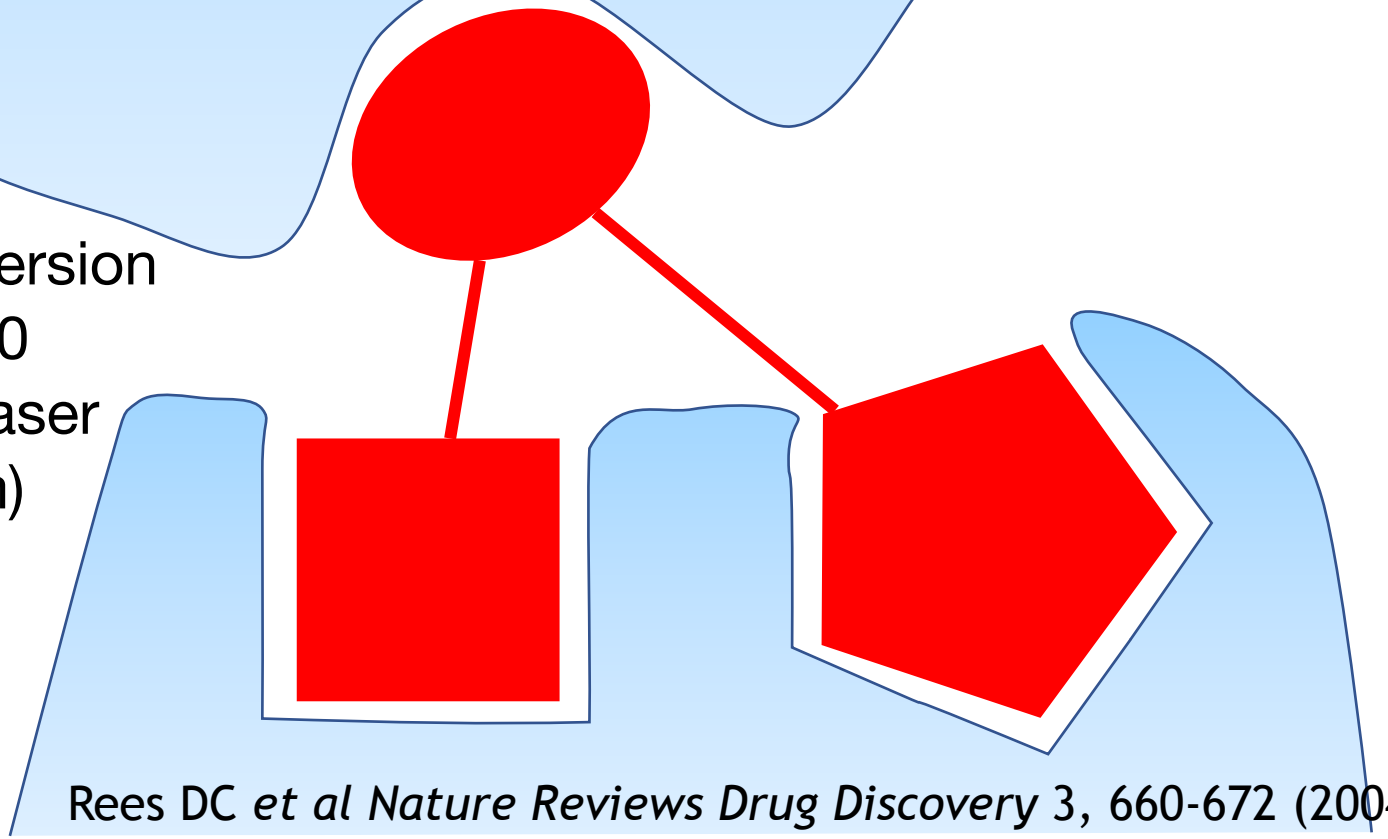
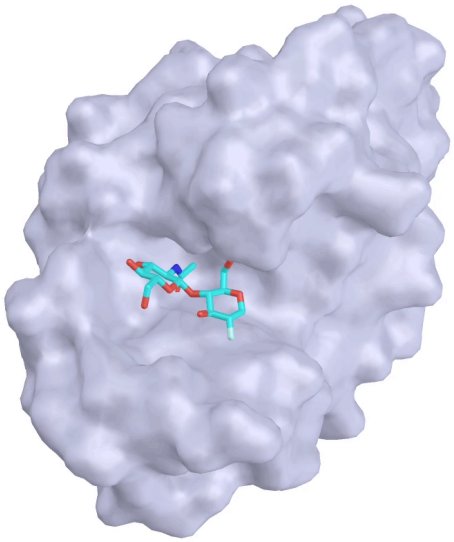
Fragments and crystallography

Inquiry Immersion
2019-20
James Fraser
(he/him)



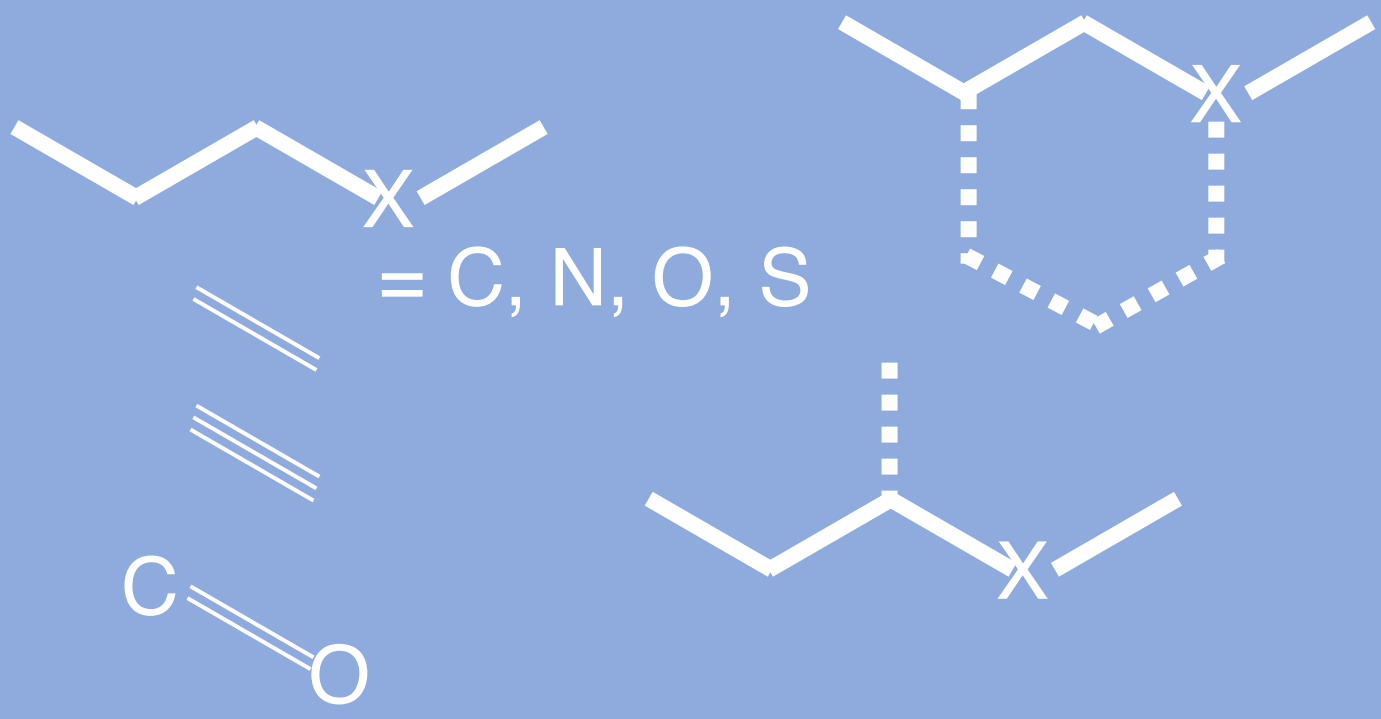
Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Lysozyme, the first enzyme with a structure determined by X-ray crystallography



Chemical space

Possible compounds with <600 Da



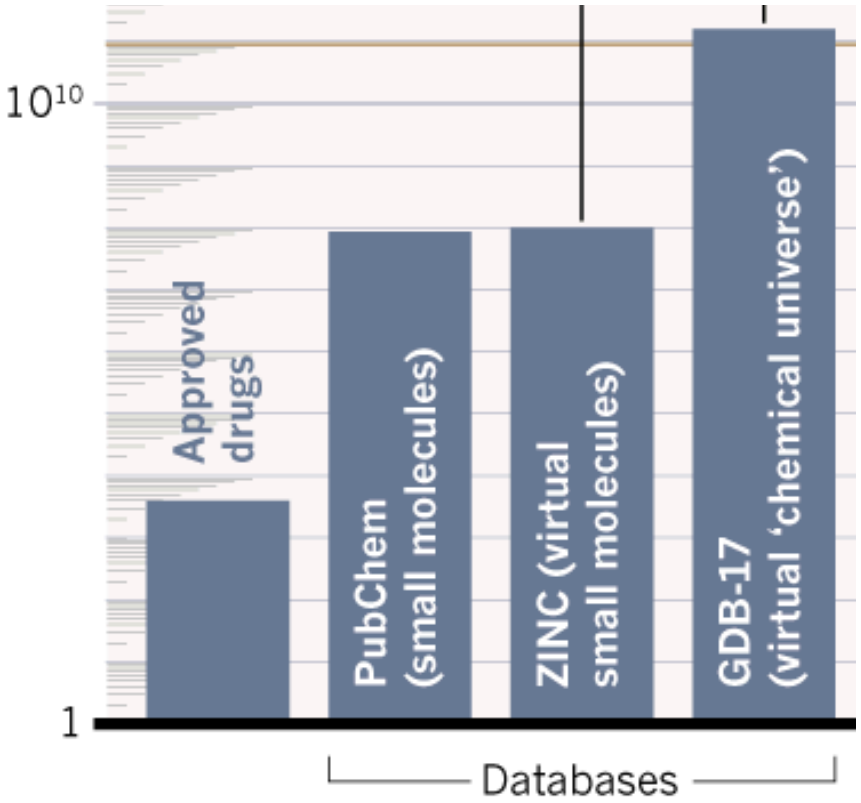
$\sim 10^{63}$

Bohacek RS *et al Molecular Research Reviews* 1,3-50 (1996)

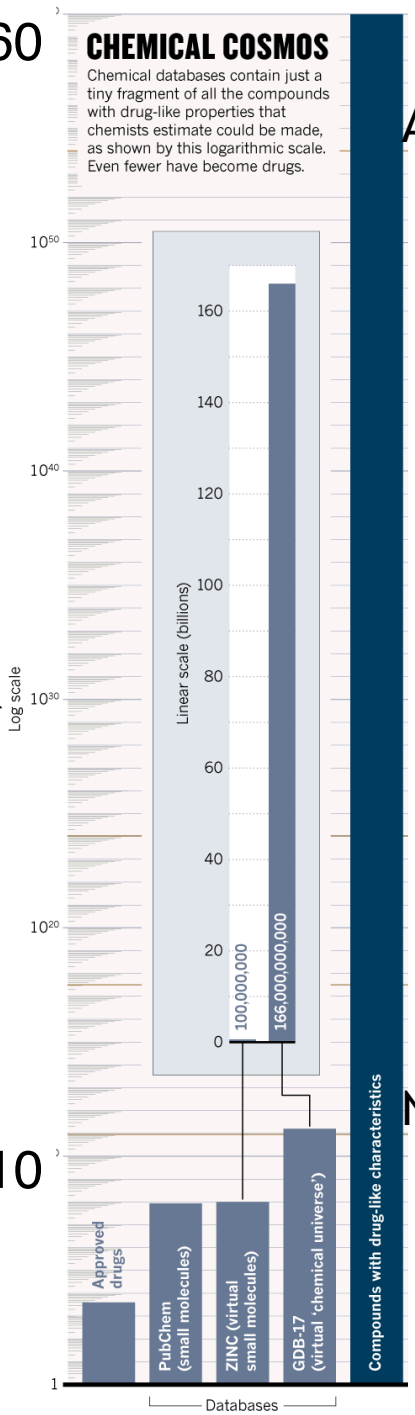
Chemical space is huge!

