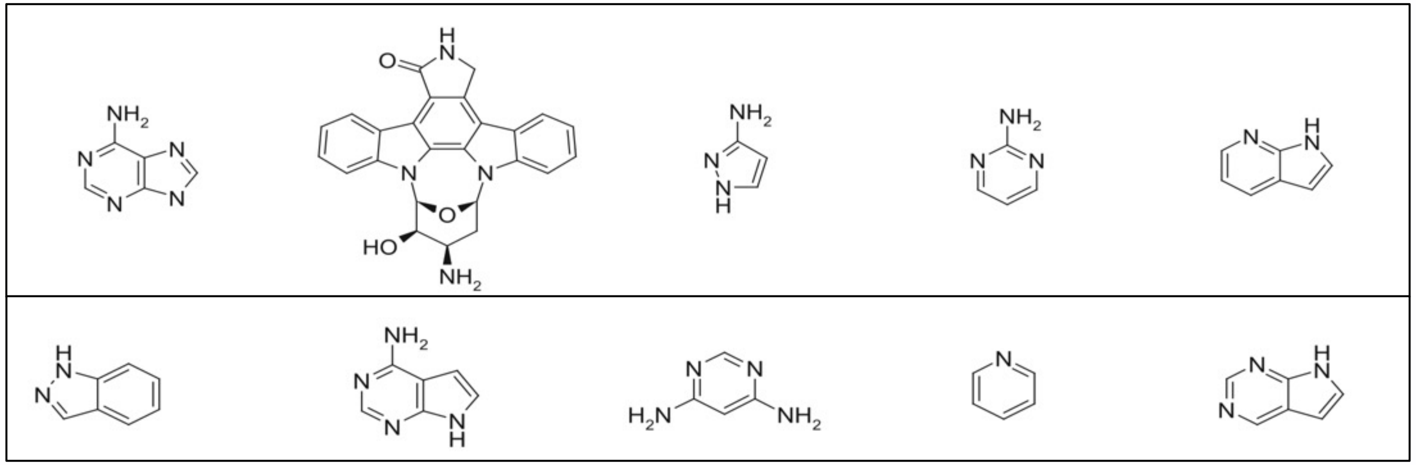
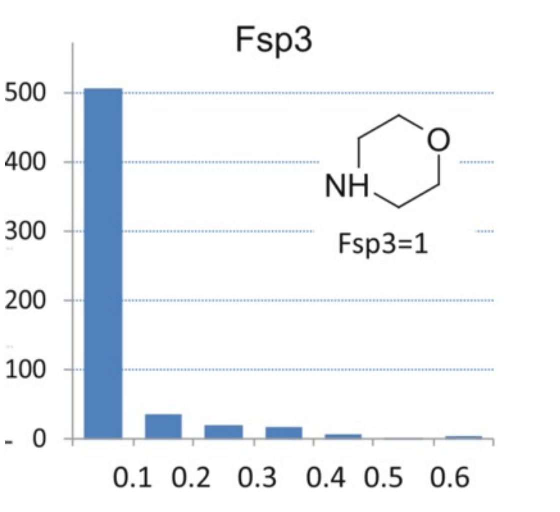
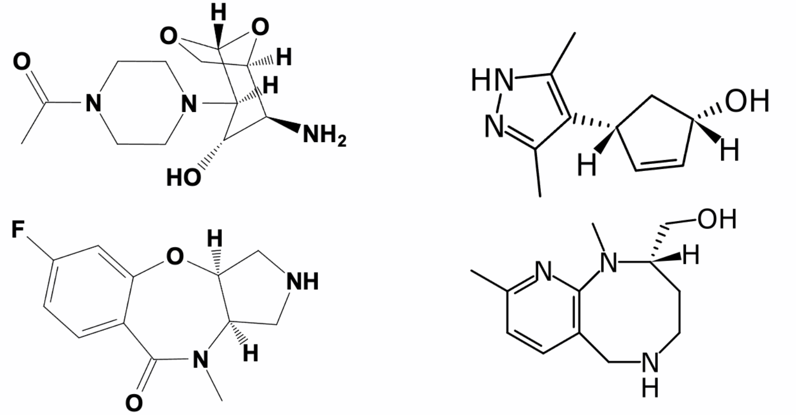
## TYPICAL HINGE BINDER

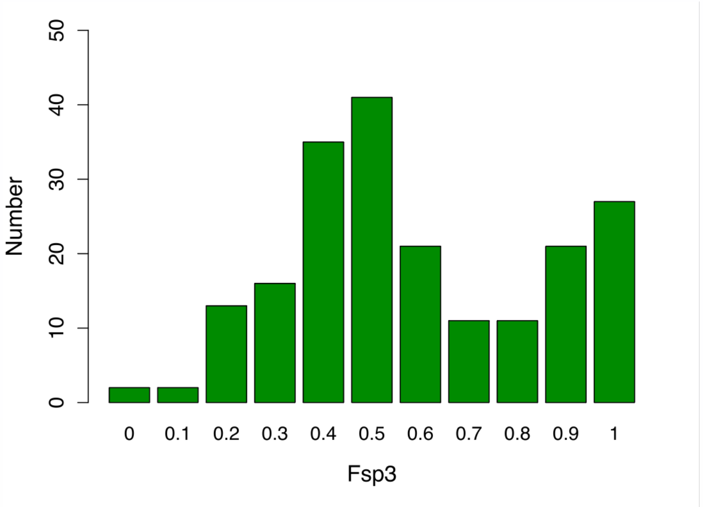





Xing, L.; et al. Bioorganic Med. Chem. 2015

## PRIMARY CRYSTALLOGRAPHIC HITS



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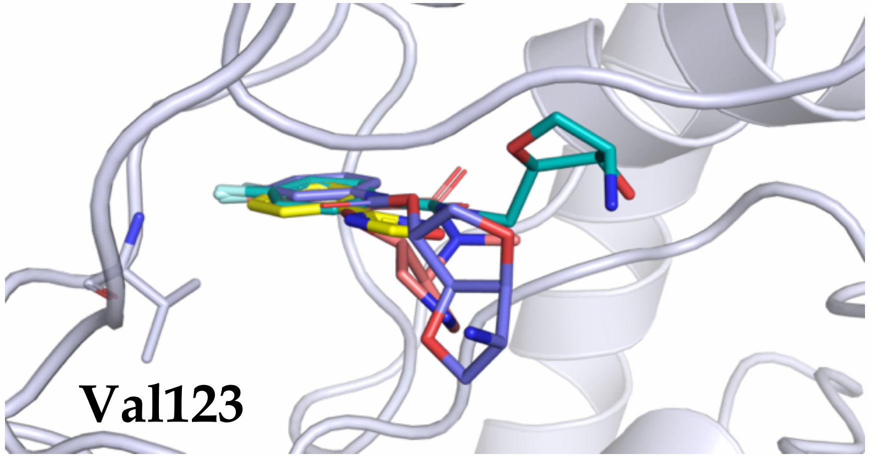
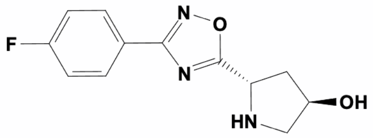
# HIGH DIVERSITY OF STRUCTURAL DATA AT HIGH HIT RATES

