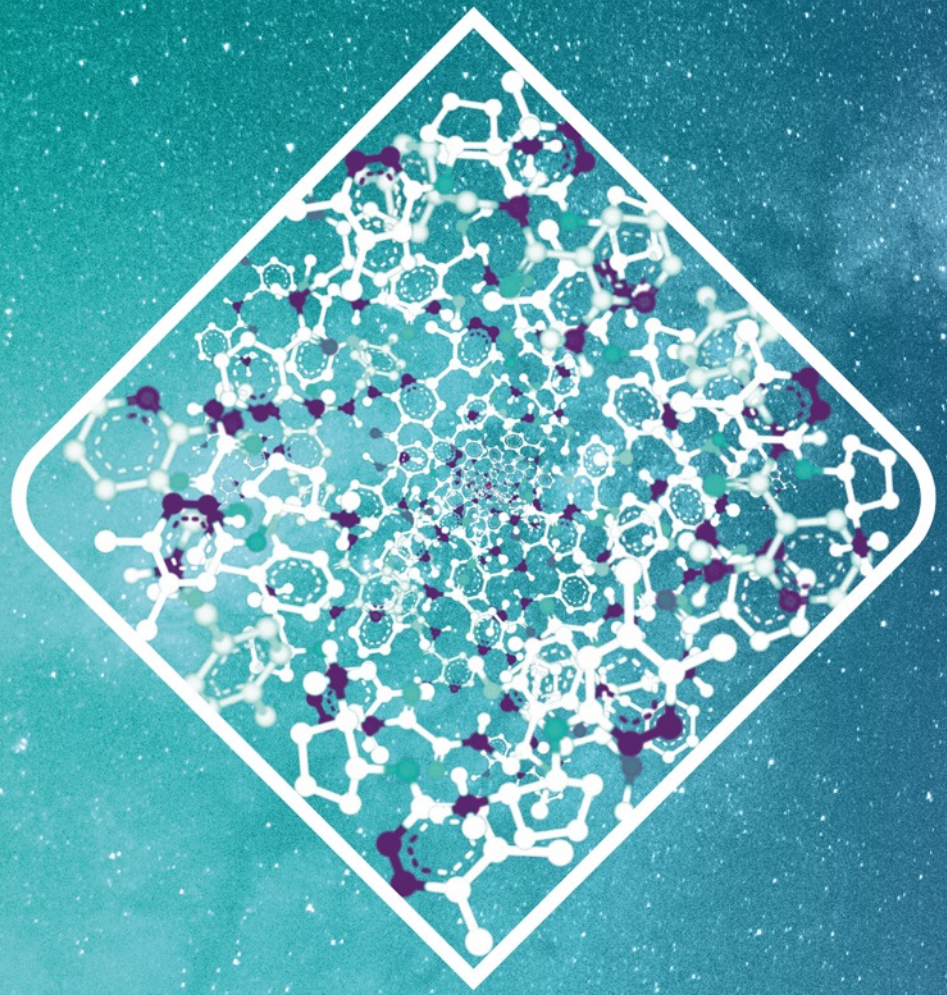


CRYSTALS FIRST

TURNING INSIGHTS INTO MEDICINES

Magnet for the needle in haystacks: Unlocking chemical matter using “crystal structure first” fragment hits

Dr. Serghei Glinca, Founder & CEO

WHO WE ARE

CRYSTALS FIRST

Structural Biology

Biophysics

Computational Modelling

Protein Production

PROF. GERHARD KLEBE

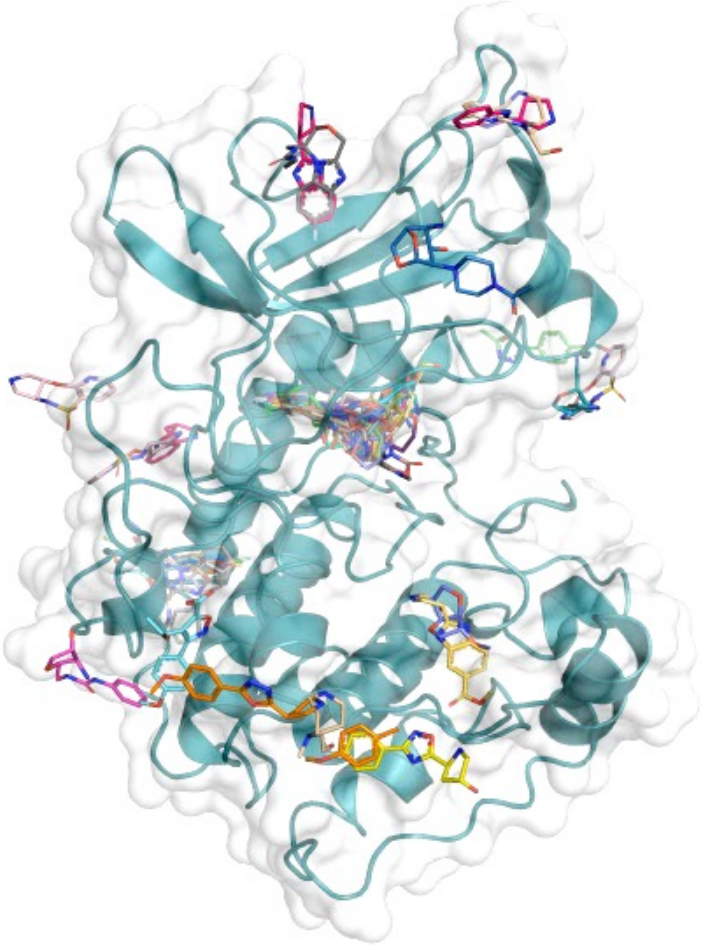
"RED BIBLE OF DRUG DESIGN"

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

WHAT WE DO

CRYSTALS FIRST

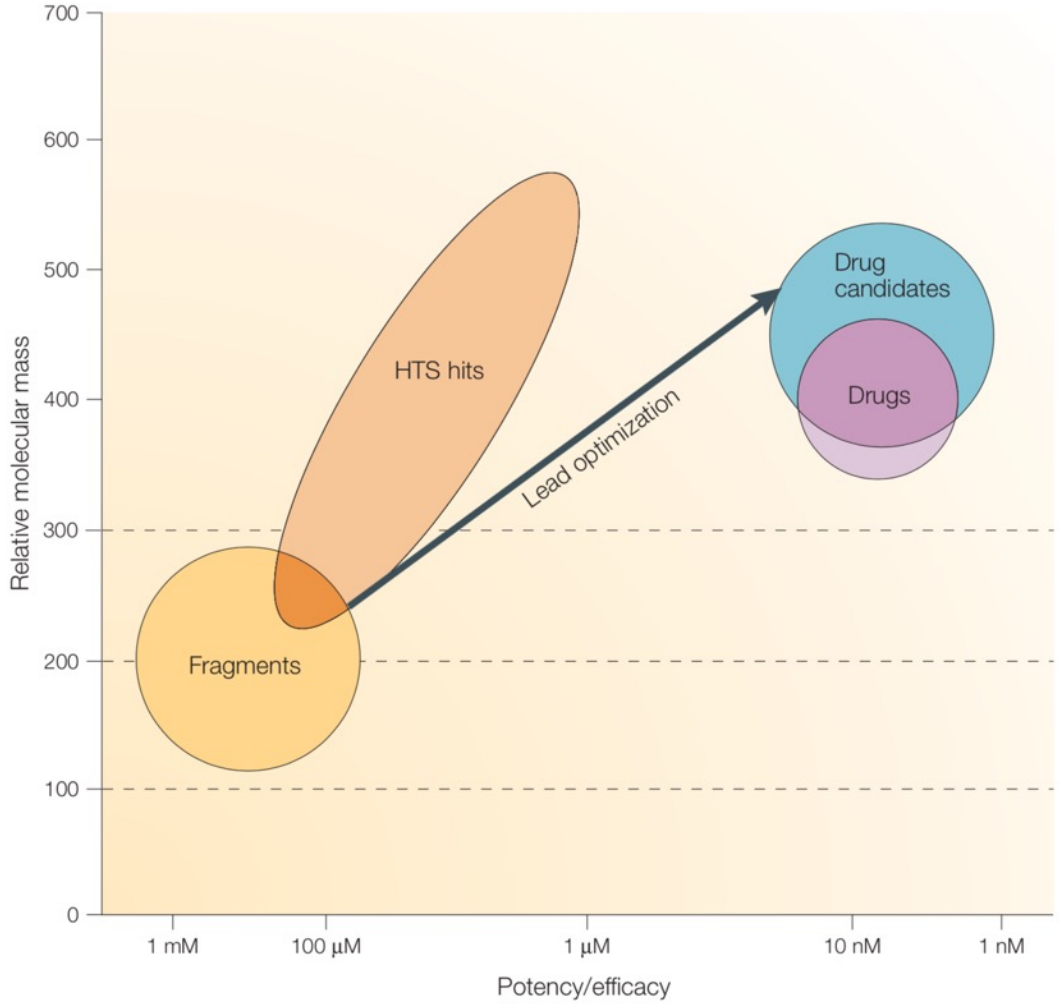
- Rapid access to structural data and large-scale computational modelling
- SmartSoak® enables an up to 10X accelerated process for soaking of protein crystals.
- The technology is target-agnostic and has been successfully applied for over 40 protein targets.
- Crystallographic screens deliver hit rates up to 30 %.