Mullard A *Nature* 549,445 (2017)

Chemical space is huge!



Mullard A *Nature* 549,445 (2017)



10^{60}

Atoms in the solar system

Log scale!

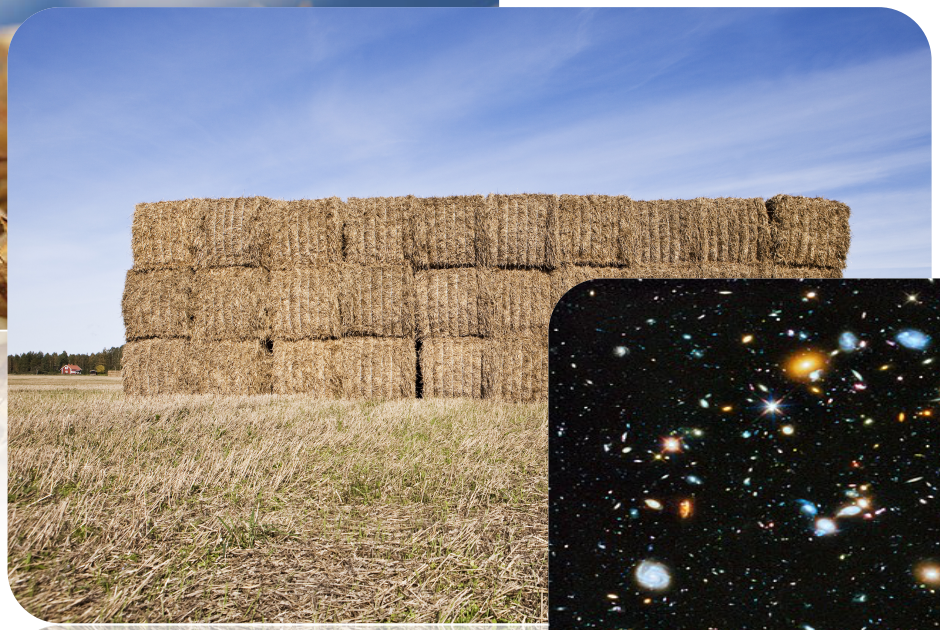
Stars in the universe

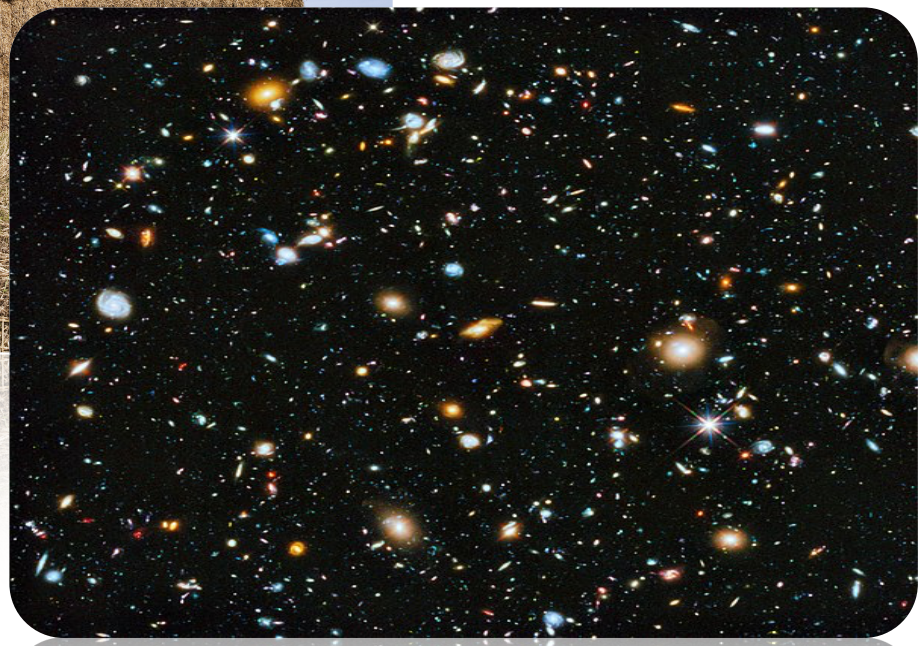
Neurons in the human brain

10^{10}

Mullard A *Nature* 549,445 (2017

Needles in enormous haystacks





Finding that rare needle…

High throughput screening

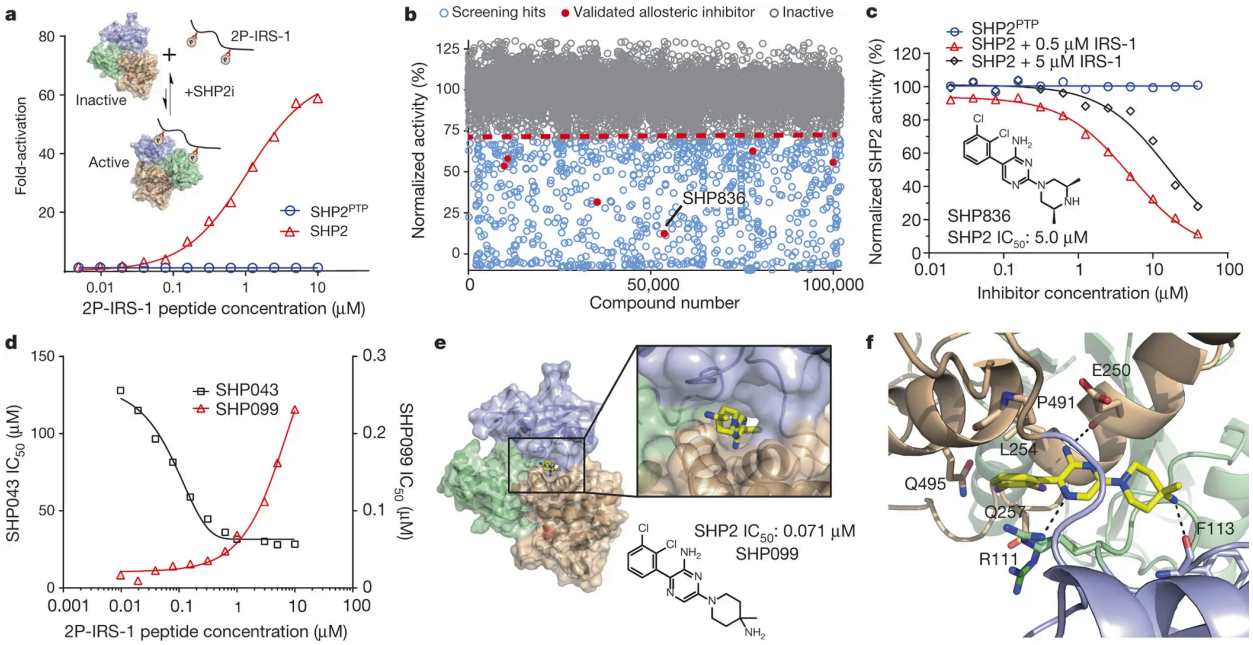
Library
30 heavy atoms
~10^6

Hit

Lead

Candidate

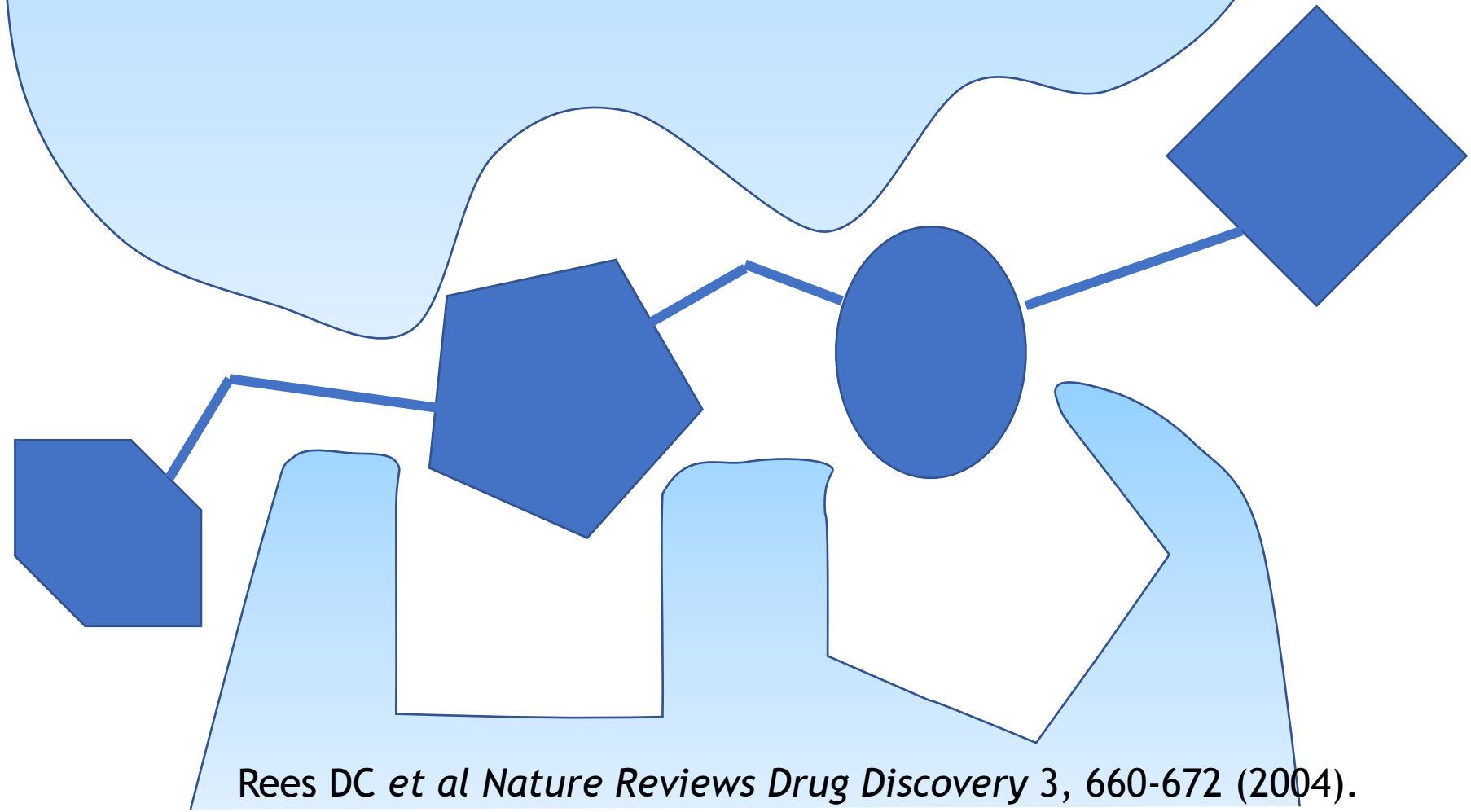
100,000 molecules screened 3 followup assays



Contrast with active site inhibitor

SHP836 - is a published ion channel inhibitor!