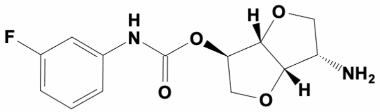
## Fluorine hinge interaction



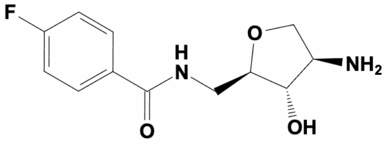

**FRGx 001**



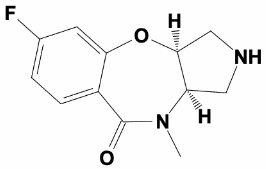
**FRGx 032**



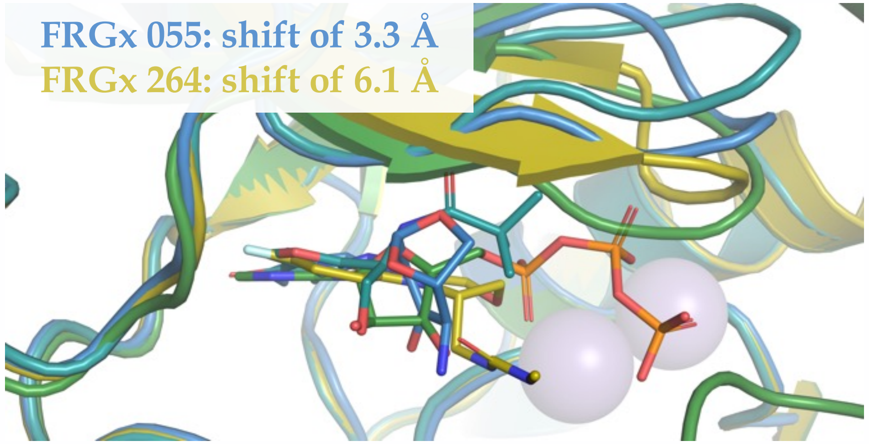
**FRGx 203**



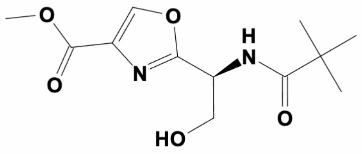
**FRGx 296**



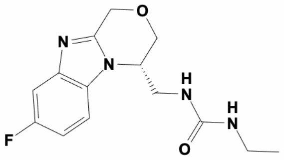
## G-loop movement



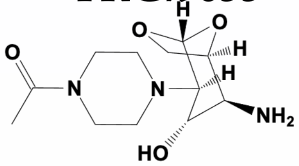

**FRGx 186**



**FRGx 264**



**FRGx 055**



**ATP**

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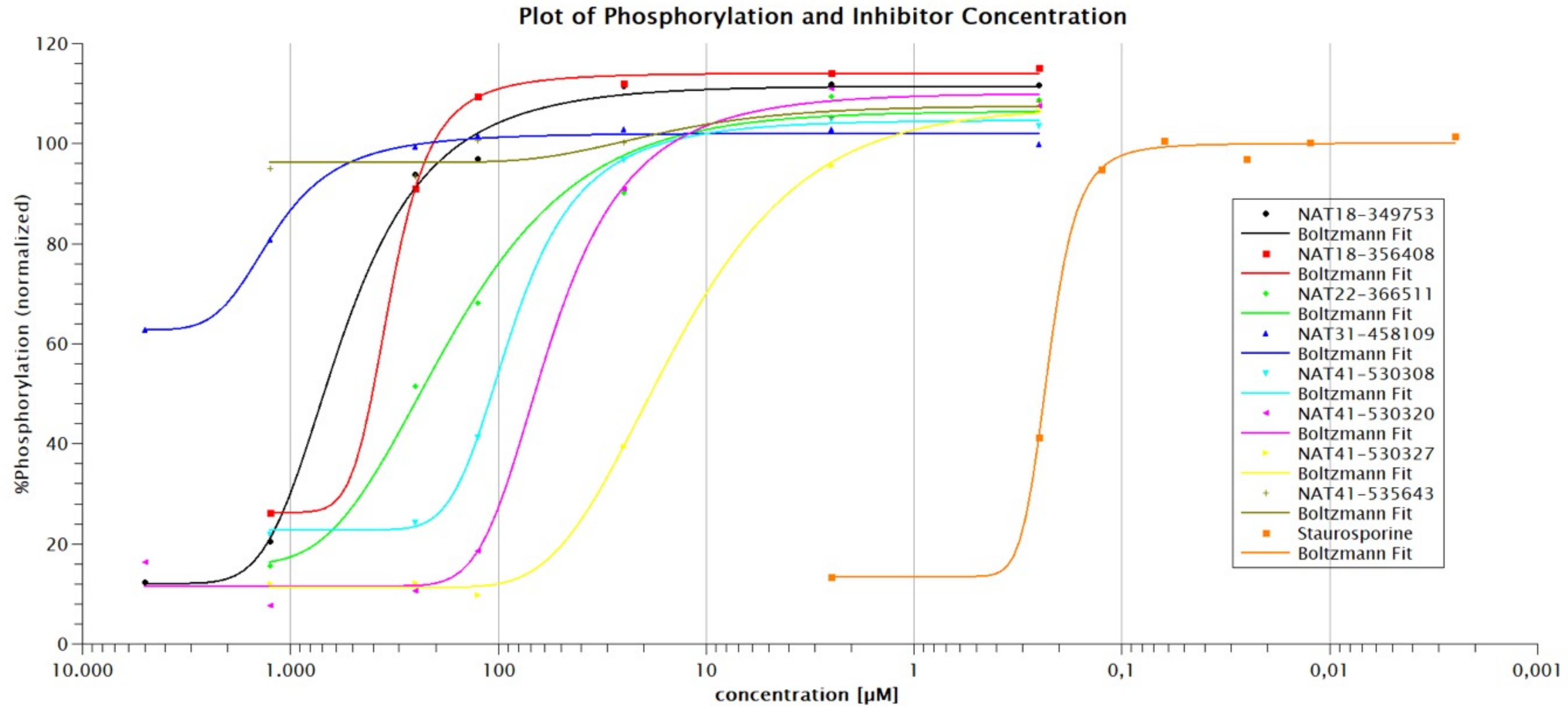
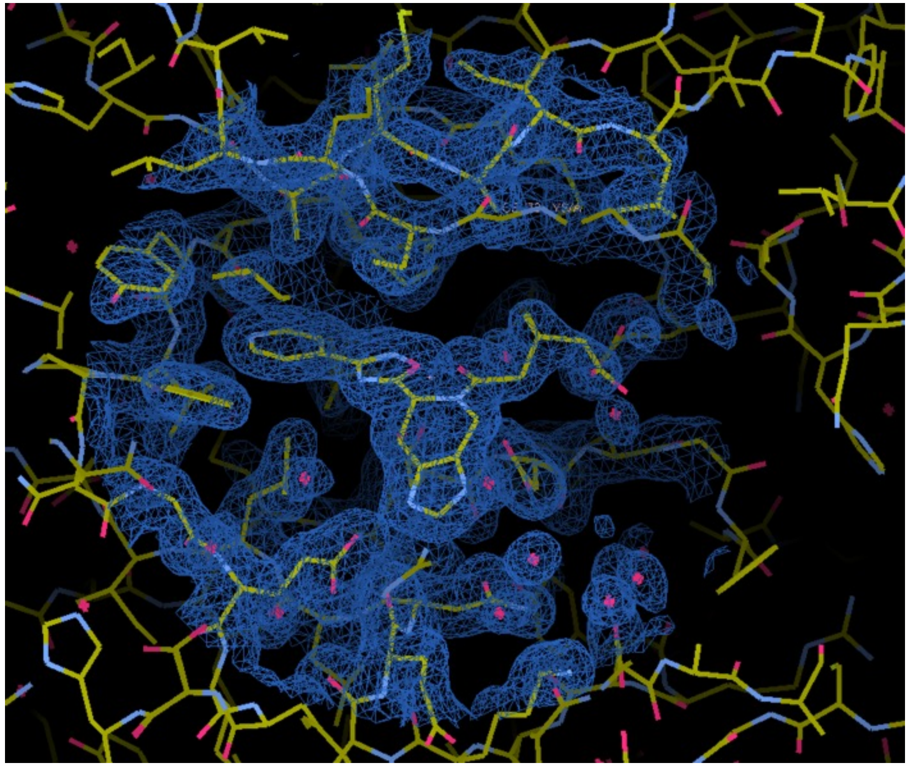
# RAPID STRUCTURALLY-ENABLED FOLLOW UP STRATEGY