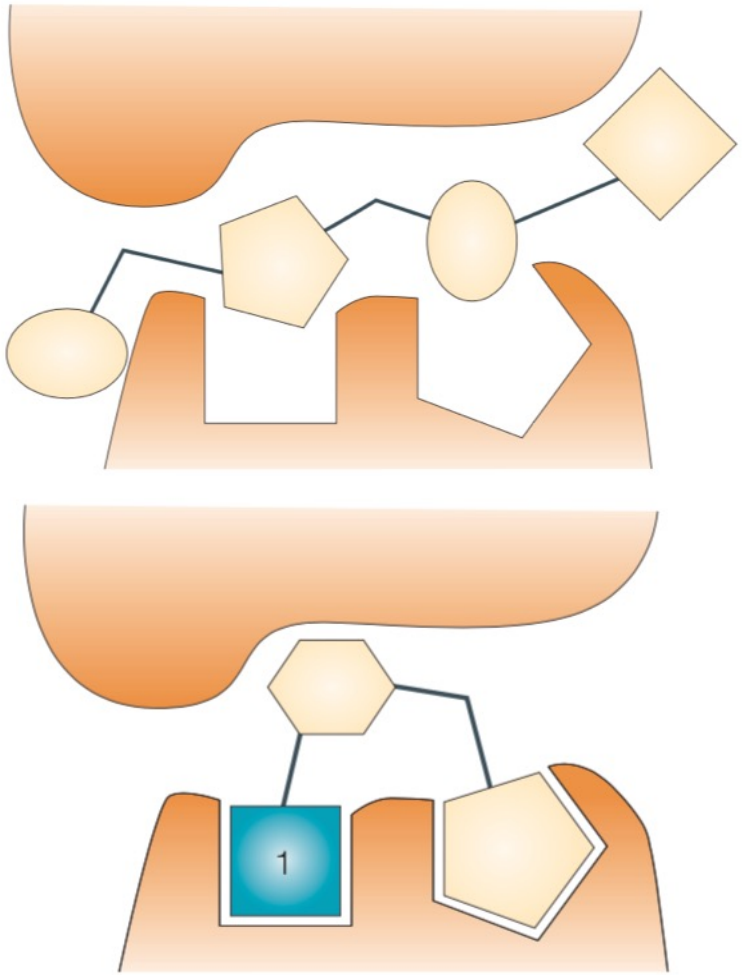


MERCK KSQ® Therapeutics AiCuris Anti-infective Cures

FRAGMENT-BASED DRUG DISCOVERY - FBDD

CRYSTALS FIRST





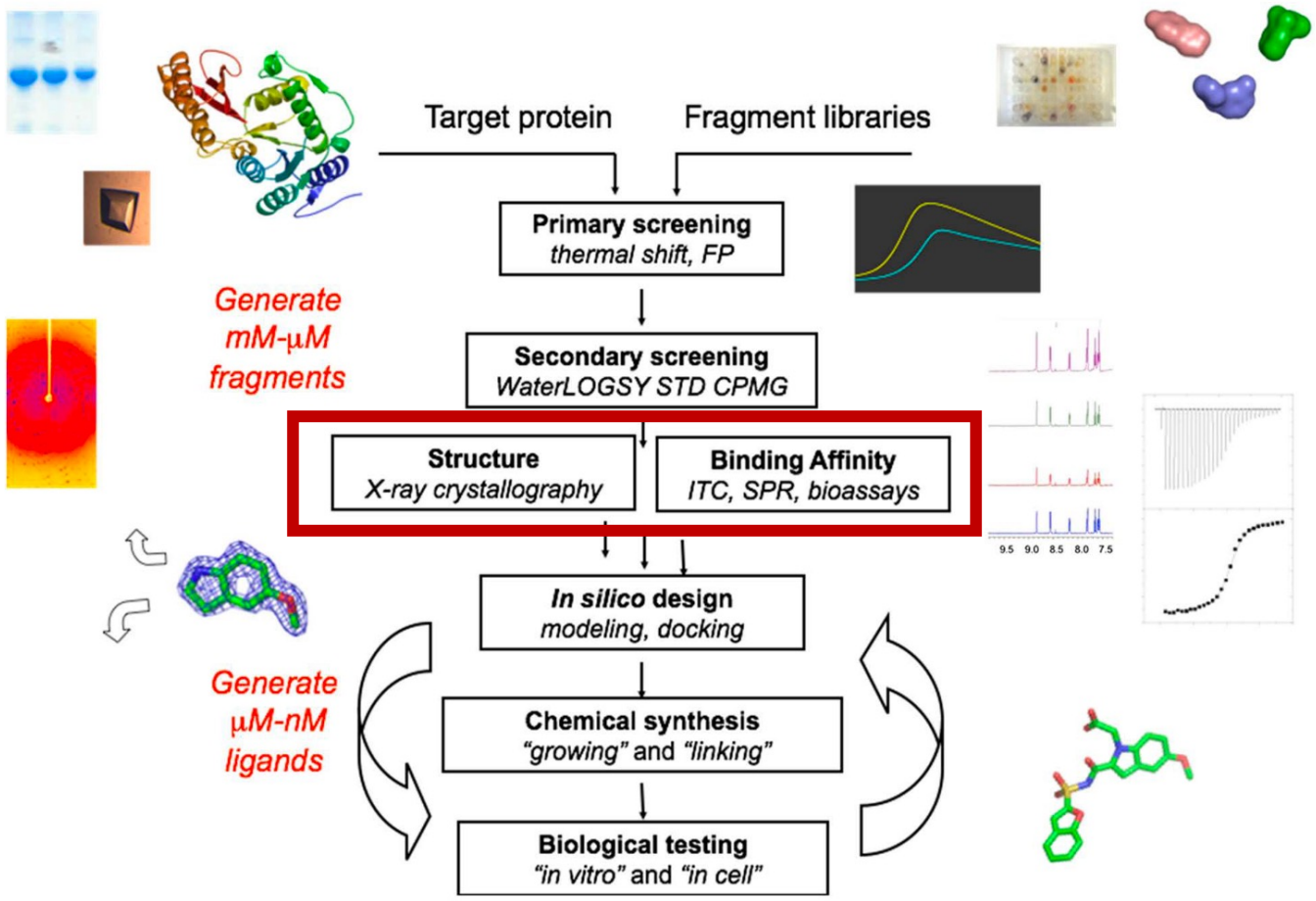
Rees, D. C.; et al. Nature Reviews Drug Discovery, 2004, 3(8), 660–672.

OPINIONS WHY NOT TO USE FBDD

CRYSTALS FIRST

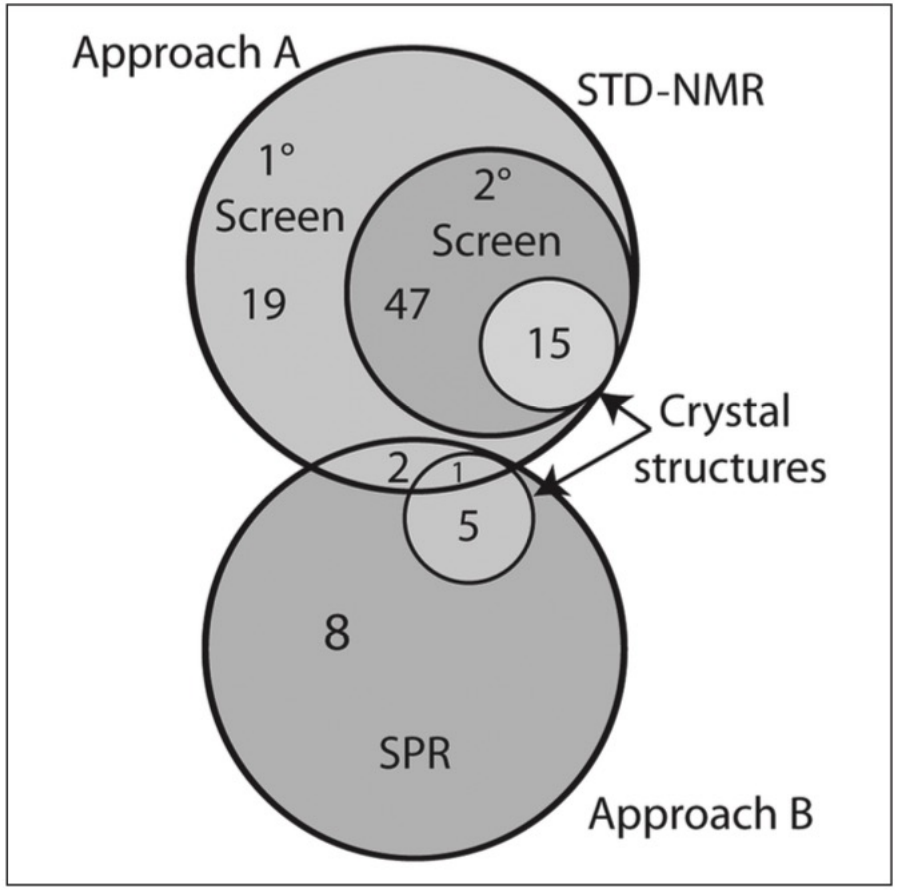
- Other hit id methods are more efficient
- Potency of fragments is too low
- Takes too long to build more potent compound
- SAR requires structural data
- Structural biology delivers empty structures
- Crystallography is a bottleneck