Drug discovery – HTS hit



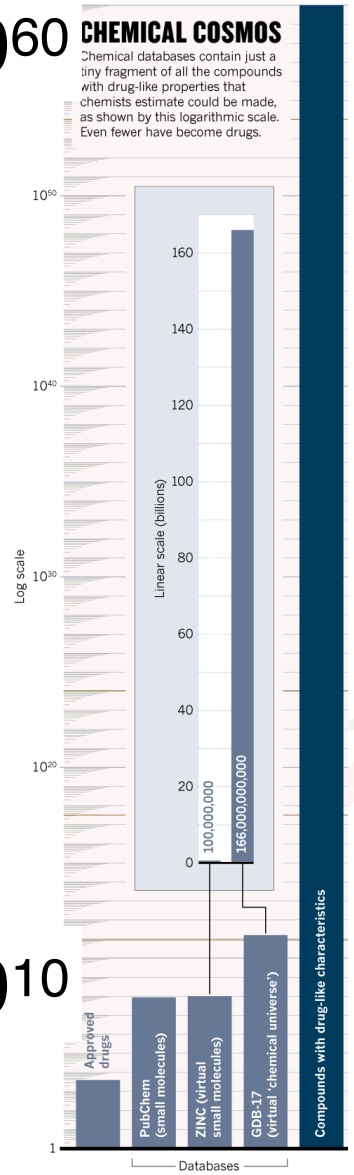
Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

High throughput screening

Library
30 heavy atoms
~10^6

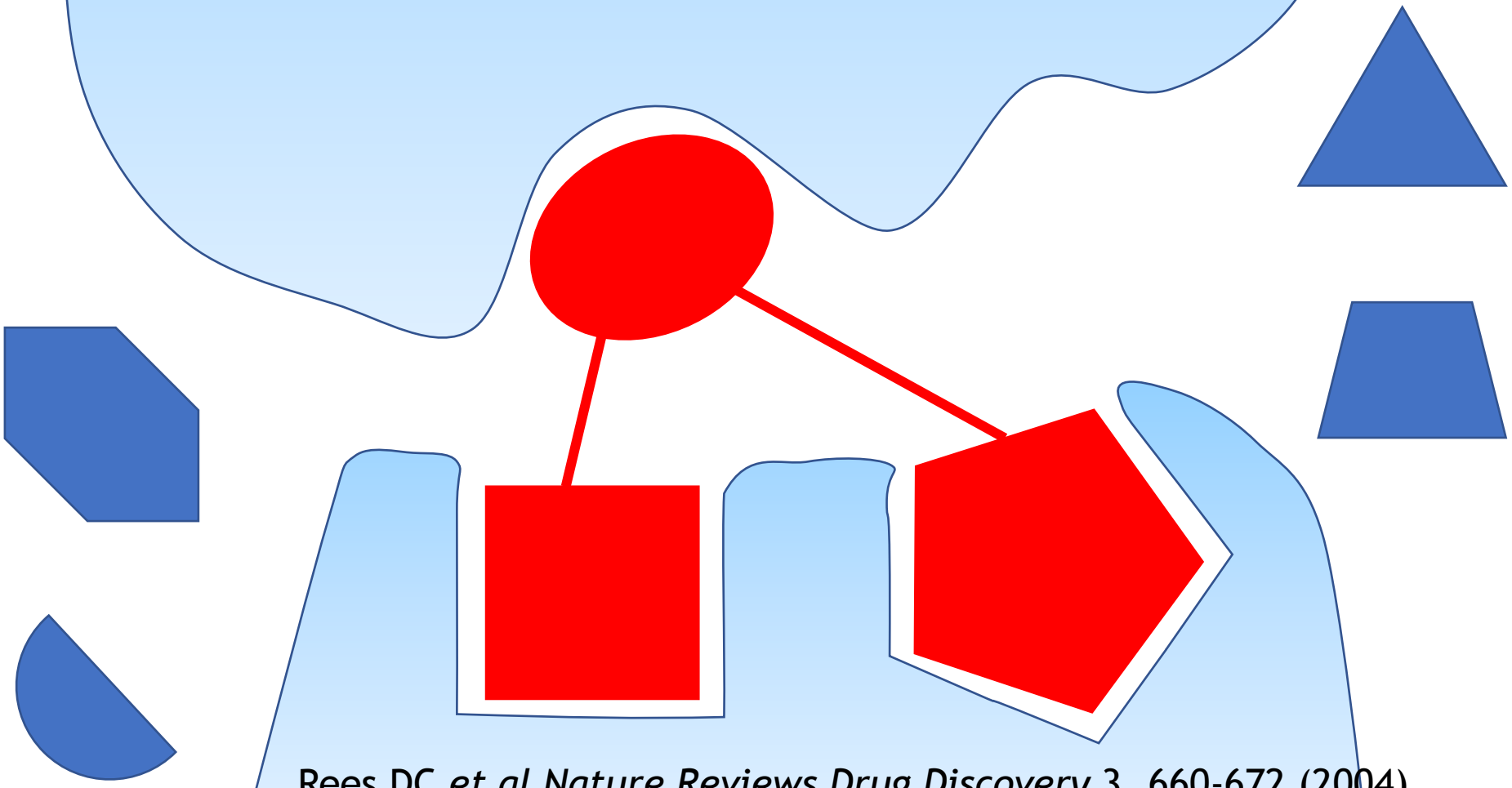
10^{60}

Vast undersampling!



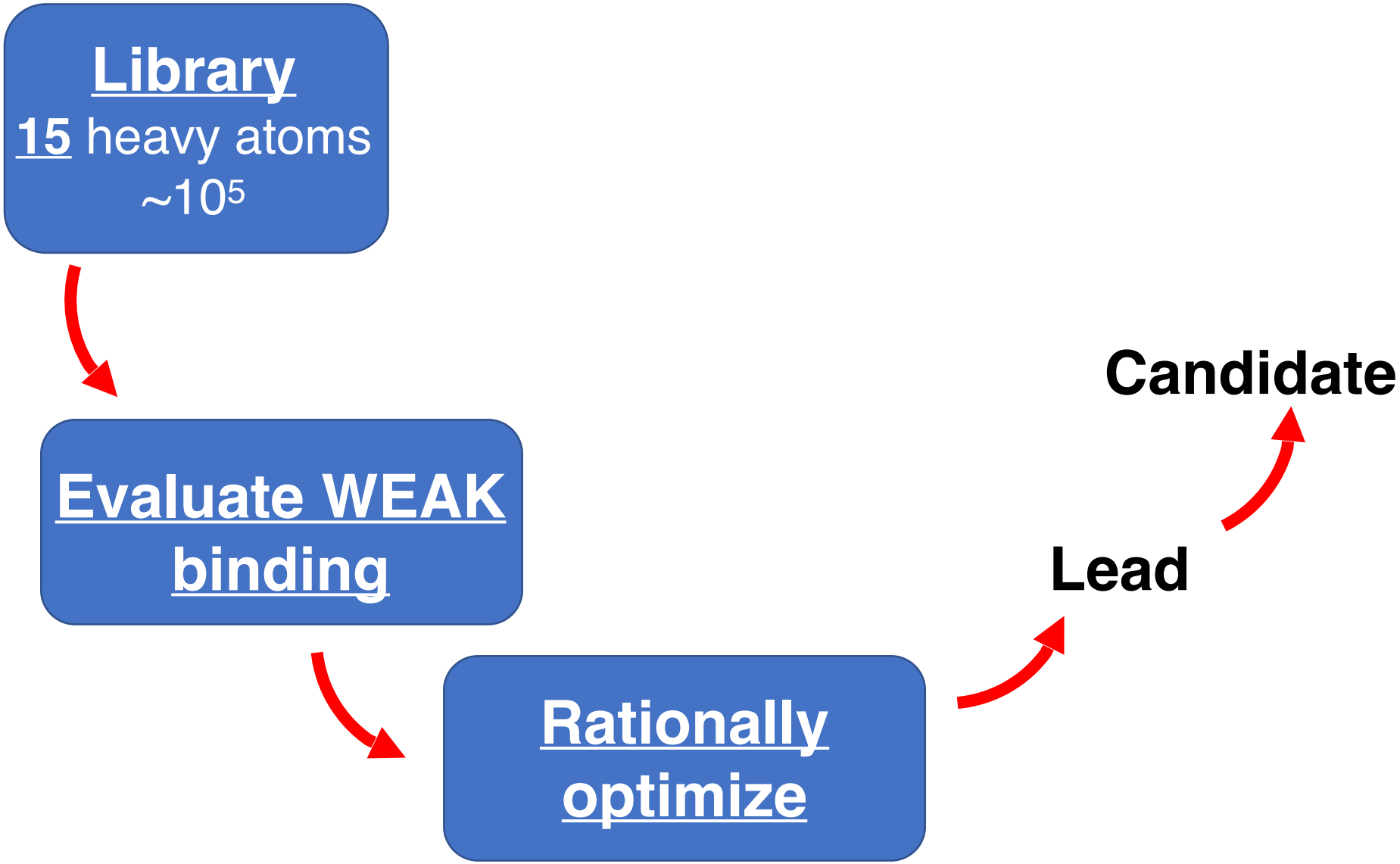
10^{10}

Fragment based drug discovery



Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Fragment based drug discovery