## ZLYTE™ FRET ASSAY – 20 follow up compounds tested

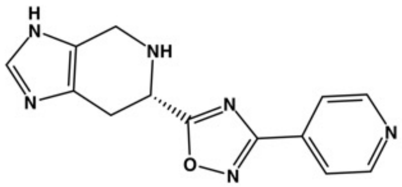
Six µMolar hits from 20 tested compounds

Success rate: 30 %

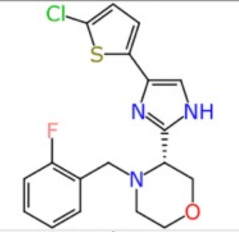
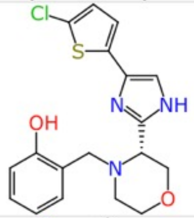
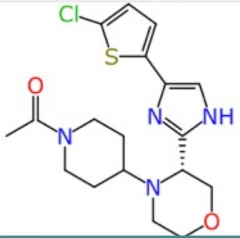
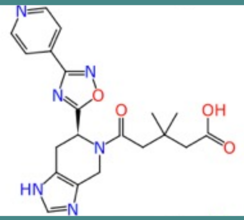
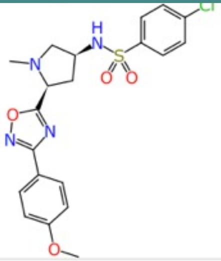
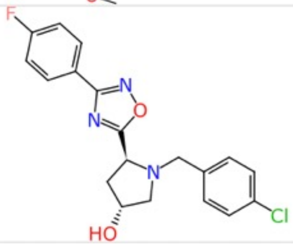




FRG-00012



Ki > 1mM

| Molecule | Name | Ki | LE |
|---|---|---|---|
|  | NAT41-530327 | 6 | 0.29 |
|  | NAT41-530320 | 22 | 0.26 |
|  | NAT41-530308 | 37 | 0.23 |
|  | NAT22-366511 | 78 | 0.18 |
|  | NAT18-356408 | 158 | 0.17 |
|  | NAT18-349753 | 198 | 0.19 |

# FRAGMENT-TO-HIT

## STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES

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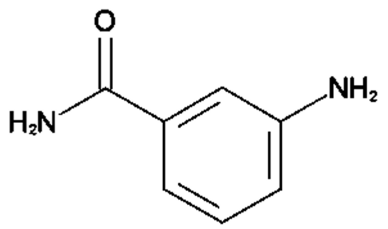
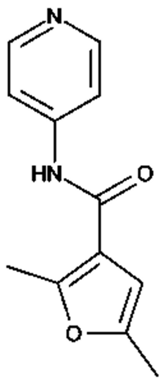
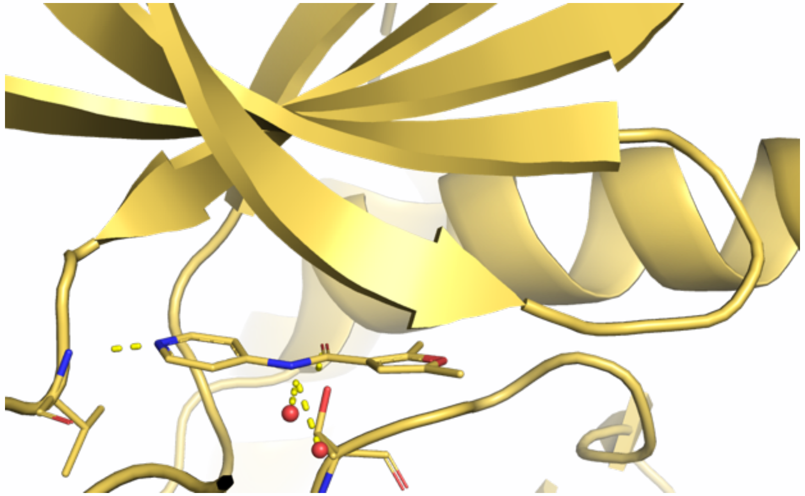
# USE CASE III: STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES

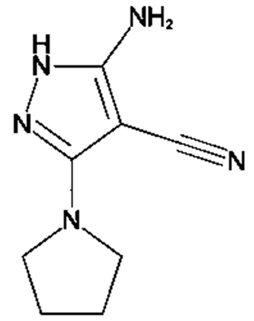



3AB – 5N3Q

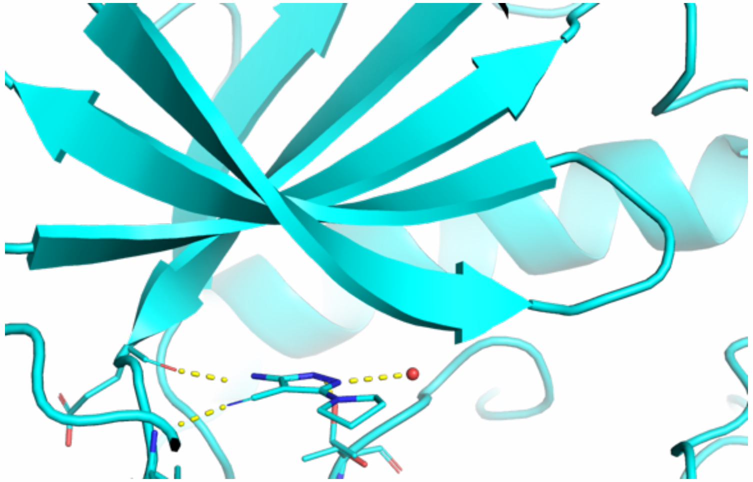


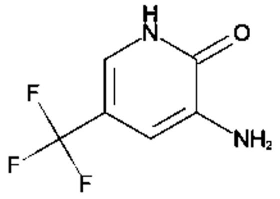

47V – 5N1L



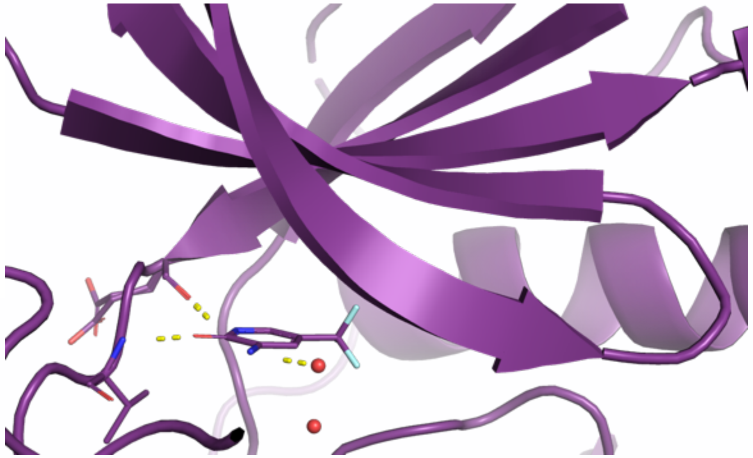



E9Q – 5N7P






8JW – 5N33



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# CHEMICAL SPACE DOCKING

- Selection of crystallographic fragments: **4** fragments **chosen**
- Template-based docking of all REAL Space fragments selection of best **190**
- Enumeration of products: **2,644,995**
- Docking (5 poses each): **10,811,842**
- Scored docking poses: **3,269,104**
- Only best pose per molecule: **1,628,163**
- Lead-like filter: **1,009,231**
- Cluster by Tanimoto similarity (best 25 of each cluster): **3,379**
- Inspection by eye: **106** selected for synthesis

No affinity data, only co-structures

Lead-like filter

Clustering

Synthesis

Philipps Universität Marburg

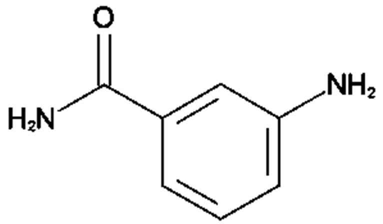
CHEM SPACE

Enamine

BioSolveIT

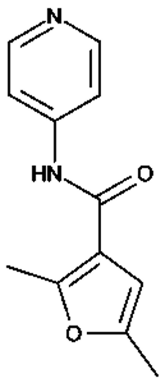
CRYSTALS FIRST

# USE CASE III: STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES



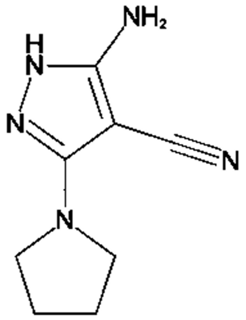
3AB – 5N3Q

- 32 compounds, 6 solubility issues, 7 active, 19 non-active
- Ki fragment: ~17 mM
- Follow up compounds stay in mM range



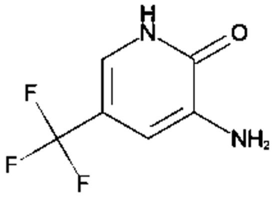
47V – 5N1L

- 16 compounds, 4 solubility issues, 4 active, 8 non-active
- Ki fragment: solubility too low; Ki (>15 mM)
- Best follow up: 139 µM (40.48 µM)
- Factor: min. 100X (370X)



E9Q – 5N7P

- 26 compounds, 1 solubility issues, 10 active, 15 non-active
- Ki fragment: ~3mM
- Best follow up: 86µM (30.5 µM)
- Factor: 36X (100X)



8JW – 5N33

- 19 compounds, 7 solubility issues, 4 active, 8 non-active
- Ki fragment: ~6mM
- Best follow up: 5.6µM (2.34 µM)
- Factor: 1100X (2500X)