FRAGMENT SCREENING CASCADE



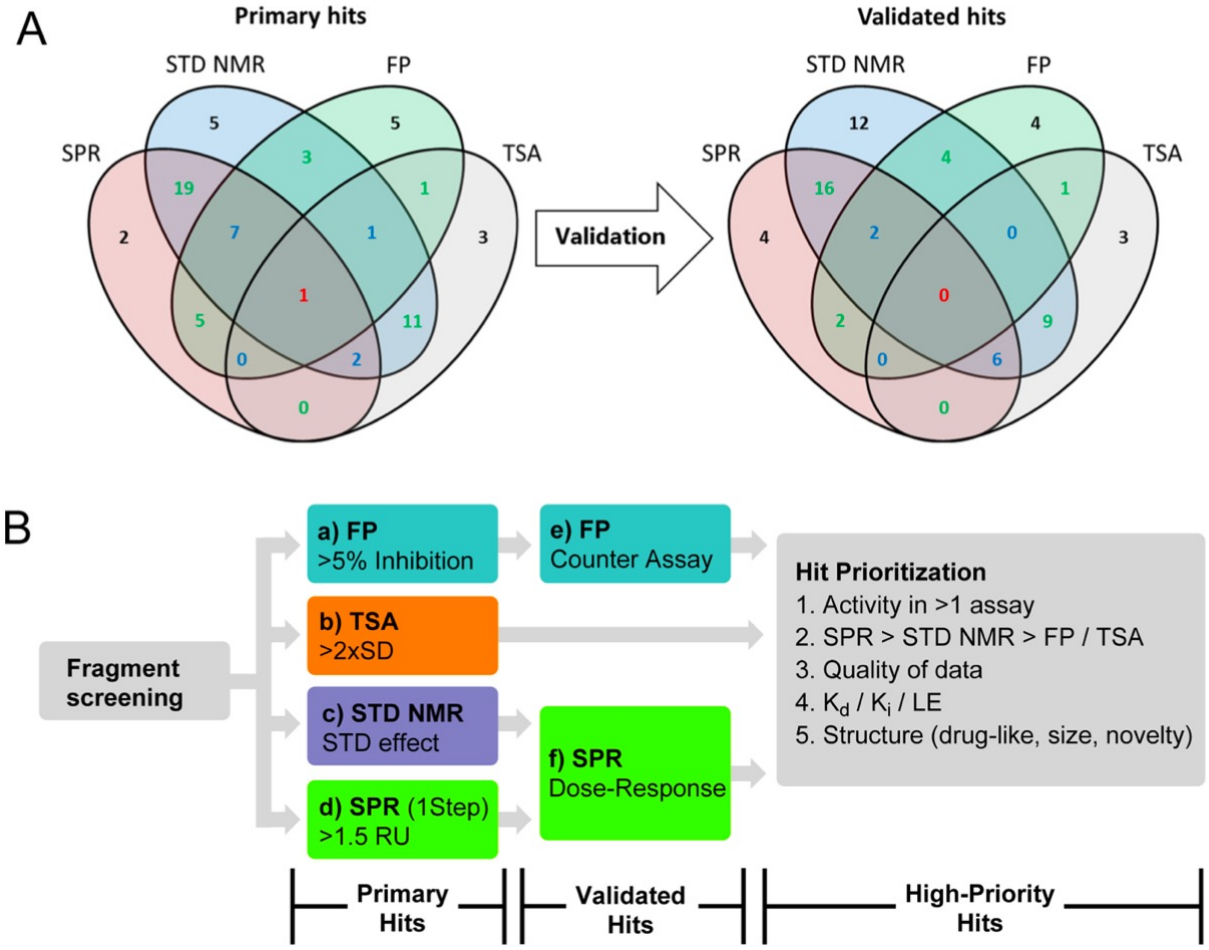
Abell, C.; Dagostin, C. RSC Drug Discov. Ser. 2015

LOW OVERLAP OF FRAGMENT HITS IN BIOPHYSICAL SCREENINGS



HIV-1 integrase core domain

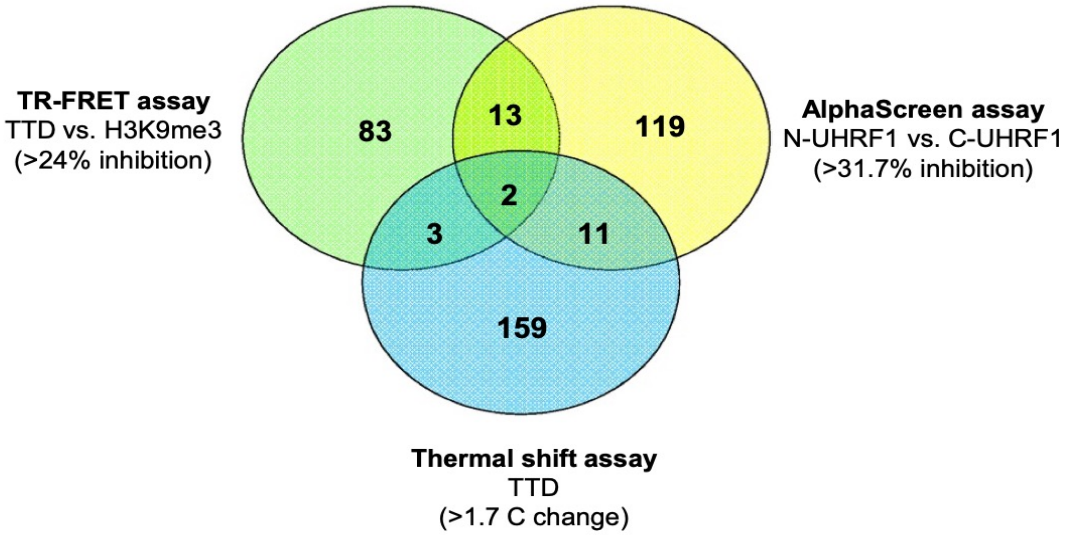
Wielens et al., 2013



Fragment-based deconstruction–reconstruction for KEAP1 – 77 frags

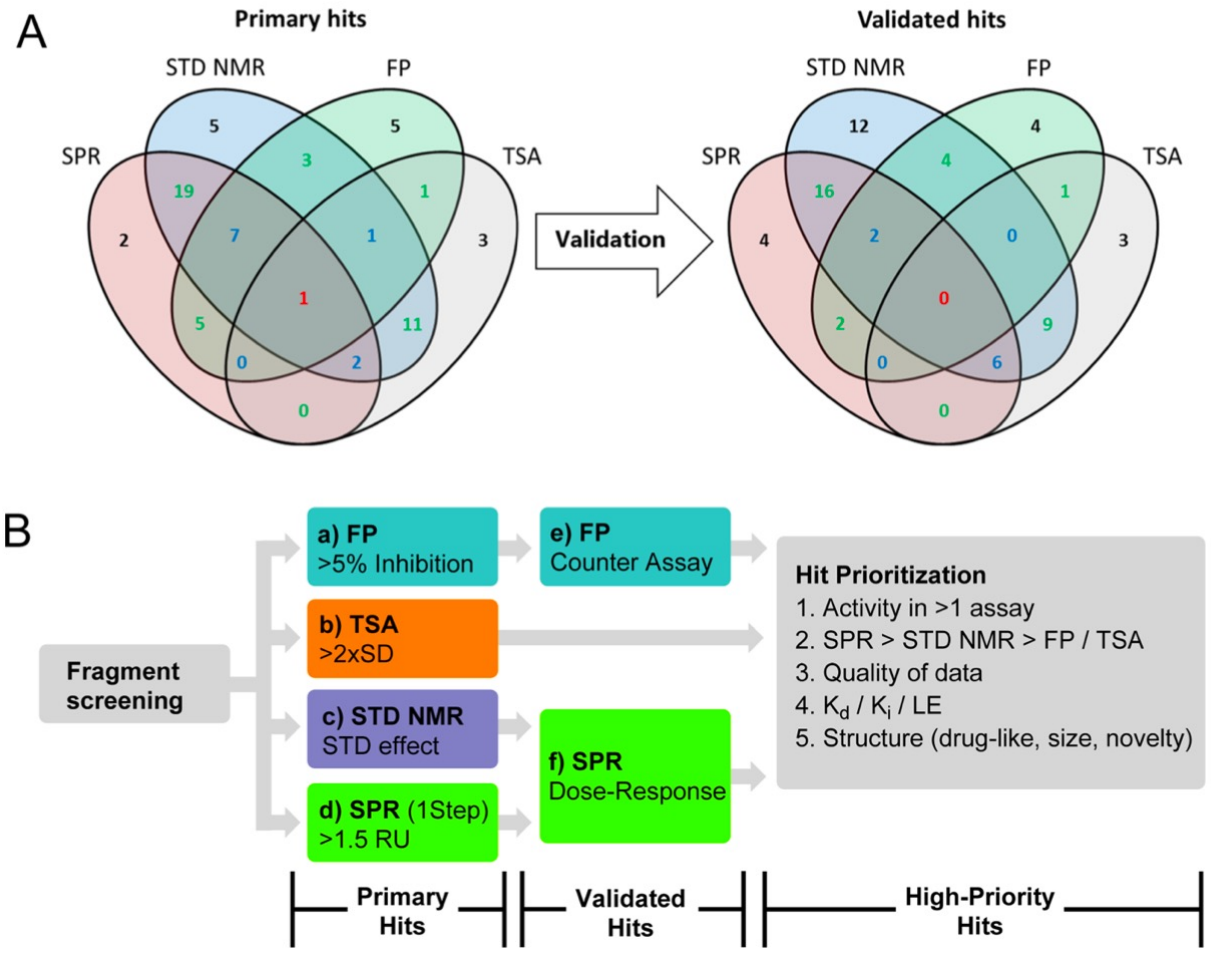
Pallesen et al., 2021

LOW OVERLAP OF FRAGMENT HITS IN BIOPHYSICAL SCREENINGS



Epigenetic factor UHRF1 – 2300 frag lib

Chang et al., 2021



Fragment-based deconstruction–reconstruction for KEAP1 – 77 frags

Pallesen et al., 2021

NOT START WITH CRYSTALLOGRAPHIC SCREENING IN FBDD?