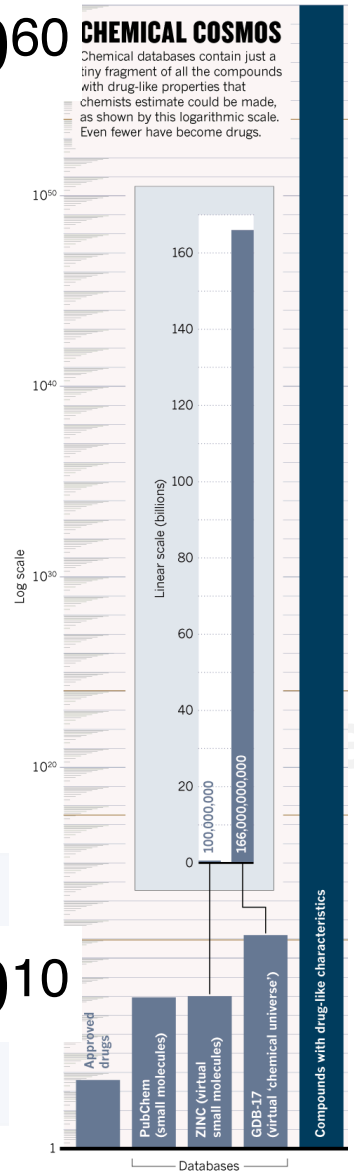


Fragment based drug discovery

Library
15 heavy atoms
~10^5

Less undersampling

10^{60}



10^{10}

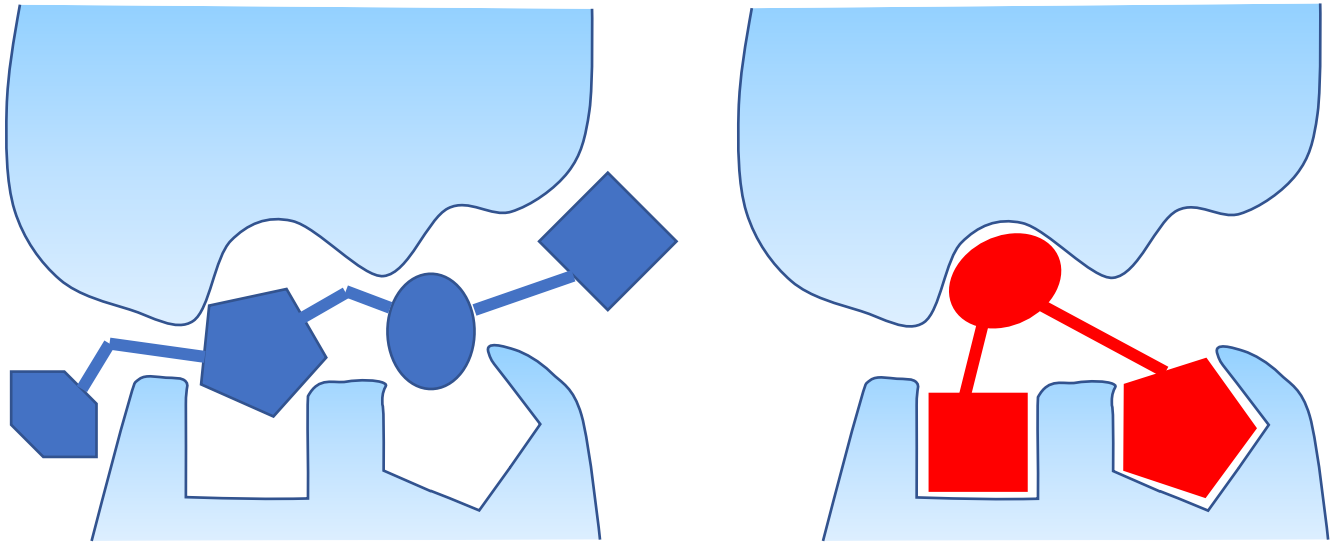
Evaluate WEAK binding

Rational optimize

Candidate

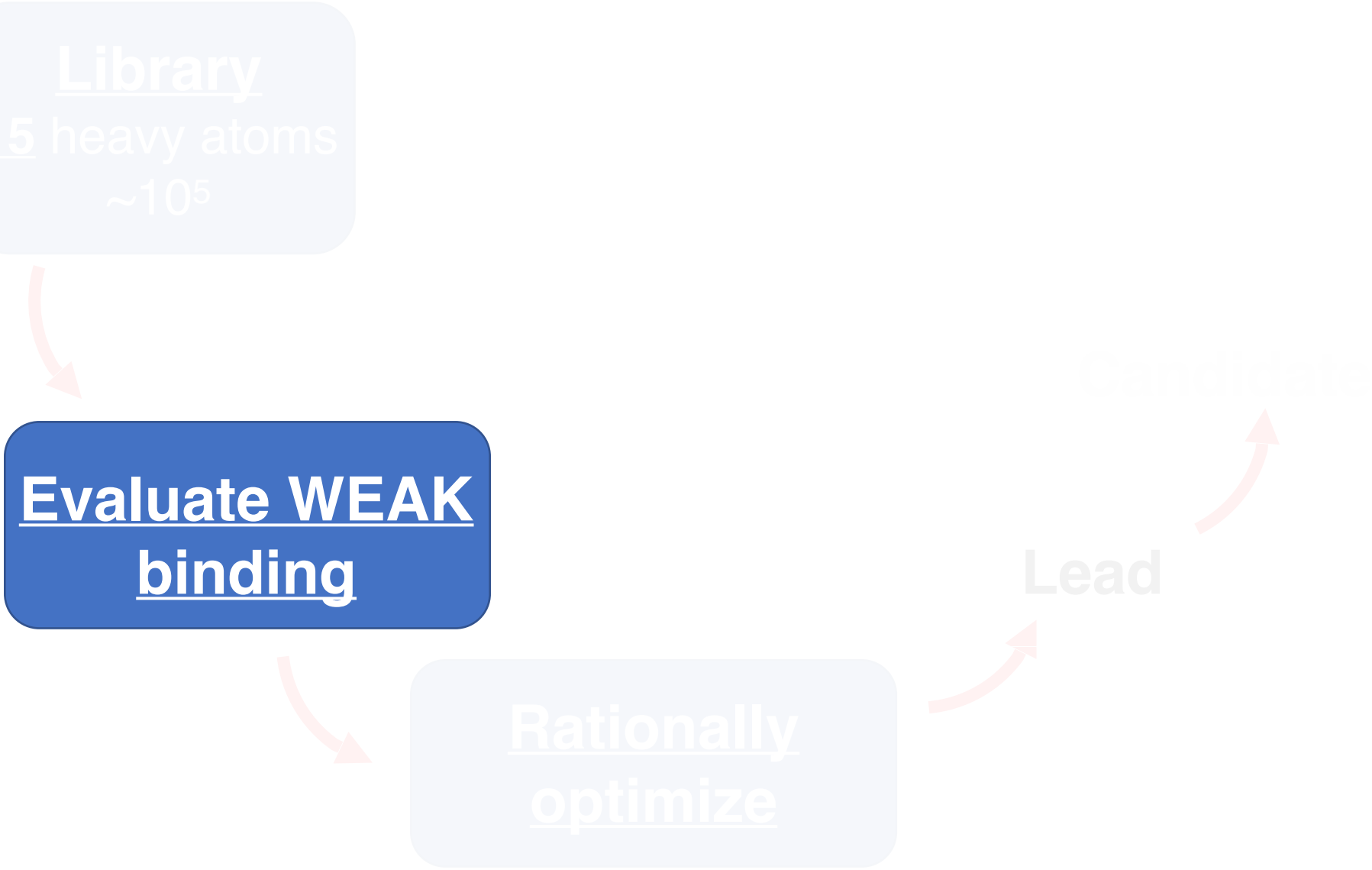
ead

HTS vs Fragment based

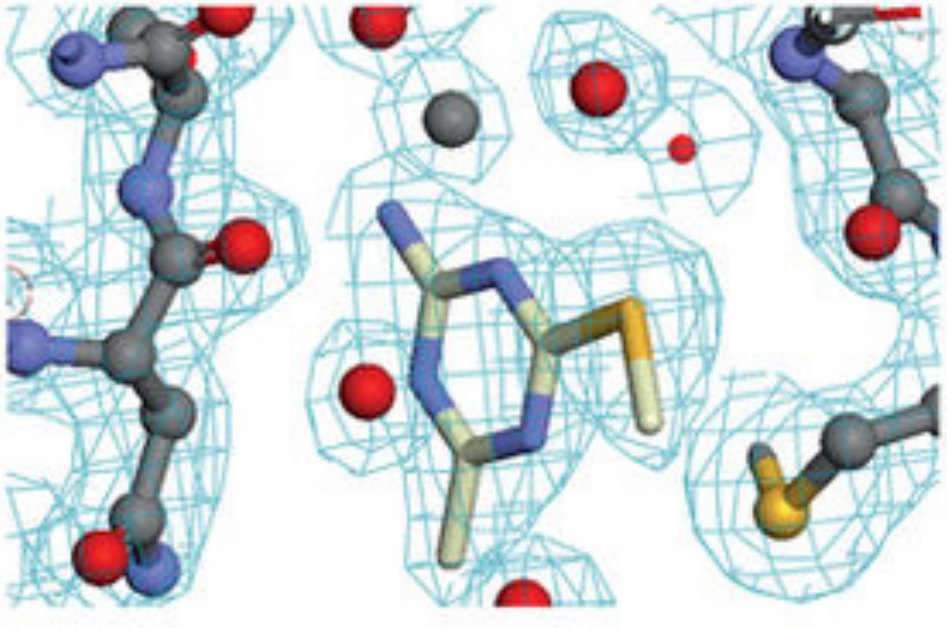


	High-throughput screening	Fragment-based
Library size	1,000,000 – 10,000,000	<10,000
Molecular weight	>300 kDa	<300 kDa
Screening	More flexible	Well characterized targets
Affinities	μM	mM
Optimization	Fixing problems, improving affinity	Iterative improvement
Main downside	Attrition, can't solve "challenging" targets	Biophysical methods are hard!

Fragment based drug discovery



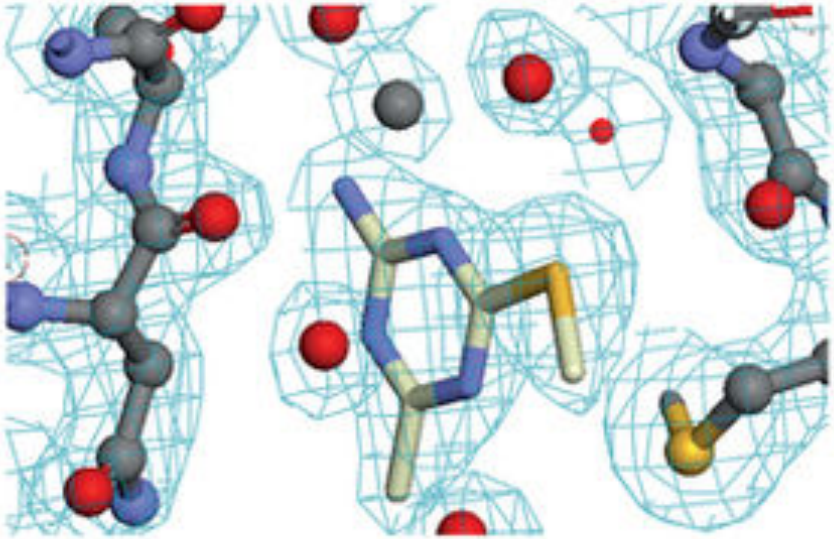
Assessing drug-target interaction



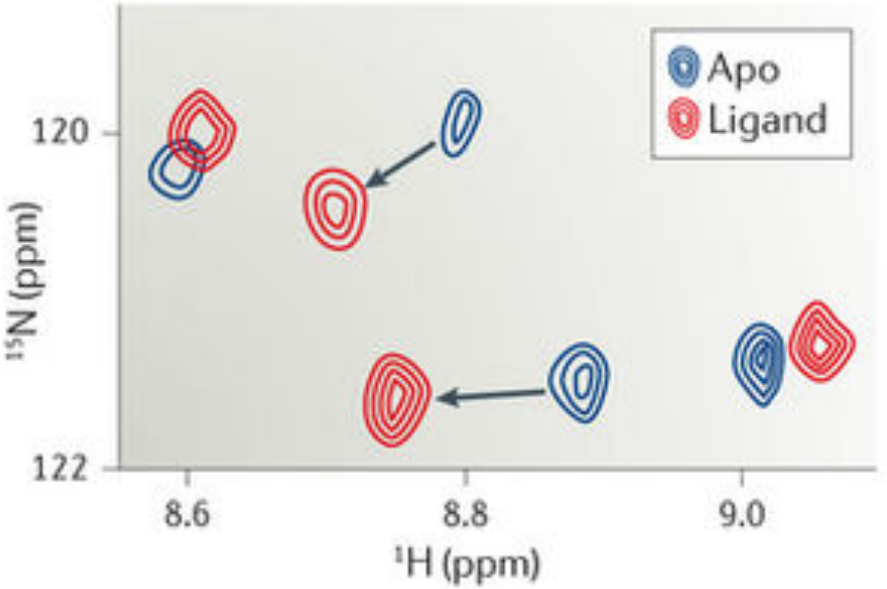
High resolution X-ray (or Cryo-EM) structure

Renaud JP *et al*. *Nature Reviews Drug Discovery* 15,679-698 (2016)

Assessing drug-target interaction



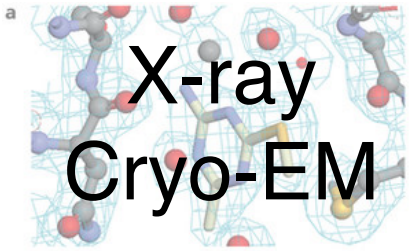
X-ray
Cryo-EM

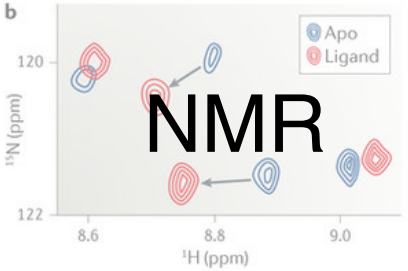


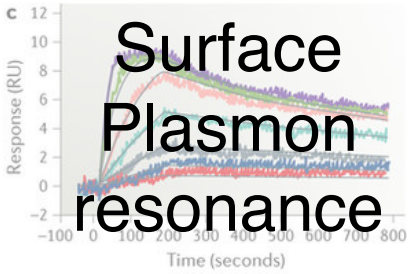
NMR

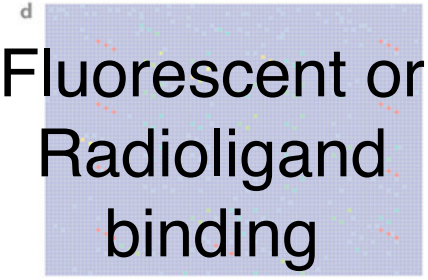
Renaud JP *et al*. *Nature Reviews Drug Discovery* 15,679-698 (2016)