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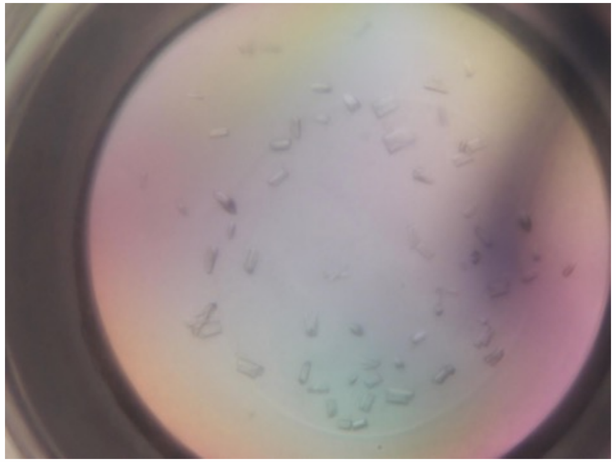
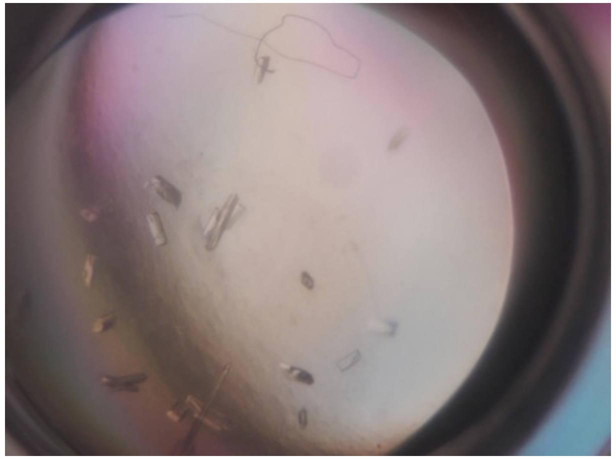
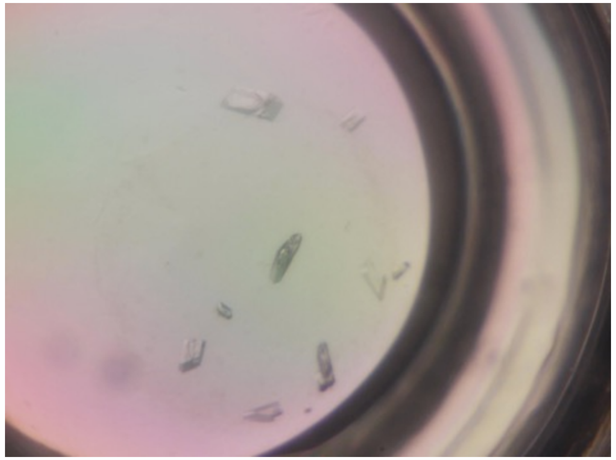
# USE CASE III: STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES

## CO-CRYSTALLIZATION

- 12 most active compounds selected
- First co-crystallization batch
- 7 compounds out of 12 produced crystals
- Data collection this Tuesday
- Co-structures determined

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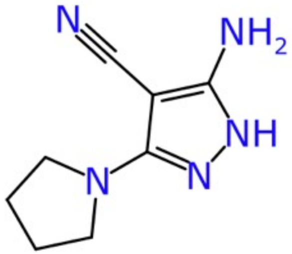
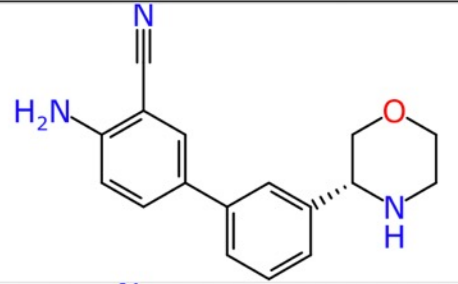
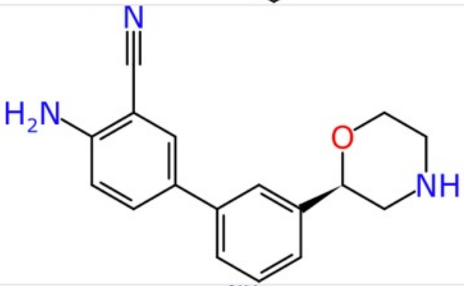
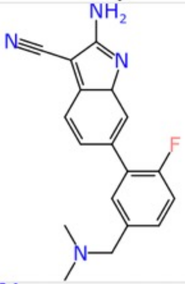
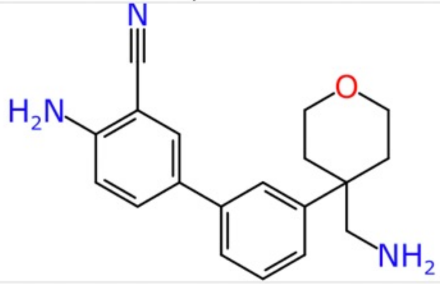
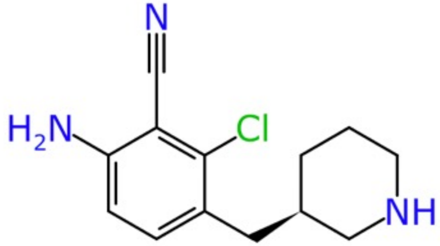
# E9Q CLUSTER