CRYSTALS FIRST

Fragment library: 361 compounds

Protein: Endothiapepsin

Study: 6 biophysical assays + X-ray

71 X-ray hits

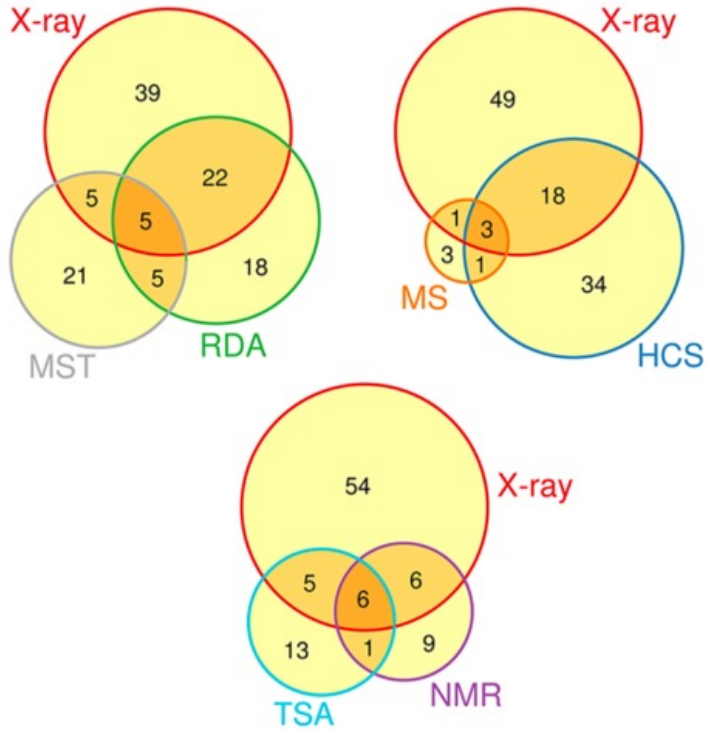
44 % (31) fragments only by X-ray

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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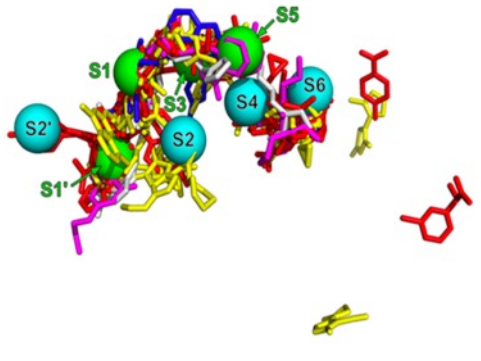
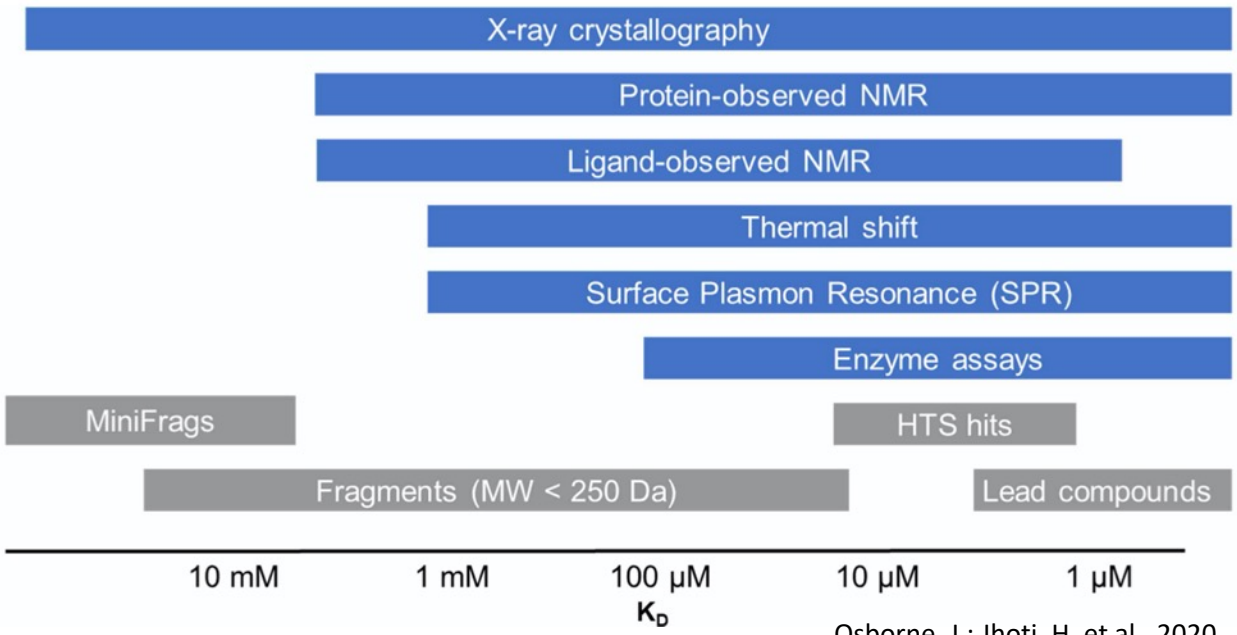
screening method or combination	fraction of X-ray binders among the hits of this method[b]	missed fraction of possible 71 structures[c]
HCS	21 out of 56 = 38%	70%
RDA	27 out of 50 = 54%	62%
STD-NMR	12 out of 22 = 55%	83%
ESI-MS	4 out of 8 = 50%	94%
TSA	11 out of 25 = 44%	85%
MST	10 out of 36 = 28%	86%
at least 1 method	40 out of 119 = 34%	44%
at least 2 methods	19 out of 41 = 46%	73%
at least 3 methods	13 out of 21 = 62%	82%
at least 4 methods	8 out of 10 = 80%	89%
at least 5 methods	5 out of 6 = 83%	93%
all 6 methods	0 out of 0 = 0%	100%



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

WHY PUT STRUCTURAL DATA FIRST?

CRYSTALS FIRST



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

Crystallographic fragment screening using SmartSoak®

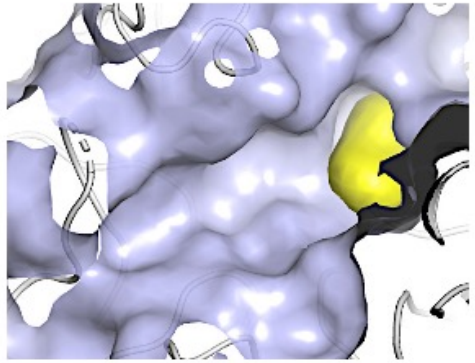
- ✓ sensitive screening method delivering binding modalities *ad hoc.*
- ✓ guidelines for further prosecution of identified hits & structurally-enabled lead design.
- ✓ access to novel chemical and IP space.
- ✓ SBDD from the beginning.

WHY PUT STRUCTURAL DATA FIRST?

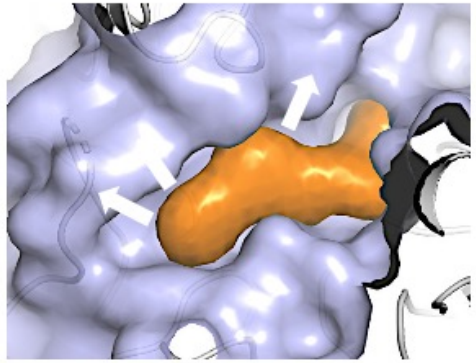
CRYSTALS FIRST

	HTRF
Fragment bound X-ray structure	IC_{50} > 1 mM
Rational design to explore SAR	IC_{50} < 30 µM
In silico library evaluation and synthesis	IC_{50} < 5 µM
3x	
Rational design to optimize library hits	IC_{50} = < 0.1 µM MW <400; clogP 0.7

Total # of virtual compounds evaluated	40K
Total # of crystal structures	18
Total # of compounds made	195



X-ray with Fragment



X-ray with Optimized Ligand

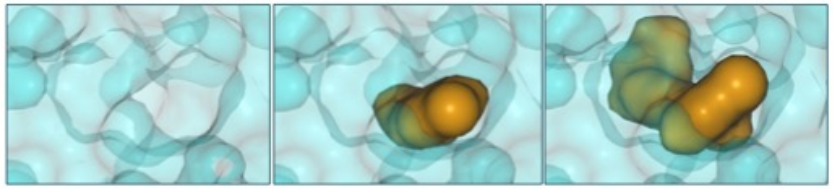
- Successfully applied SBDD to rapidly identify diverse E3 ligase ligands
- Multiple exit vectors identified and confirmed via chemistry, molecular modeling and X-ray
- Degraders synthesized for BRD4 + additional Kymera targets including STAT3 and IRAK4

KYMERA

https://www.kymeratx.com/wp-content/uploads/2021/05/E3-ligase-drug-targeting-May-26-2021-FINAL.pdf

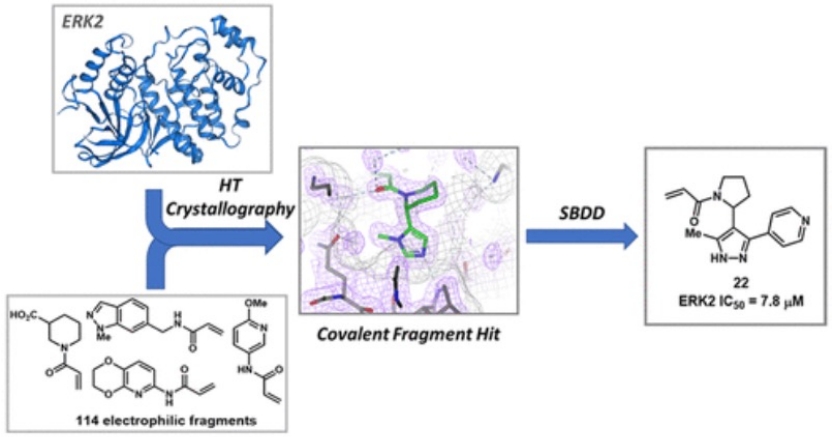
WHY PUT STRUCTURAL DATA FIRST?

CRYSTALS FIRST



Apo Tunnel Site	Fragment	Early Lead
SHP2 ITC K_D/µM	1000	1.1
Ligand Efficiency	0.34	0.39

- Isothermal titration calorimetry (ITC) used to characterise affinity of weakly binding compounds
- Structure-guided growing of fragment hit into adjacent pockets gave an Early Lead with ~ 1000-fold increase in affinity



Screening for covalent fragments

astex®

https://astx.com/2020-aacr-fragment-based-drug-discovery-to-identify-small-molecule-allosteric-inhibitors-of-shp2/
Denis et al. 2022, J.Med.Chem.