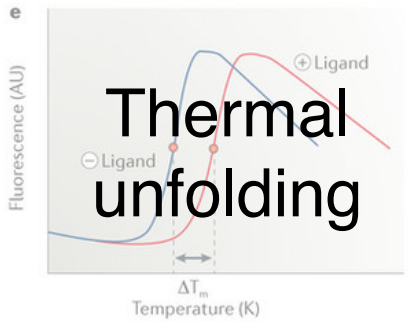
Assessing drug-target interaction

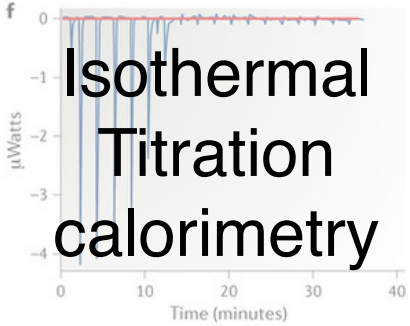


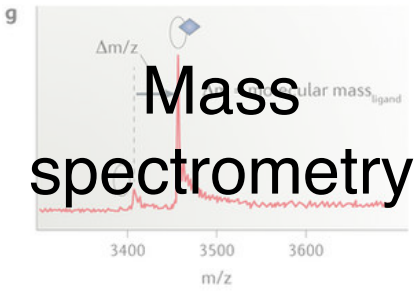


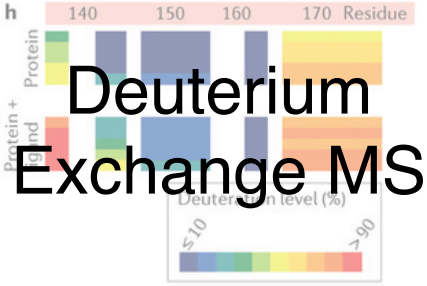


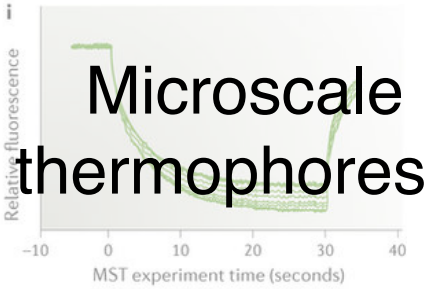




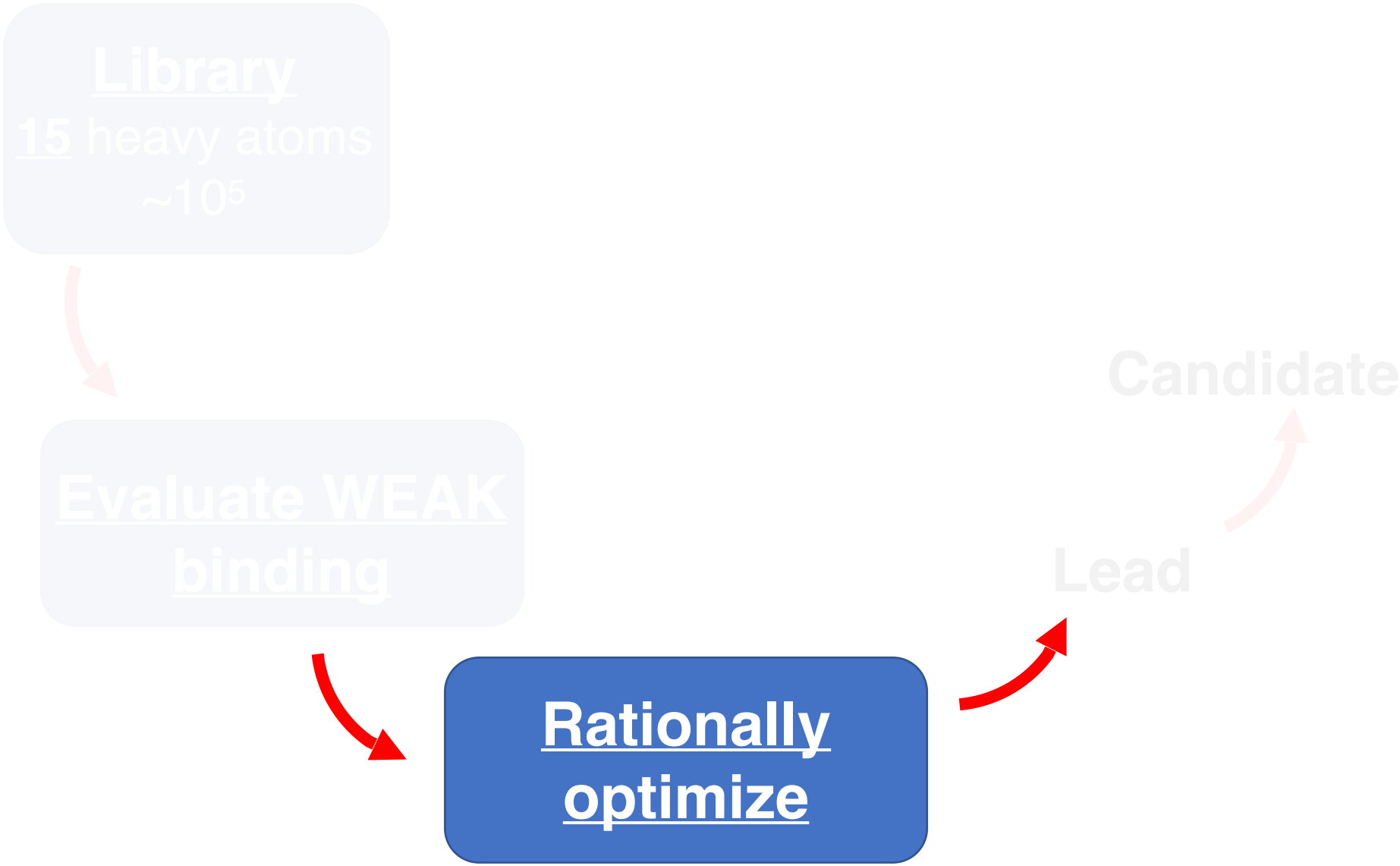




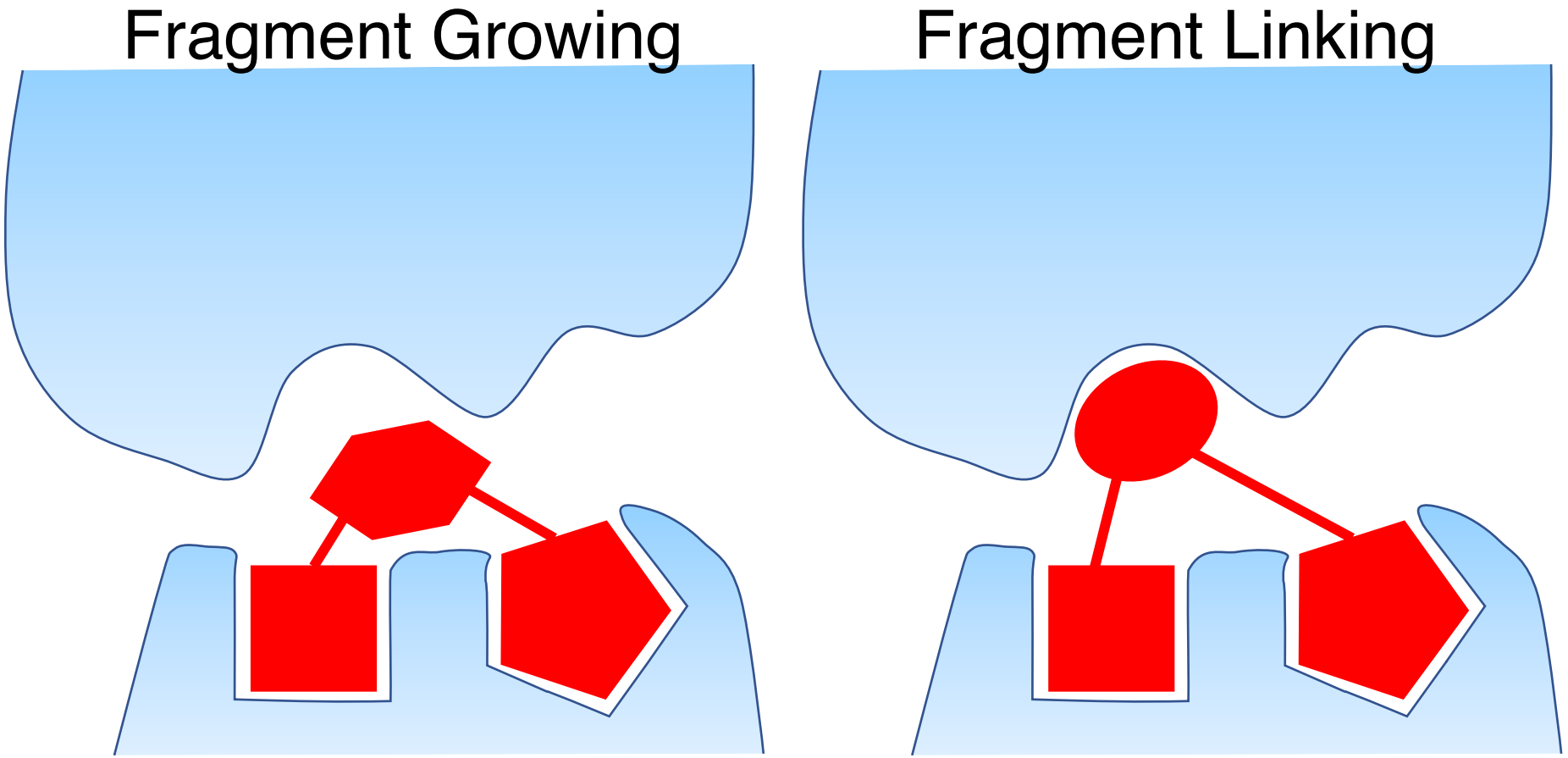




Fragment based drug discovery

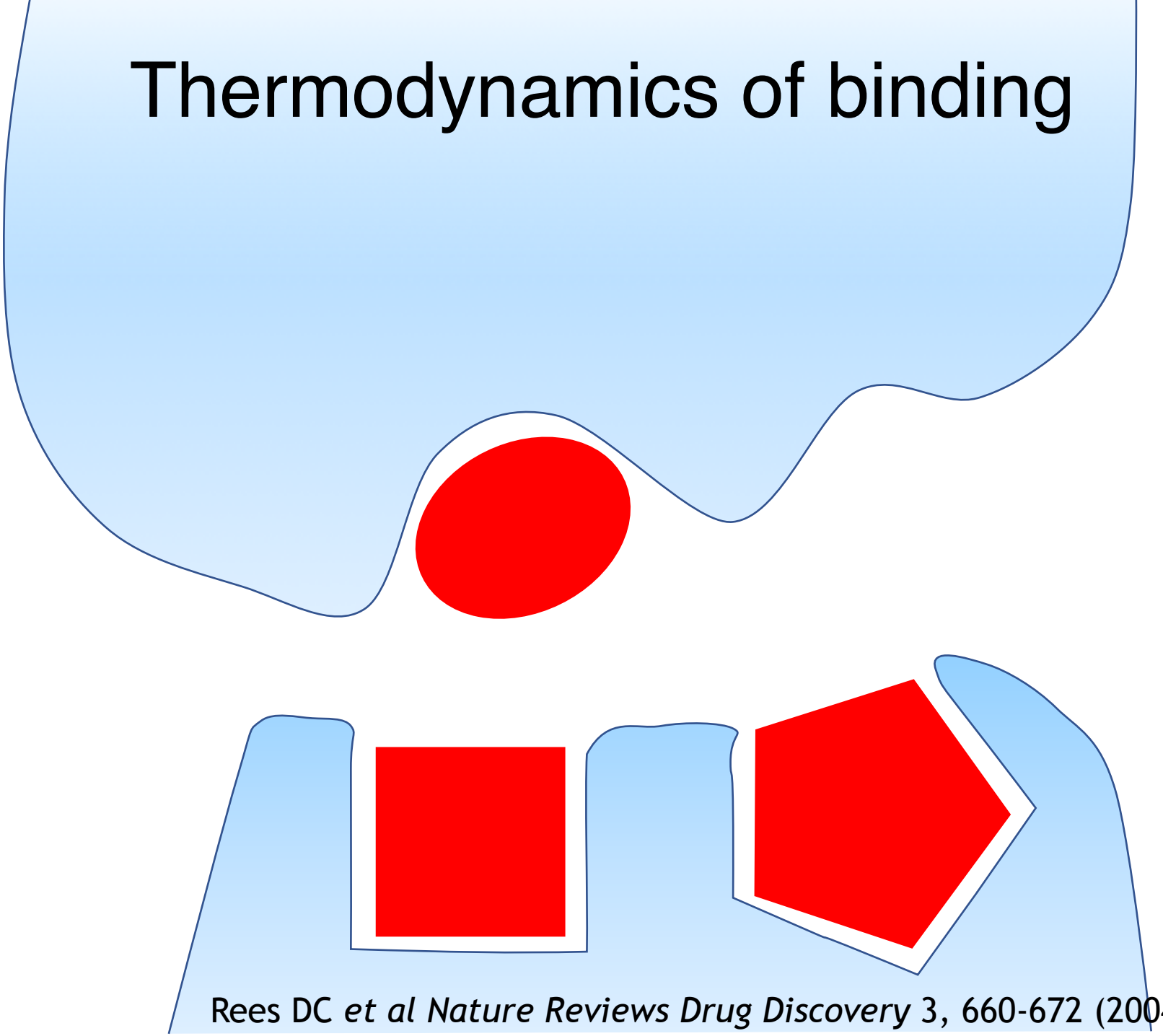


Increasing fragment potency



Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

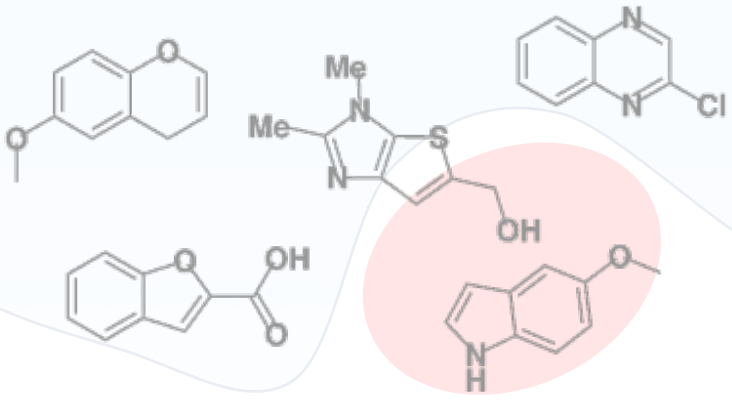