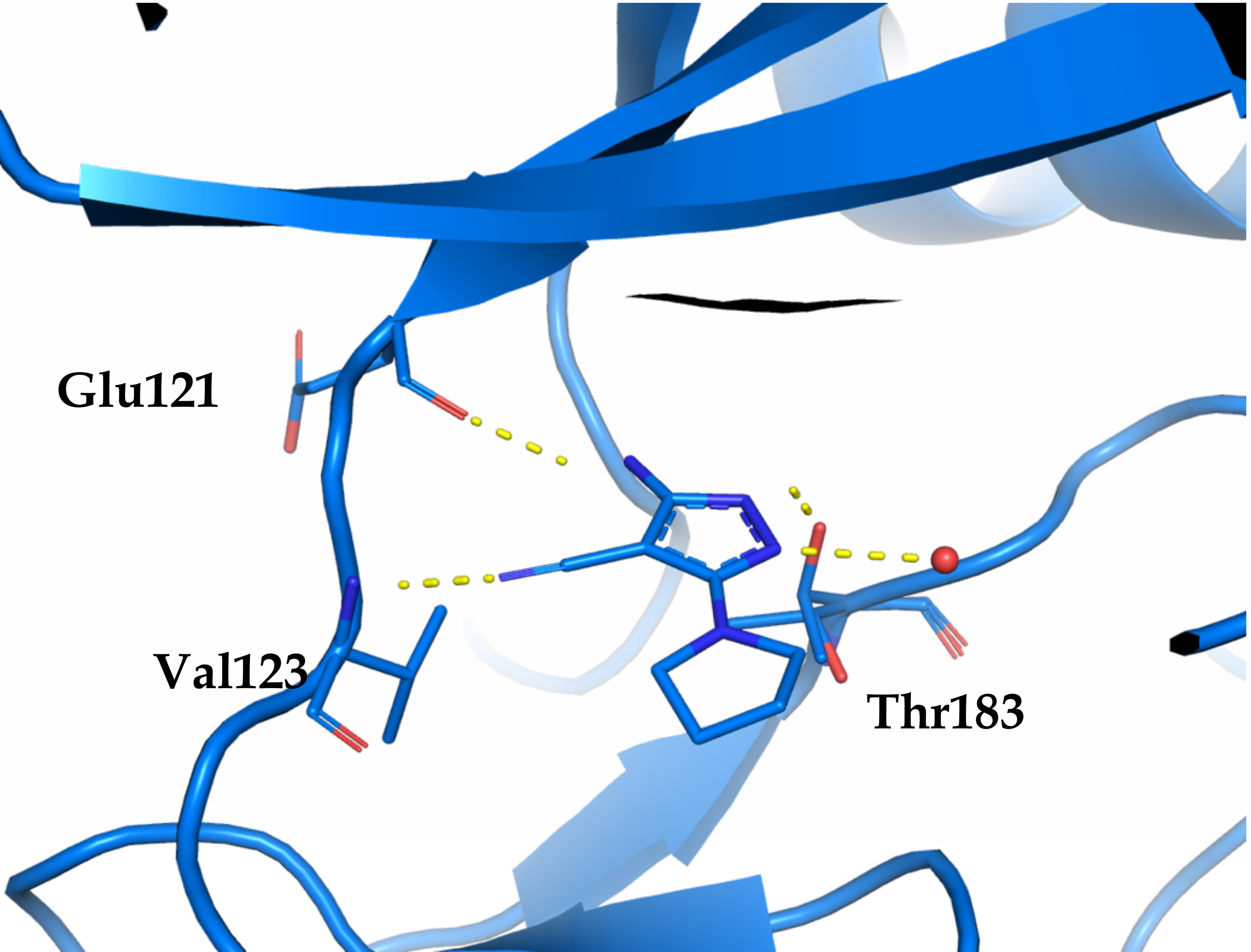


- 26 compounds, 1 solubility issues, 10 active, 15 non-active
- Ki fragment: ~3mM
- Best follow up: 86μM (30.5 μM)
- Factor: 36X (100X)
- 5 co-structures

| Molecule | Name | Ki | LE |
|---|---|---|---|
|  | 0060 | 956 μM | 0.20 |
|  | 0068 | 2102 μM | 0.17 |
|  | 0081 | 174 μM | 0.22 |
|  | 0086 | 390μM | 0.20 |
|  | 0088 | 86 μM | 0.33 |