WHY PUT STRUCTURAL DATA FIRST?

BOEHRINGER INGELHEIM PUTS “X-RAY FIRST”

CRYSTALS 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]

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

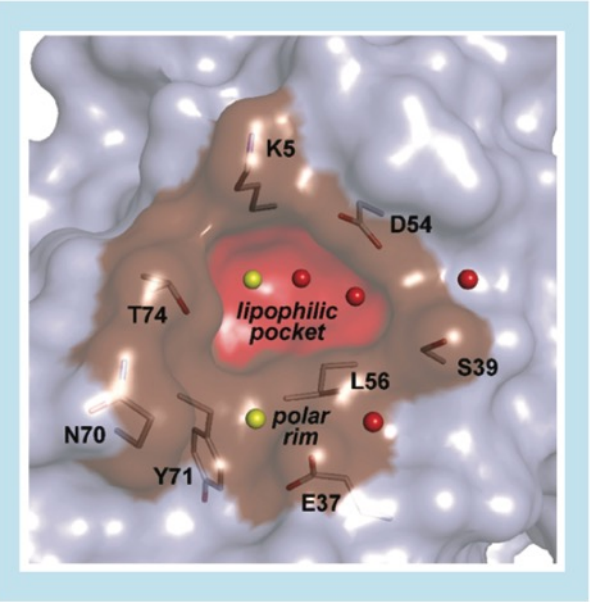
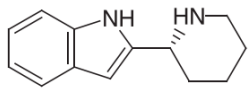
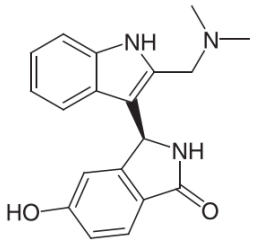


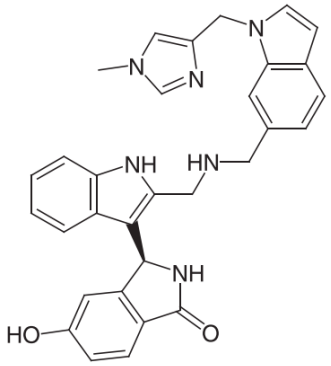
Figure 3. The lipophilic hot spot of switch I/II.
SI/II-pocket with the relevant crystallographic water molecules and amino acids in the small lipophilic pocket and the shallow polar rim surrounding the small cavity.



Compound **13**
NMR K_D $KRAS^{G12D}_{On}$ = 1.9 M



Compound **18**
ITC K_D $KRAS^{G12D}_{On}$ = 22 µM



BI-2852
ITC K_D $KRAS^{G12D}_{On}$ = 740 nM

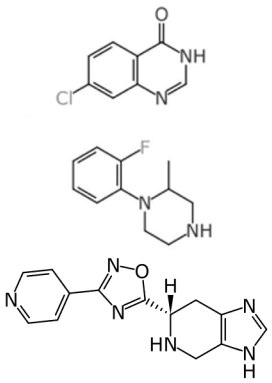
x-ray crystal structures elucidating in detail how ligands bind to the SI/II-pocket in KRAS, NRAS and HRAS in both the on and off states. The establishment of robust cocrystallization systems [26] and high throughput soaking systems [29] has allowed us to generate a high coverage of relevant RAS crystal 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.

DETERMINATION OF PROTEIN-LIGAND COMPLEXES

CO-CRYSTALLIZATION VS. SOAKING

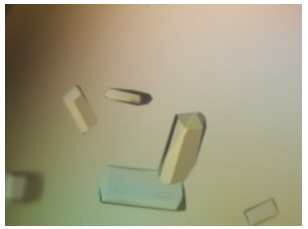
CRYSTALS FIRST

COMPOUNDS



CO-CRYSTALLIZATION

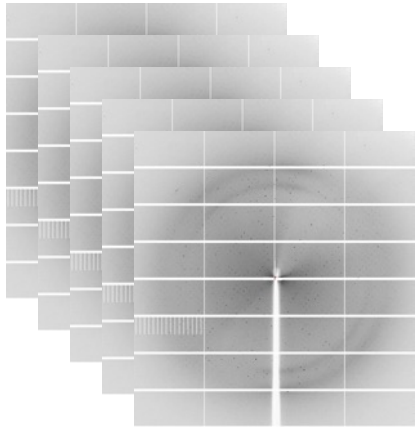
Compound is added to the protein during crystallization setup and the pre-formed protein-ligand complex is crystallized.



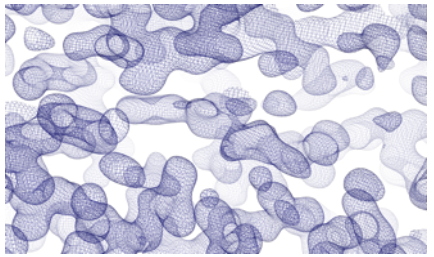
SOAKING

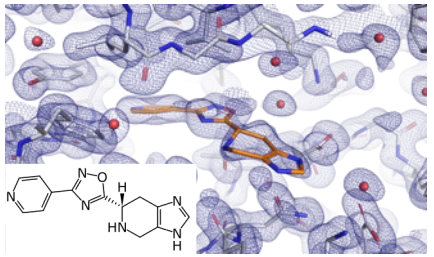
Soaking an apo-crystal in a ligand solution after the crystallisation has taken place.

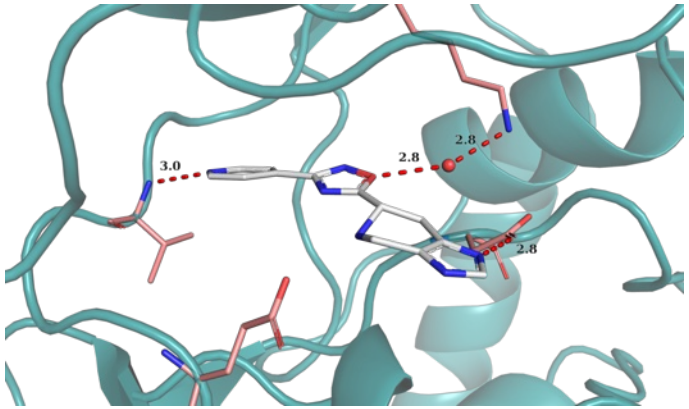
DATA COLLECTION



PROCESSING & REFINEMENT



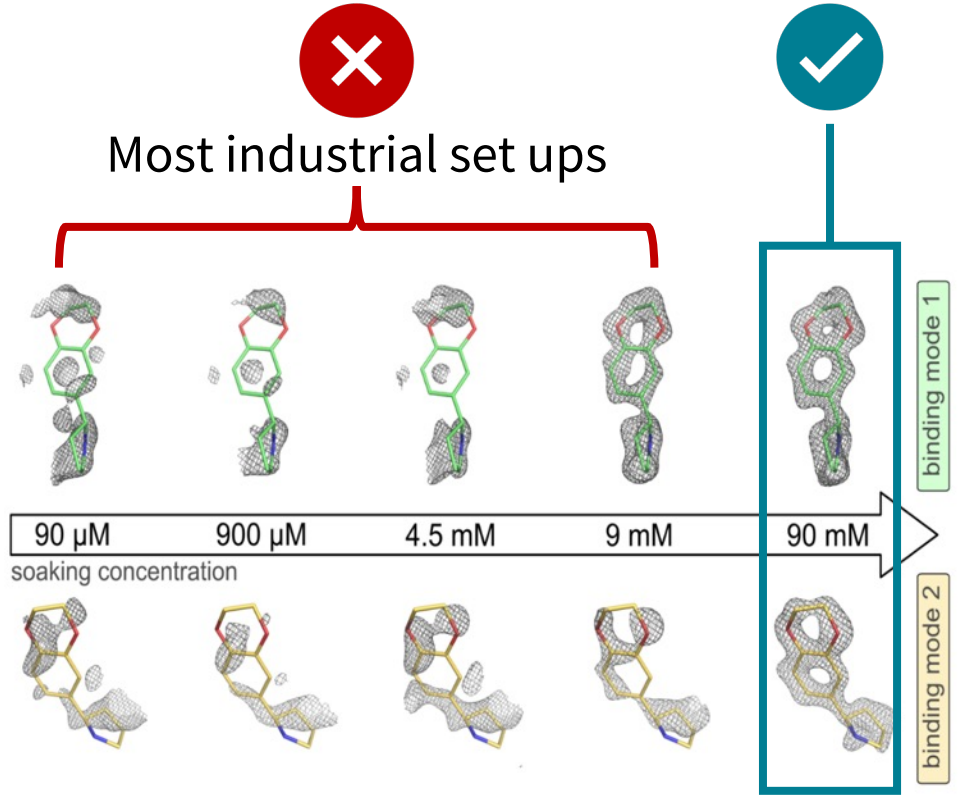




SMARTSOAK® - High-performance soaking systems

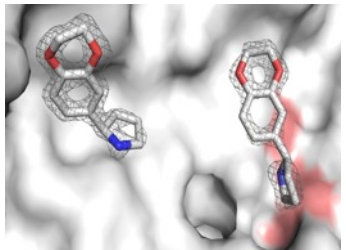
CRYSTALS FIRST

- 24 h instead on weeks/months
- does not require additional trial & error optimizations soaking conditions.
- overcomes solubility problems of fragments.
- standard concentrations are 100 mM.
- enables fragment concentrations up to 250 mM.
- enables long soaking times up to 24 hours.



soaking concentration

two copies bound



THE MAGNET FOR THE NEEDLE

CRYSTALS FIRST

Journal of Medicinal Chemistry

pubs.acs.org/jmc Article

Magnet for the Needle in Haystack: "Crystal Structure First" Fragment Hits Unlock Active Chemical Matter Using Targeted Exploration of Vast Chemical Spaces