Thermodynamics of binding

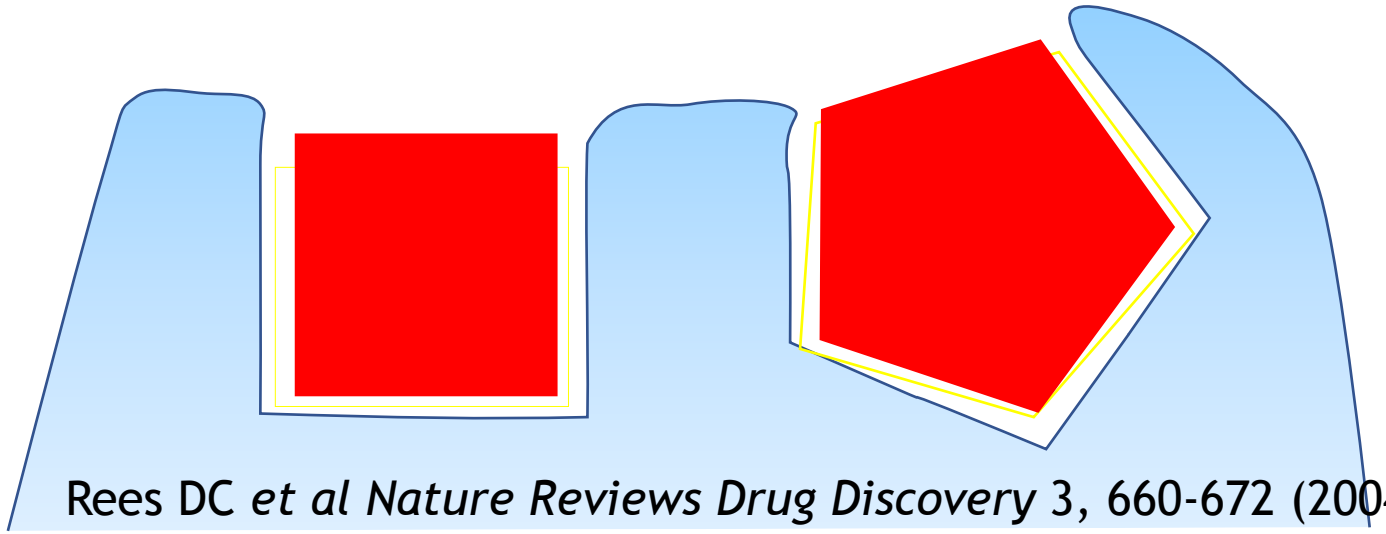


Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Thermodynamics of binding

$$\Delta G = \Delta H - T\Delta S$$

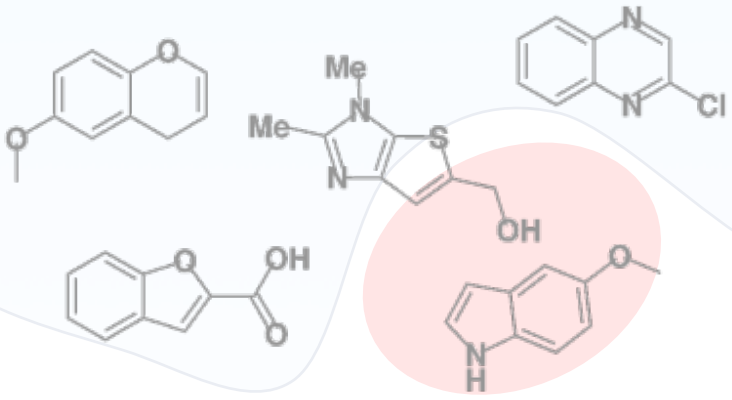




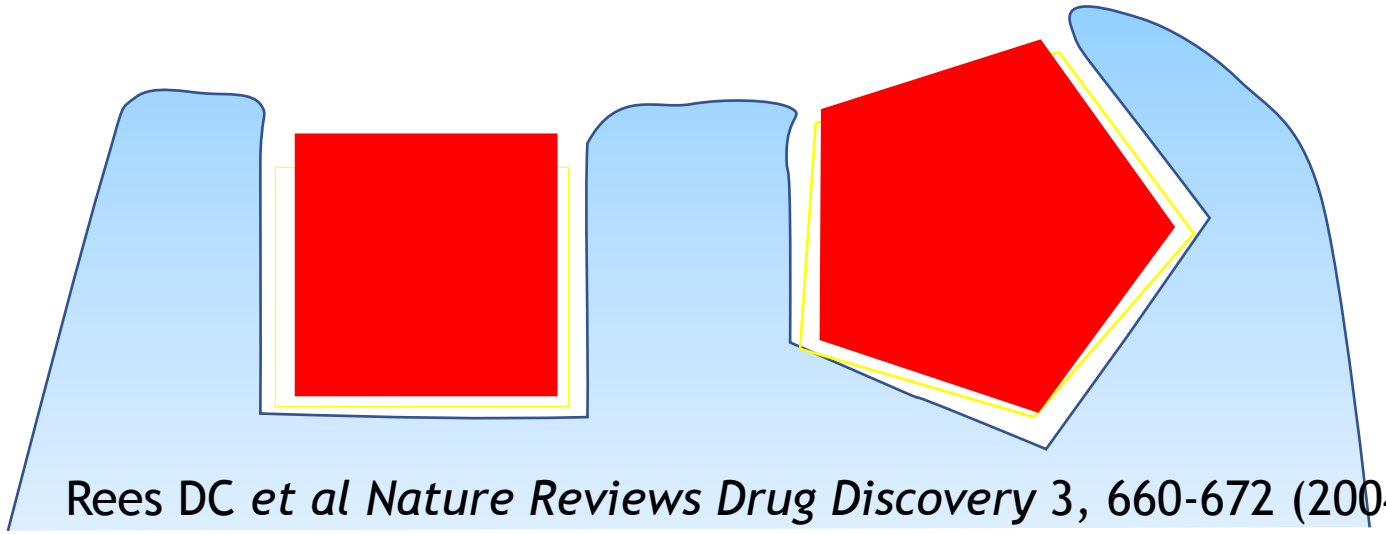
Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Thermodynamics of binding

$$\Delta G = \mathbf{\Delta H} - T\Delta S$$



Fragments primarily exploit enthalpy



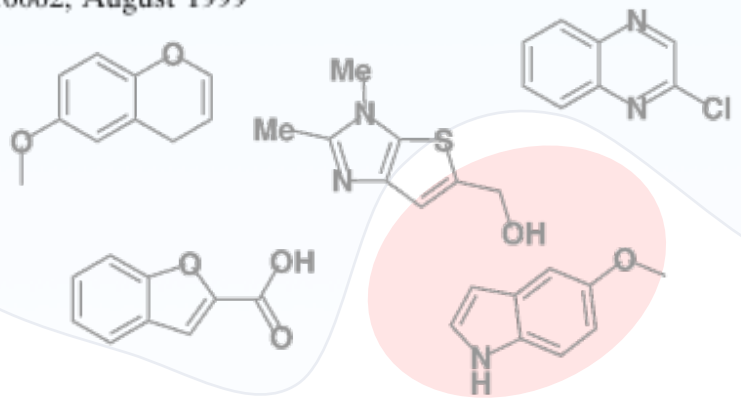
Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