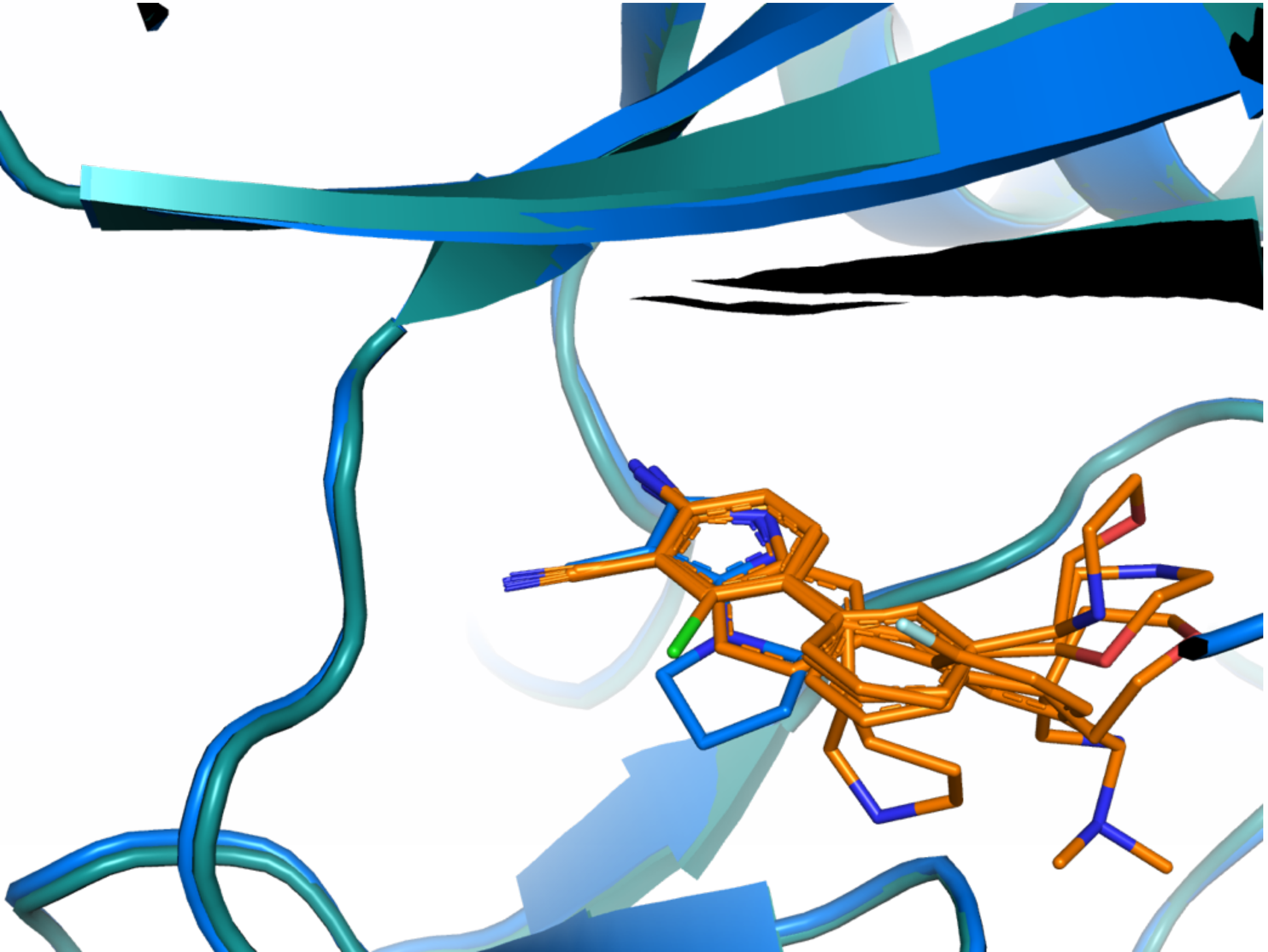
# E9Q CLUSTER

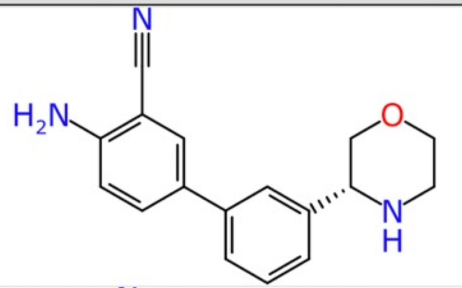
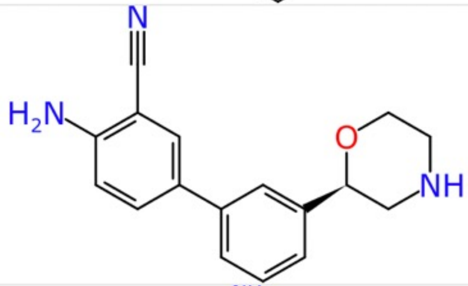
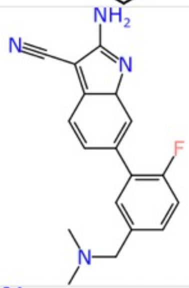
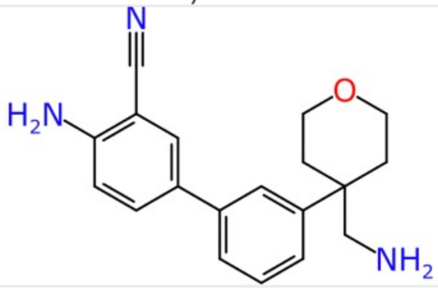
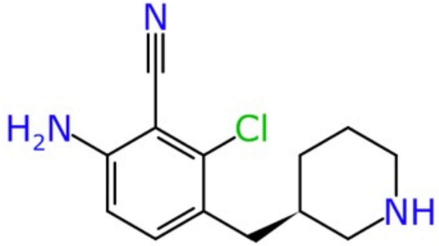
## BINDING MODE OF THE FRAGMENT



# E9Q CLUSTER

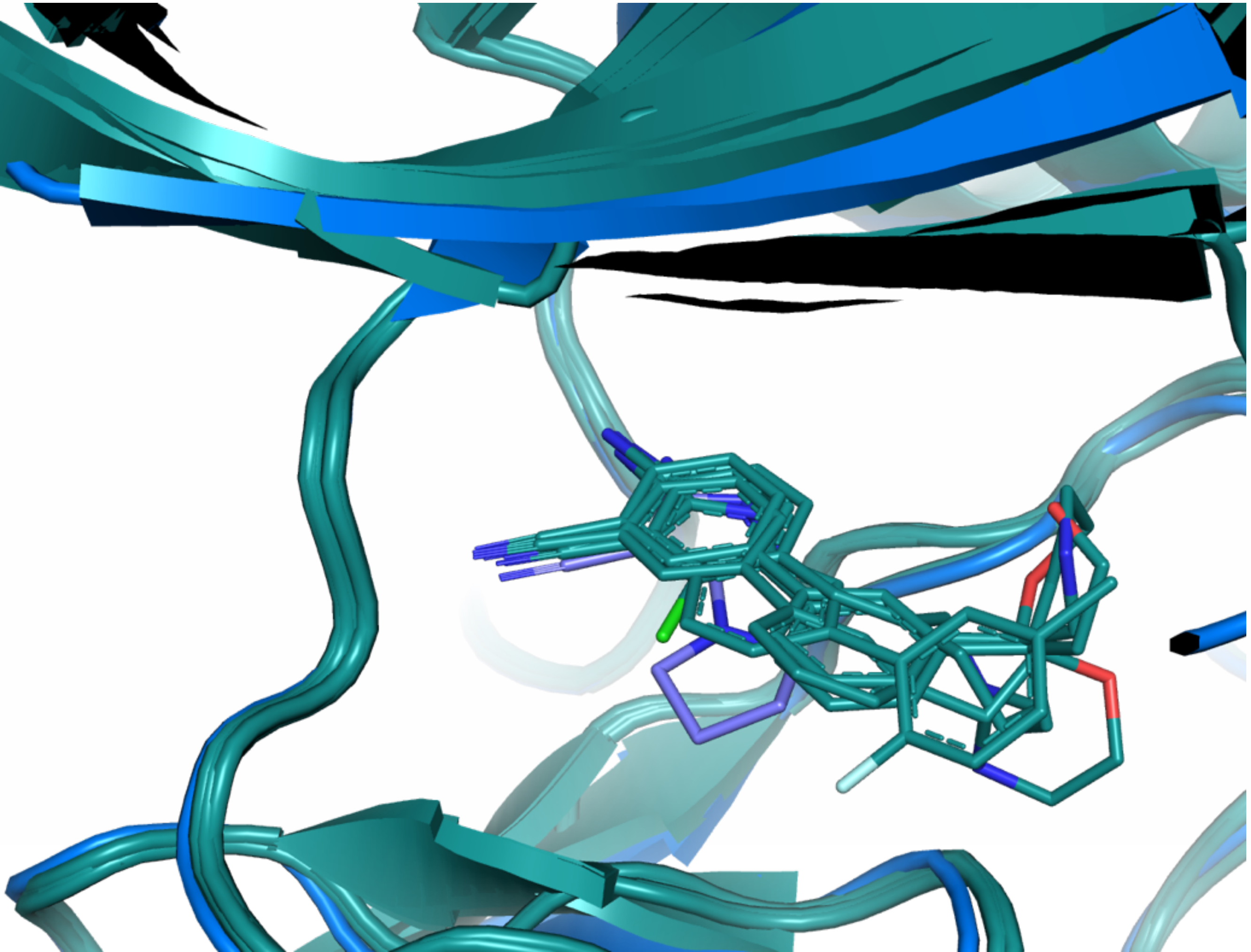
## DOCKING POSES OF ENUMERATED COMPOUNDS

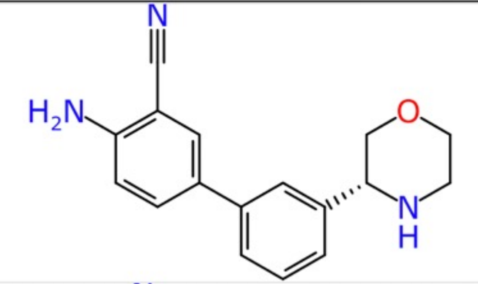
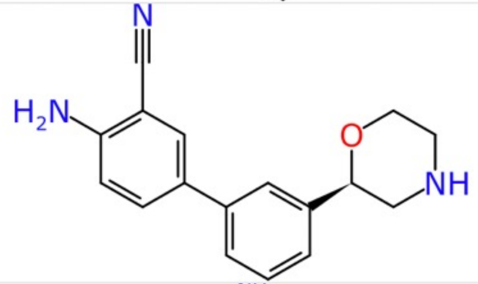
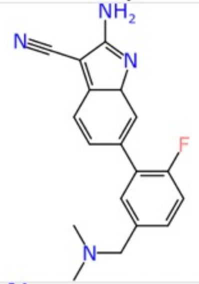
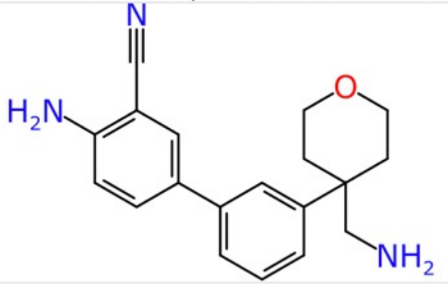
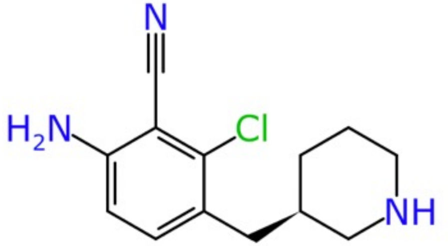


| Molecule | Name | Ki | LE |
|---|---|---|---|
|  | 0060 | 956 µM | 0.20 |
|  | 0068 | 2102 µM | 0.17 |
|  | 0081 | 174 µM | 0.22 |
|  | 0086 | 390µM | 0.20 |
|  | 0088 | 86 µM | 0.33 |