Janis Müller,[▽] Raphael Klein,[▽] Olga Tarkhanova, Anastasiia Gryniukova, Petro Borysko, Stefan Merkl, Moritz Ruf, Alexander Neumann, Marcus Gastreich, Yurii S. Moroz, Gerhard Klebe, and Serghei Glinca*

Cite This: https://doi.org/10.1021/acs.jmedchem.2c00813 Read Online

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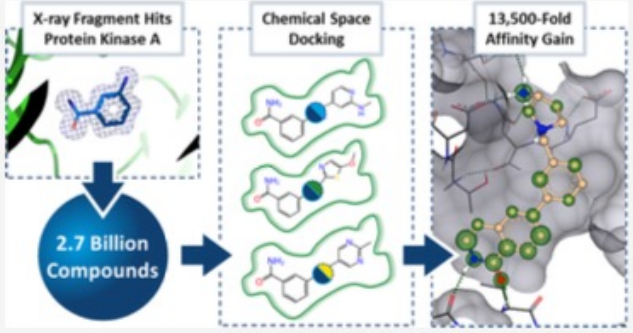
ABSTRACT: Fragment-based drug discovery (FBDD) has successfully led to approved therapeutics for challenging and "undruggable" targets. In the context of FBDD, we introduce a novel, multidisciplinary method to identify active molecules from purchasable chemical space. Starting from four small-molecule fragment complexes of protein kinase A (PKA), a template-based docking screen using Enamine's multibillion REAL Space was performed. A total of 93 molecules out of 106 selected compounds were successfully synthesized. Forty compounds were active in at least one validation assay with the most active follow-up having a 13,500-fold gain in affinity. Crystal structures for six of the most promising binders were rapidly obtained, verifying the binding mode. The overall success rate for this novel fragment-to-hit approach was 40%, accomplished in only 9 weeks. The results challenge the established fragment prescreening paradigm since the standard industrial filters for fragment hit identification in a thermal shift assay would have missed the initial fragments.



IN THE PIPELINE

Derek Lowe's commentary on drug discovery and the pharma industry. An editorially independent blog, all content is Derek's own, and he does not in any way speak for his employer.

FILTERS

Crystal Fragments

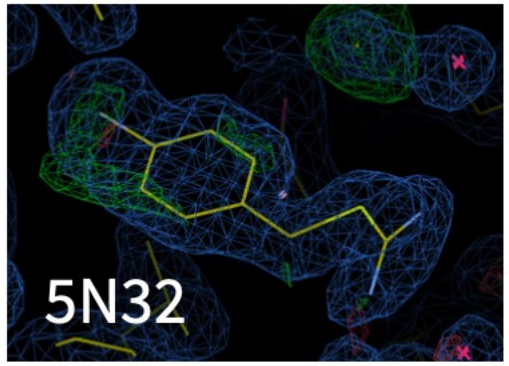
BY DEREK LOWE | 22 SEP 2022

I started doing a good deal of fragment-based drug discovery ten or fifteen years ago, and I still have a lot of respect for the technique. For those outside the business, the idea behind FBDD is to not start off with a big screen of drug-sized molecules that you might have in your general screen collection or various focused libraries, but rather to do a smaller screen (generally in th...

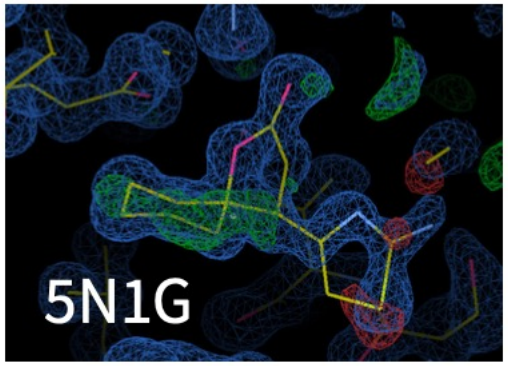
Philipps Universität Marburg BioSolveIT CHEMSPACE Enamine

”CRYSTALLOGRAPHIC LIGAND CONFIDENCE“

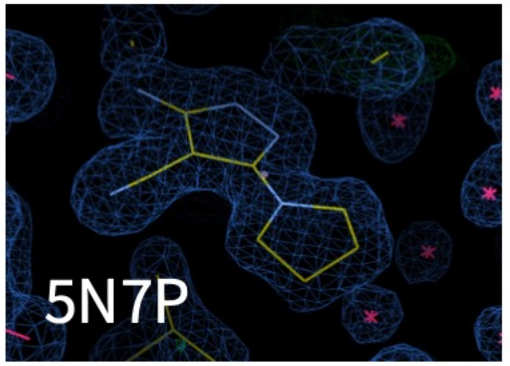
CRYSTALS FIRST



Too high B-factors
Ligand disordered
Not selected



Ligand not fully resolved
Not a hinge binder
Not selected

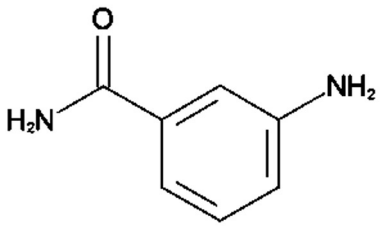


Ligand fully resolved
Optimal fit of e-density
Selected!

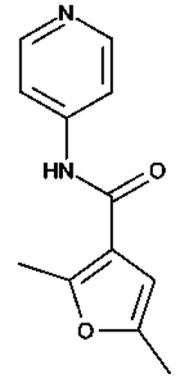
- high occupancy
- well- defined electron density
- optimal fit of the model for both the ligand and the binding pocket
- minimal ligand disorder

SELECTED FRAGMENTS FOR CHEMICAL SPACE DOCKING

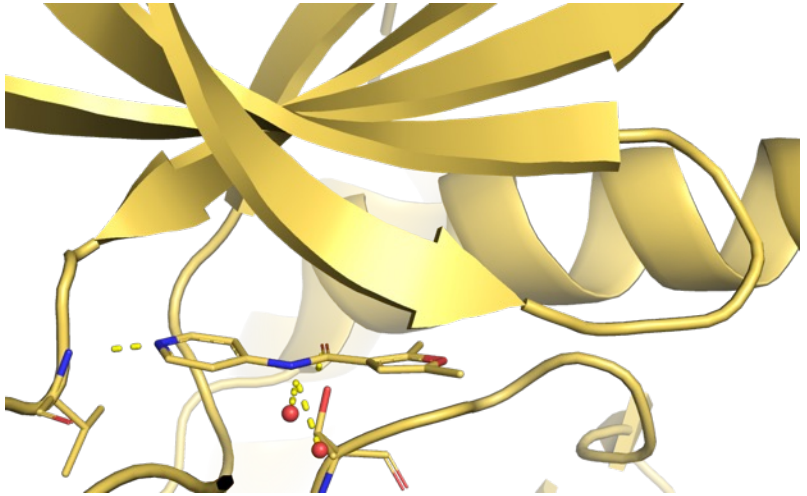
CRYSTALS FIRST

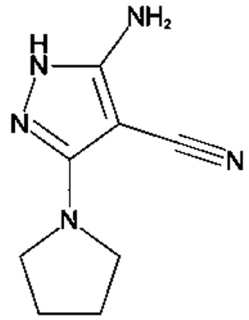


5N3Q

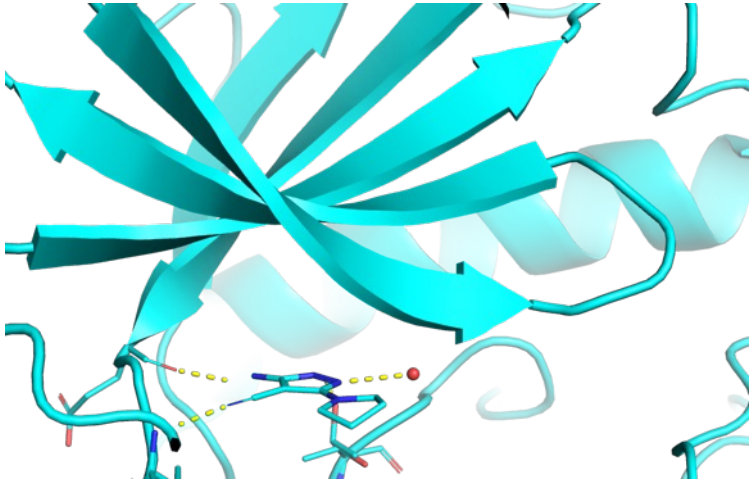


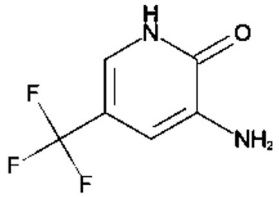
5N1L



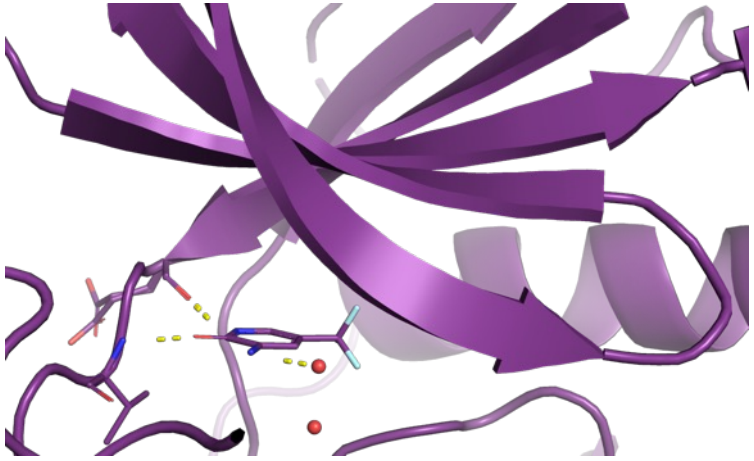


5N7P

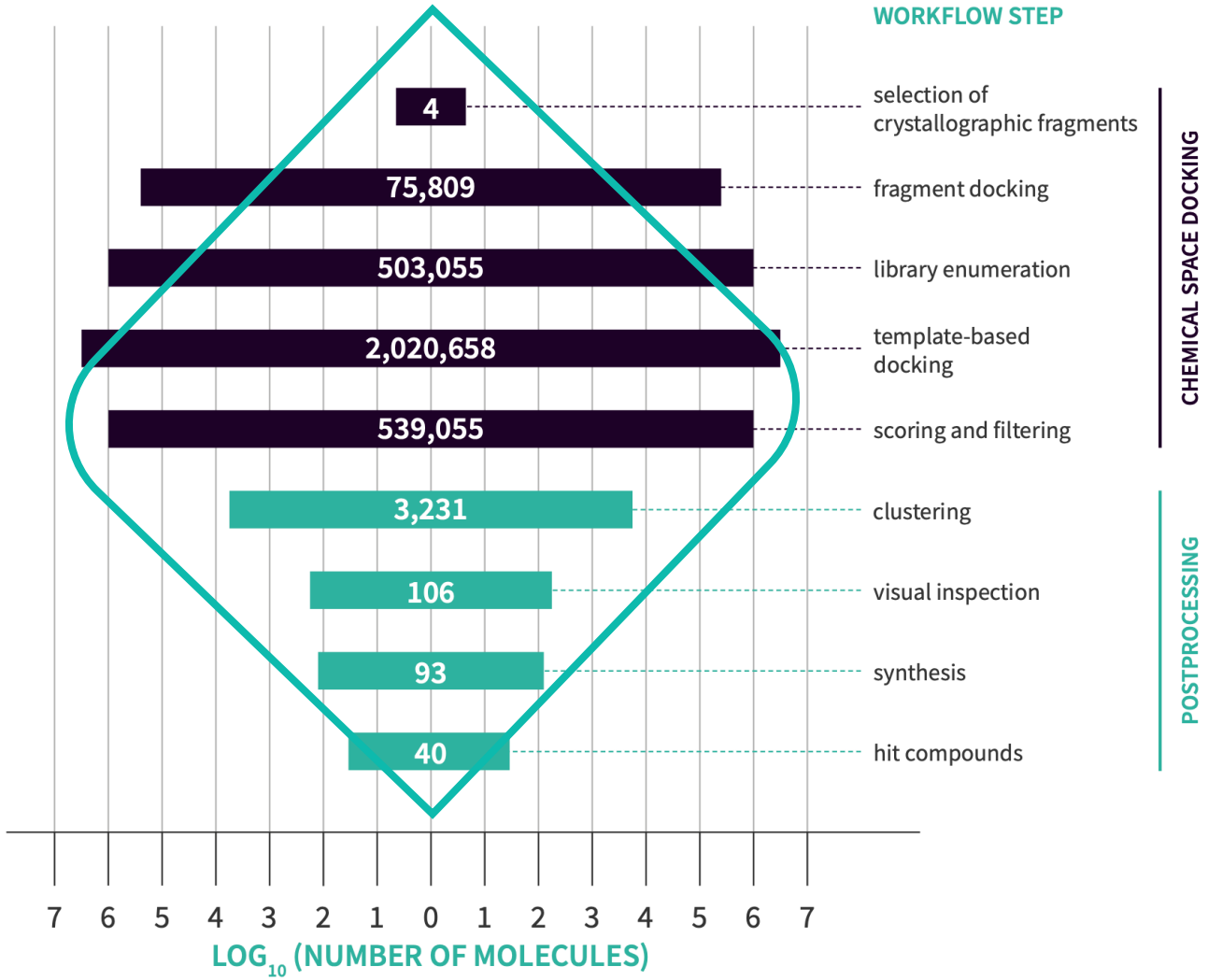




5N33



CHEMICAL SPACE DOCKING

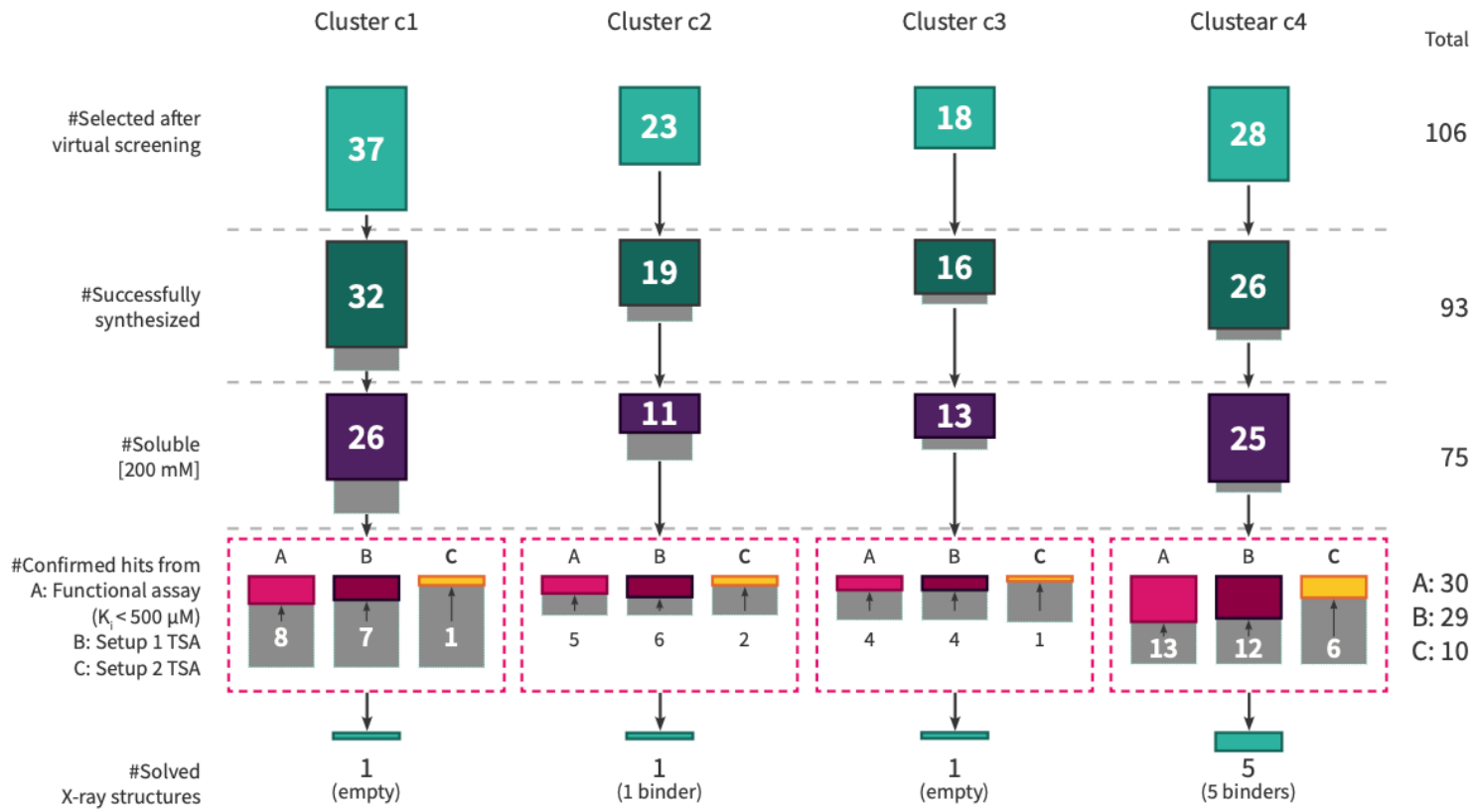


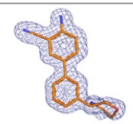
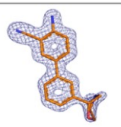
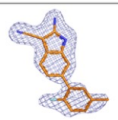
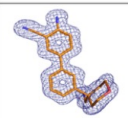
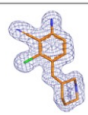
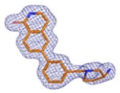
two-component reaction
Enamine REAL Space
resulting in 2.6 billion virtual
product molecules