# E9Q CLUSTER

## BINDING MODES IN CRYSTAL STRUCTURES



| Molecule | Name | Ki | LE | Res. |
|---|---|---|---|---|
|  | 0060 | 956 µM | 0.20 | 1.4 Å |
|  | 0068 | 2102 µM | 0.17 | 1.4 Å |
|  | 0081 | 174 µM | 0.22 | 1.6 Å |
|  | 0086 | 390µM | 0.20 | 1.4 Å |
|  | 0088 | 86 µM | 0.33 | 1.4 Å |

# USE CASE III: STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES

## SYNERGIES OF TOP EXPERTISE

| CHEMICAL SPACE DOCKING | SYNTHESIS | KI DETERMINATION & STRUCTURE DETERMINATION |
|---|---|---|
| BioSolveIT | CHEM SPACE, Enamine | CRYSTALS FIRST |
| 3 weeks | 3 weeks | 3 weeks |
| 4 co-structures of fragments<br>106 compounds selected | ~87% success rate<br>93 synthesized | 75 tested -> **25 actives**<br>12 co-crystallized -> **5 structures** |
| success rates 24 % | 27 % | 33 % |

# SUMMARY

## STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES

### SMARTER

- start FBDD with crystals
- multiple starting points derived from multiple crystal structures
- initial hit rate in 1st round ~ 30 % & follow up structures still in fragment-like range
- several µM candidates, one of them 1100x Ki increase compared to the fragment
- a priori meaningful SAR (through actives and non-actives)

### FASTER

- very fast fragment-to-hit strategy – 9 weeks
- crystal structures are the most important asset for decision making.
- multiple horses in the race

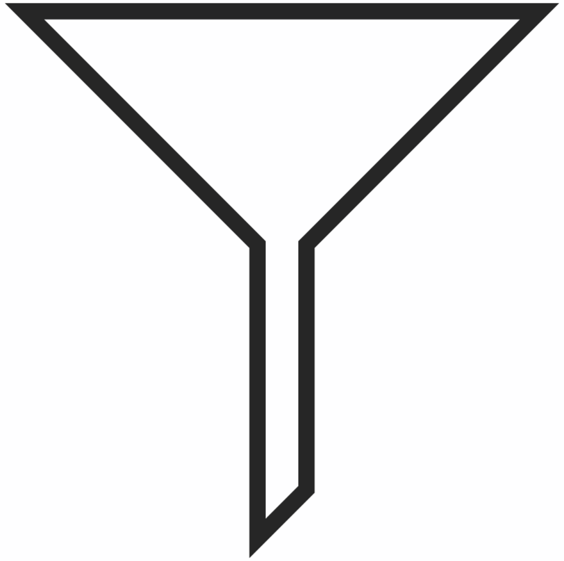
### CHEAPER

- Purchase compounds not for synthesis, but for direct SAR and structural biology
- One supervising medicinal chemist is empowered to submit several projects to LO stage

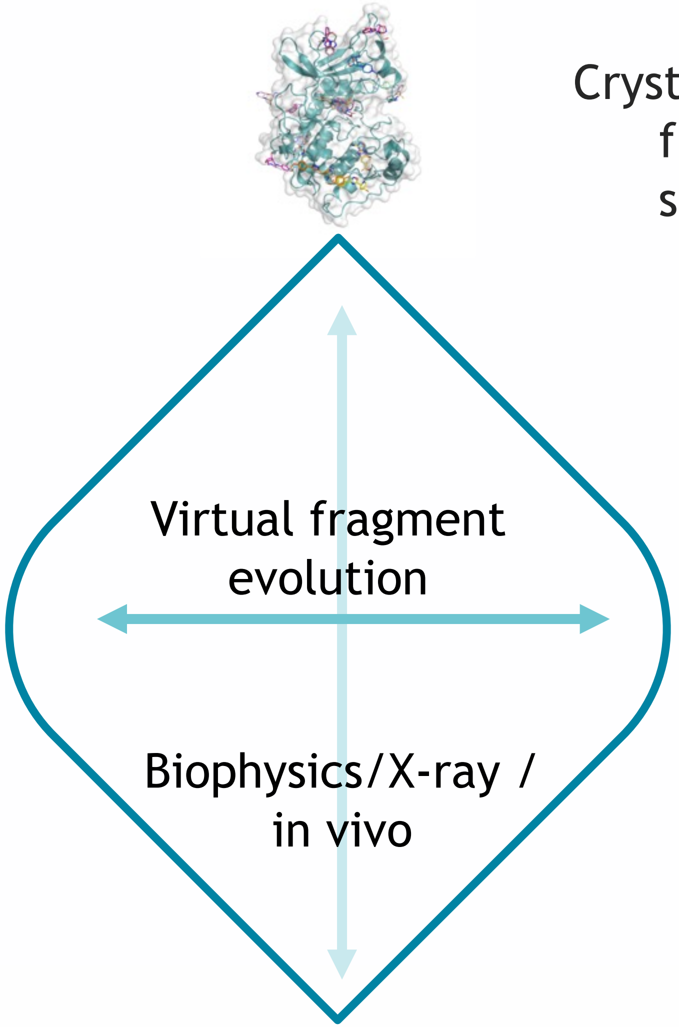
CRYSTALS FIRST

# A NEW EMERGING SCREENING PROCESS?

Typical screening process
HTS / FBLD



Crystallographic fragment screening




Active compounds

CRYSTALS FIRST

# AKNOWLEDGEMENTS

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Moritz Ruf
Christian Rötz


Philipps Universität Marburg

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Dr. Christof Siefker

AnalytiCon discovery

Dr. Lars Ole Haustedt

DESY

Oliver Wendt
Eva Crosas
Johanna Hakanpää

CHEM SPACE
Enamine

Yurii Moroz, PhD
Olga Tarkhanova

BioSolveIT

Alexander Neumann
Dr. Raphael Klein
Dr. Christian Lemmen
Dr. Marcus Gastreich

CRYSTALS FIRST

TURNING INSIGHTS INTO MEDICINES

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