No affinity data required,
only co-structures

FROM CO-STRUCTURE TO 40 ACTIVES nM-µM IN 9 WEEKS

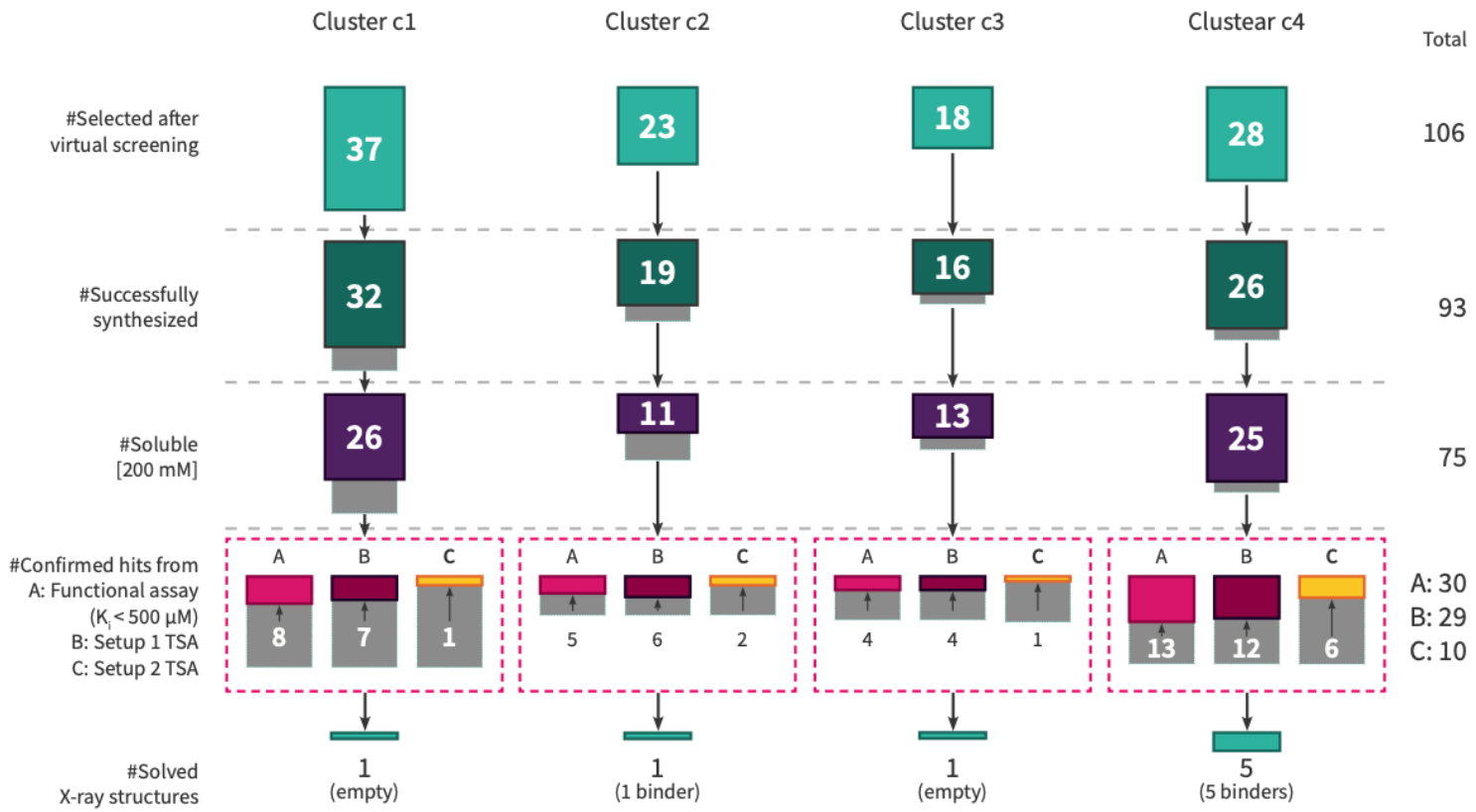
CRYSTALS FIRST

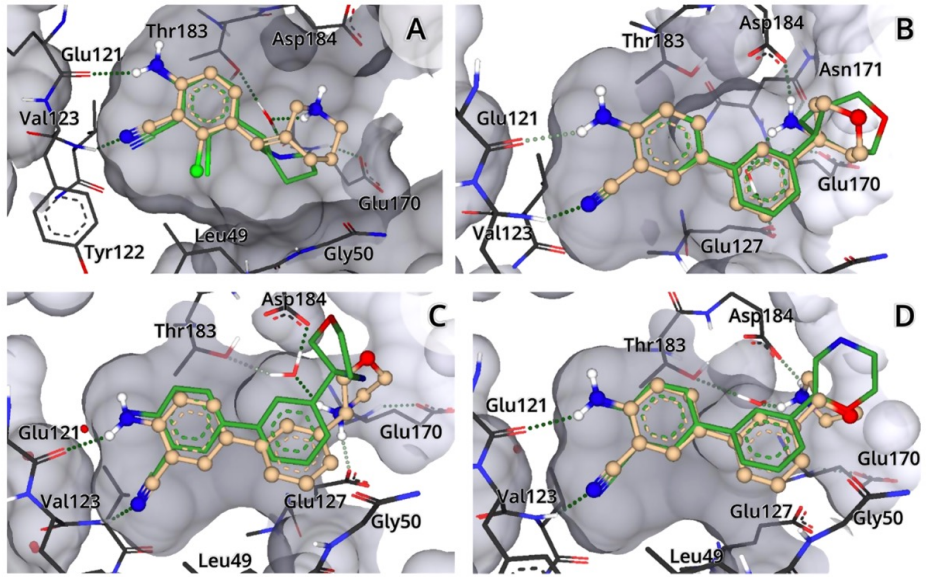


ID	EN060	EN068	EN081	EN086	EN088	EN093
PDB-ID	7PID	7PIE	7PNS	7PIF	7PIG	7PIH
Resolution	1.49 Å	1.43 Å	1.85 Å	1.39 Å	1.55 Å	1.37 Å
						

FROM CO-STRUCTURE TO 40 ACTIVES nM-µM IN 9 WEEKS

CRYSTALS FIRST





Binding mode preservation e.g. c4 docking vs. X-ray

STRUCTURE-GUIDED FRAGMENT EVOLUTION USING CHEMICAL SPACES

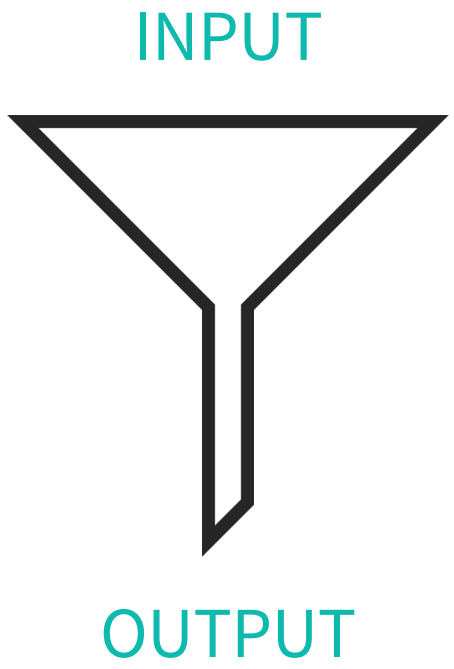
CRYSTALS FIRST

- Starting points: crystallographic fragment hits
- High ”ligand confidence” and the ”magnet”
- No prioritization based on affinity
- Fragment-to-hit success ~40 %
  - Entropy vs. enthalpy
- Only 9 weeks
- From mM to low µM and even nM in one cycle
  - 13,500-fold gain in affinity

THE INDUSTRY‘S CHALLENGE: THE NEEDLE IN THE HAYSTACK

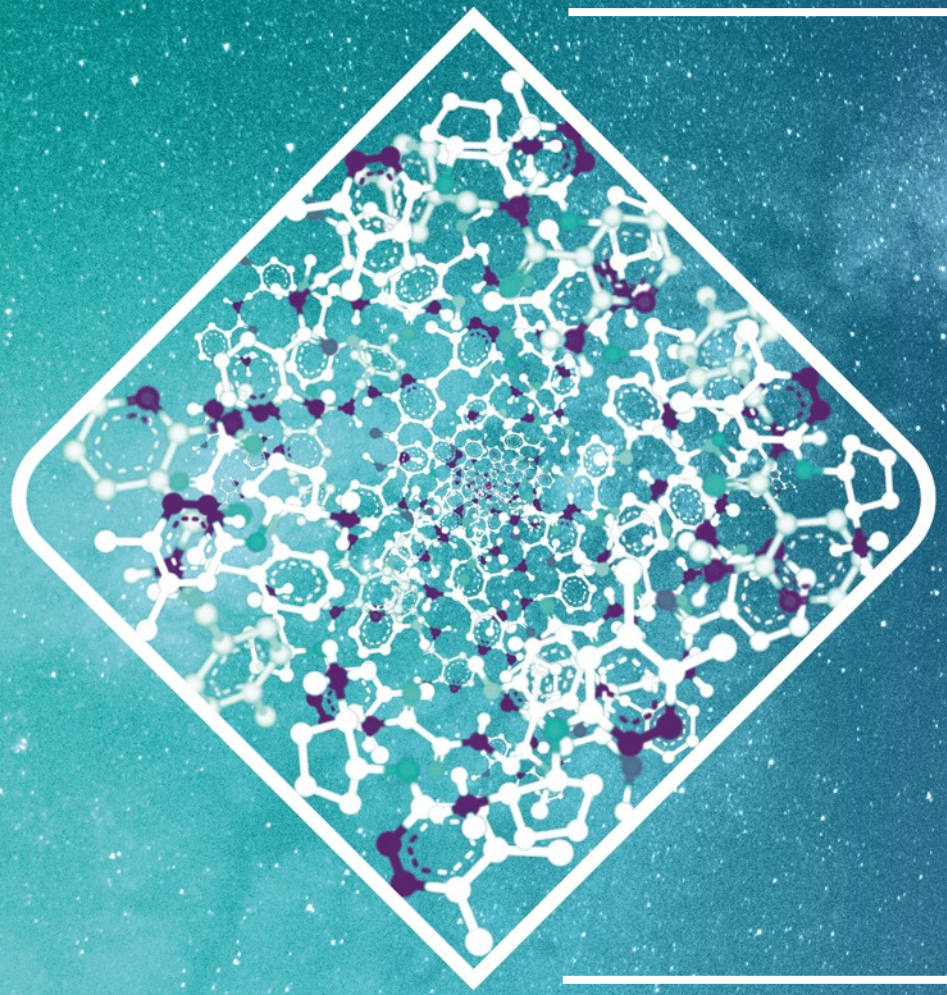
CRYSTALS FIRST

- Difficult targets
- Screening technologies
  - DELs, FBDD, MS, HTS, ML/AI
- Structural enablement for SBDD
- Non-covalent vs. covalent mode of action
- Bifunctional molecules
- Molecular glues



INCREASING COMPLEXITY FOR THE TYPICAL SCREENING SETUP

THE MAGNET PLATFORM



Structure First Approach

FragAI

Synthesized hits

PROPRIETARY TECH
SmartSoak®
FastForward
FragAI

COMPLEMENTARY APPROACHES
NMR
DEL screening
LC-MS

CRYSTALS FIRST

TURNING INSIGHTS INTO MEDICINES

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