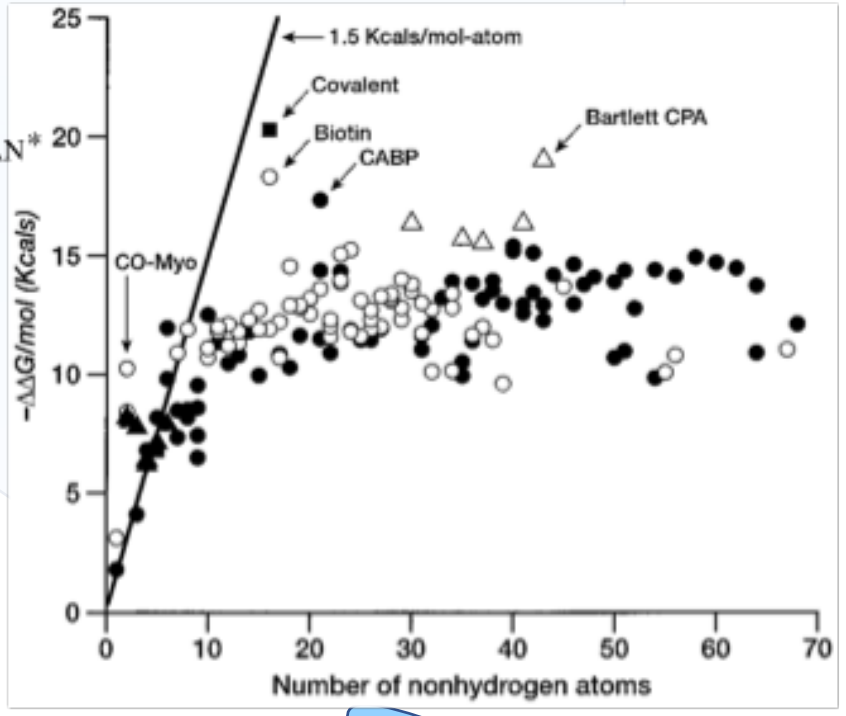
Fragments optimize binding interactions

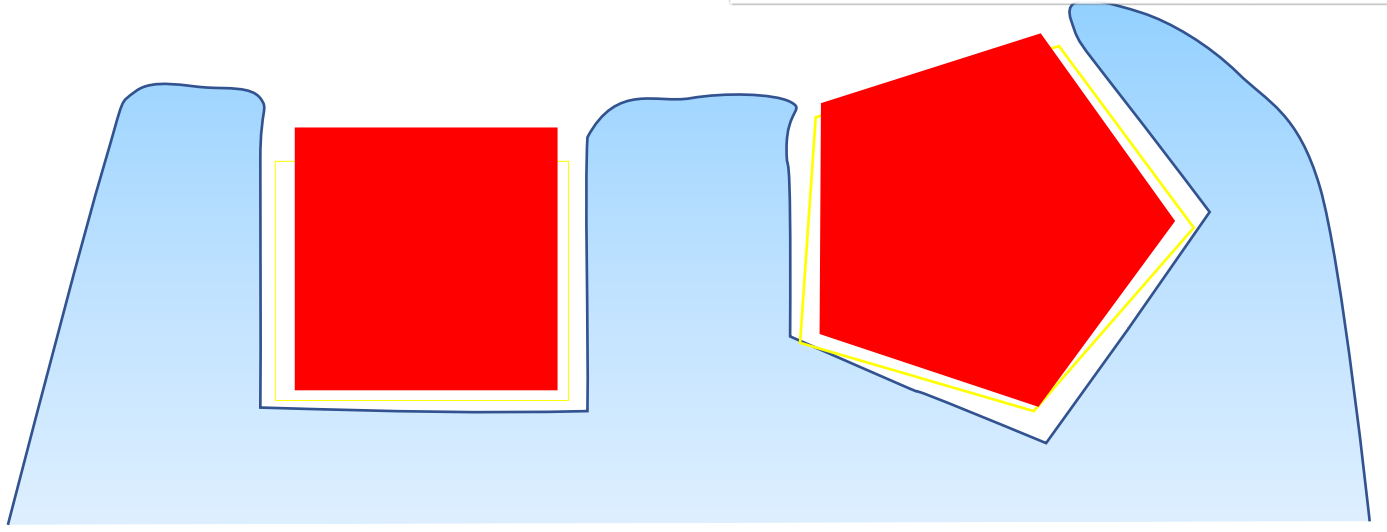
The maximal affinity of ligands

I. D. KUNTZ*†, K. CHEN*, K. A. SHARP‡§, AND P. A. KOLLMAN*

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 9997–10002, August 1999
Chemistry

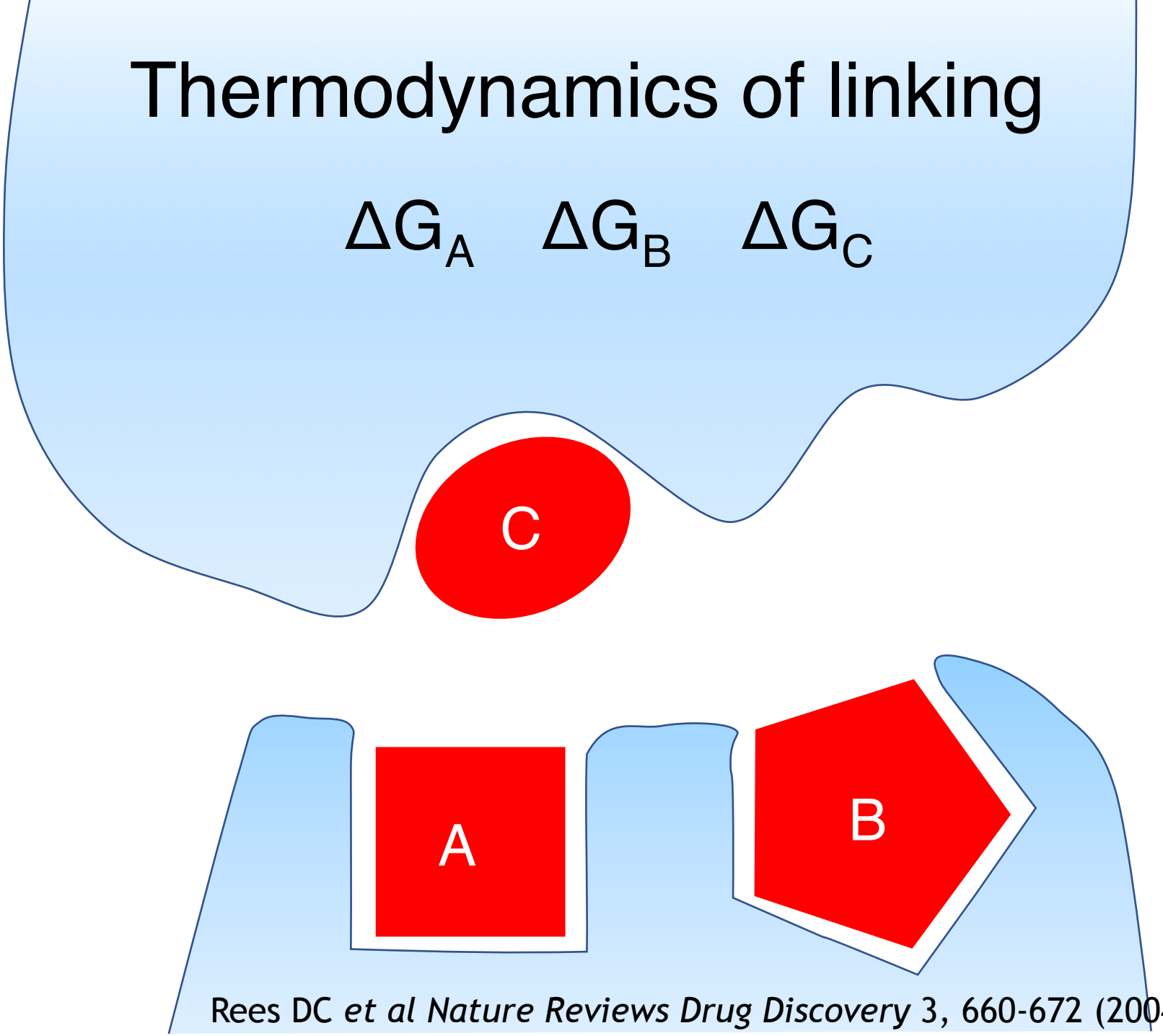






Thermodynamics of linking

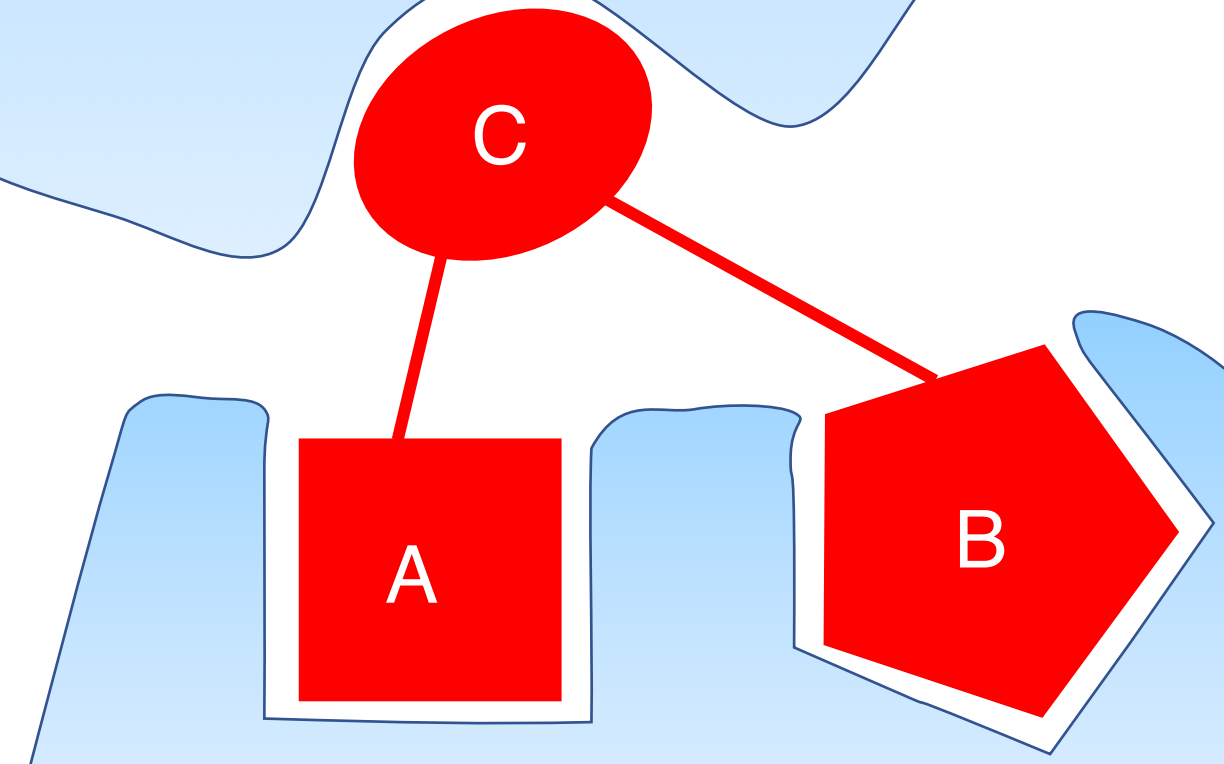
$\Delta G_A \quad \Delta G_B \quad \Delta G_C$



Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Thermodynamics of linking

$$\Delta G_{sum} = \Delta G_A + \Delta G_B + \Delta G_C$$

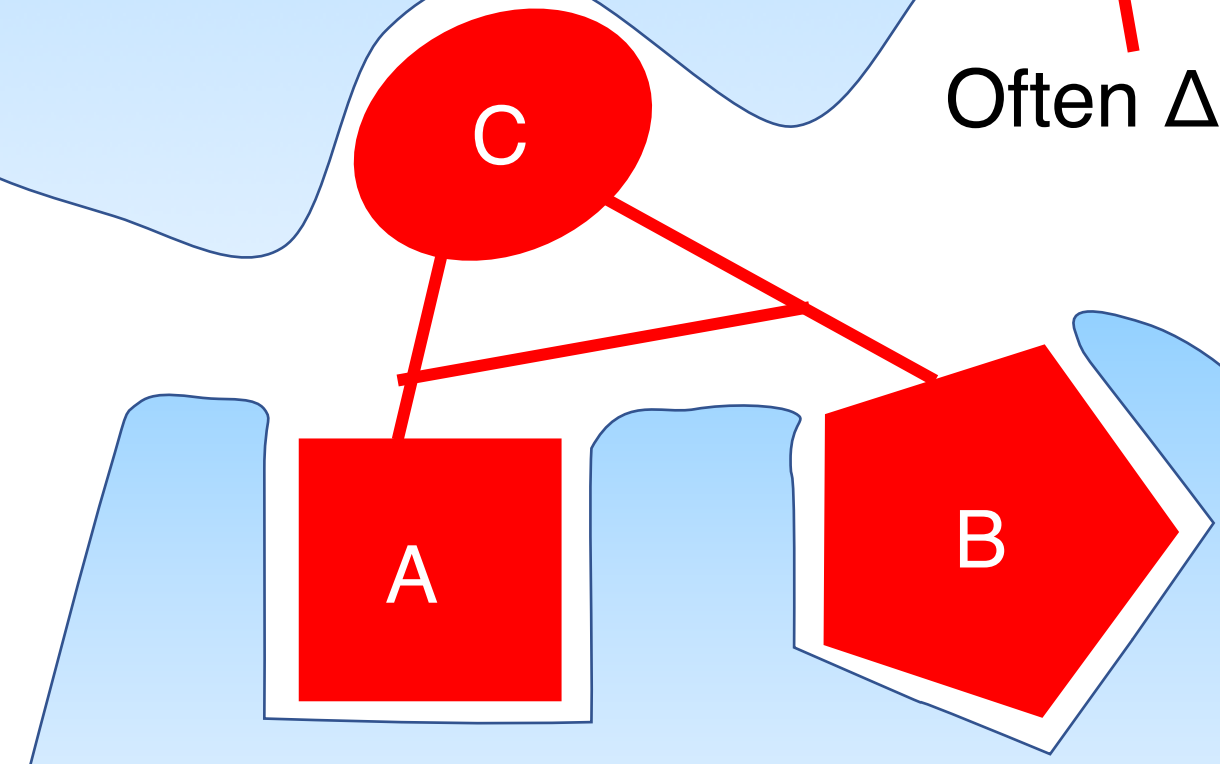


Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Thermodynamics of linking

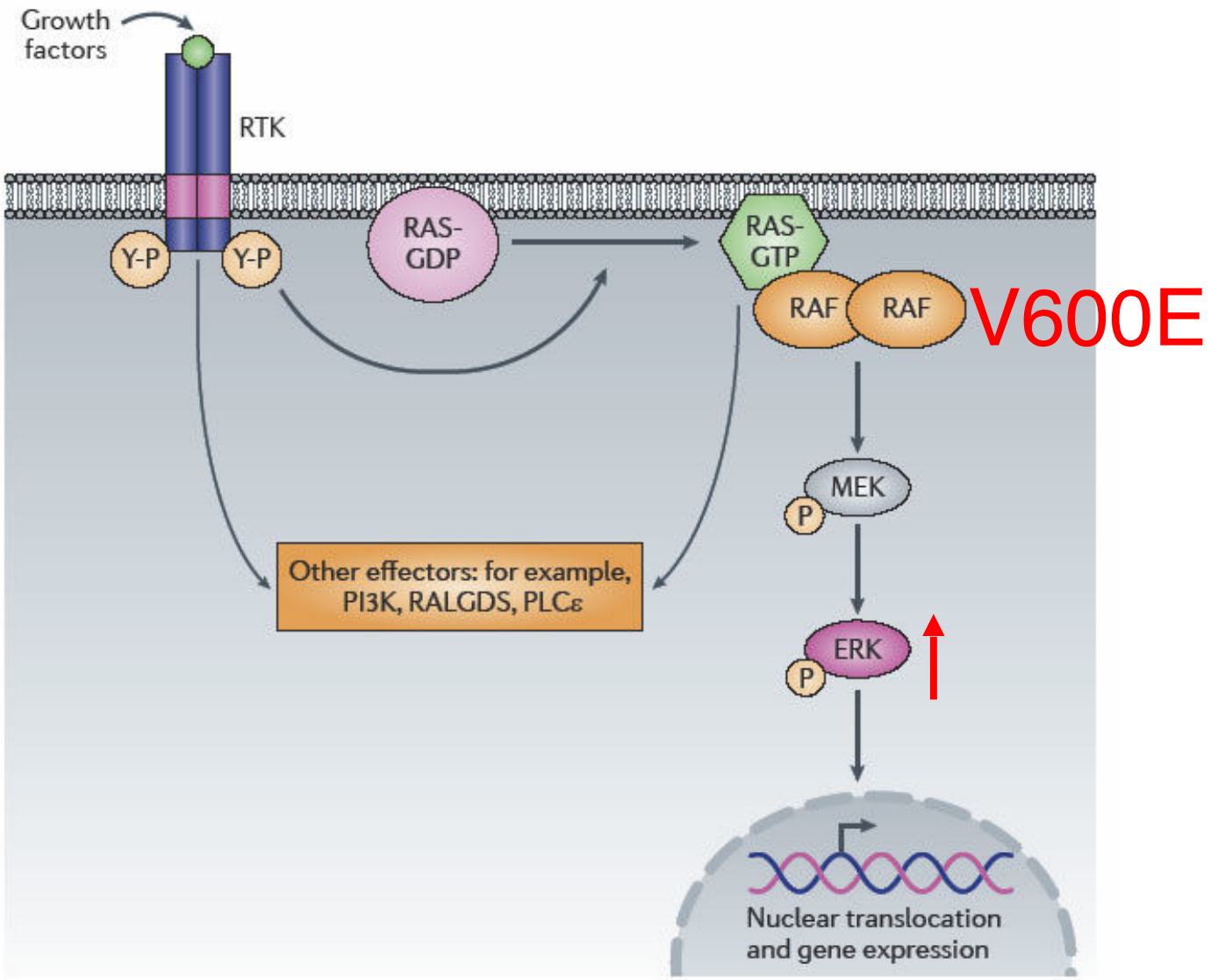
$$\Delta G_{sum} = \Delta G_A + \Delta G_B + \Delta G_C + \Delta G_{other}$$

Often ΔS driven



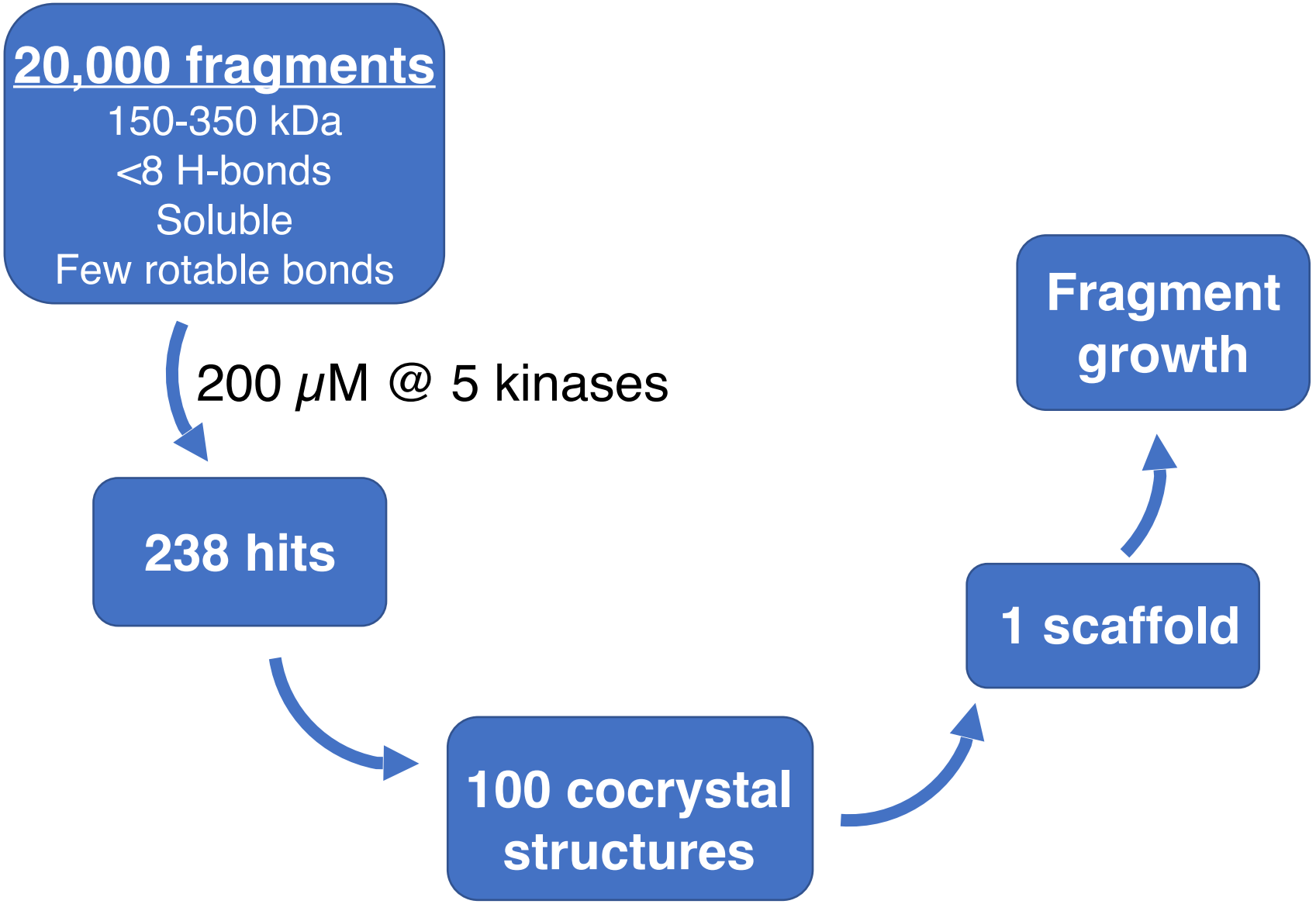
Rees DC *et al Nature Reviews Drug Discovery* 3, 660-672 (2004).

Discovery of vemurafenib

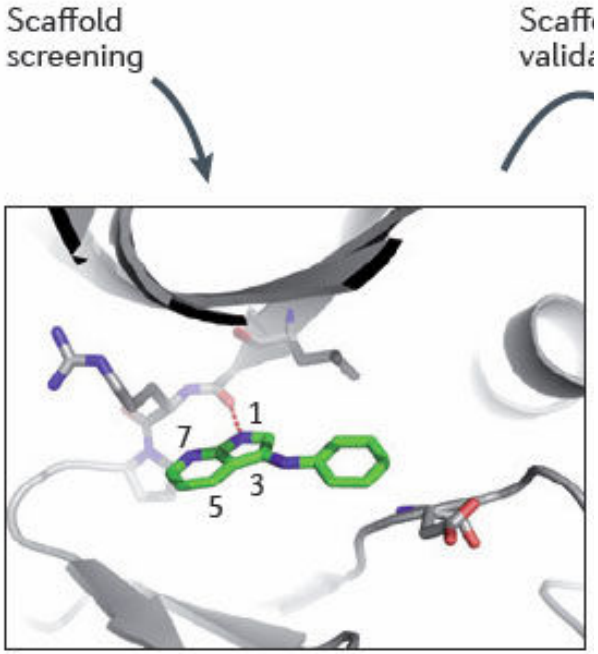


Bollag G *et al. Nature Reviews Drug Discovery* 11, 873-886 (2012)

Vemurafenib (**V**600**E** **mu**tated B-**raf** **inhib**itor



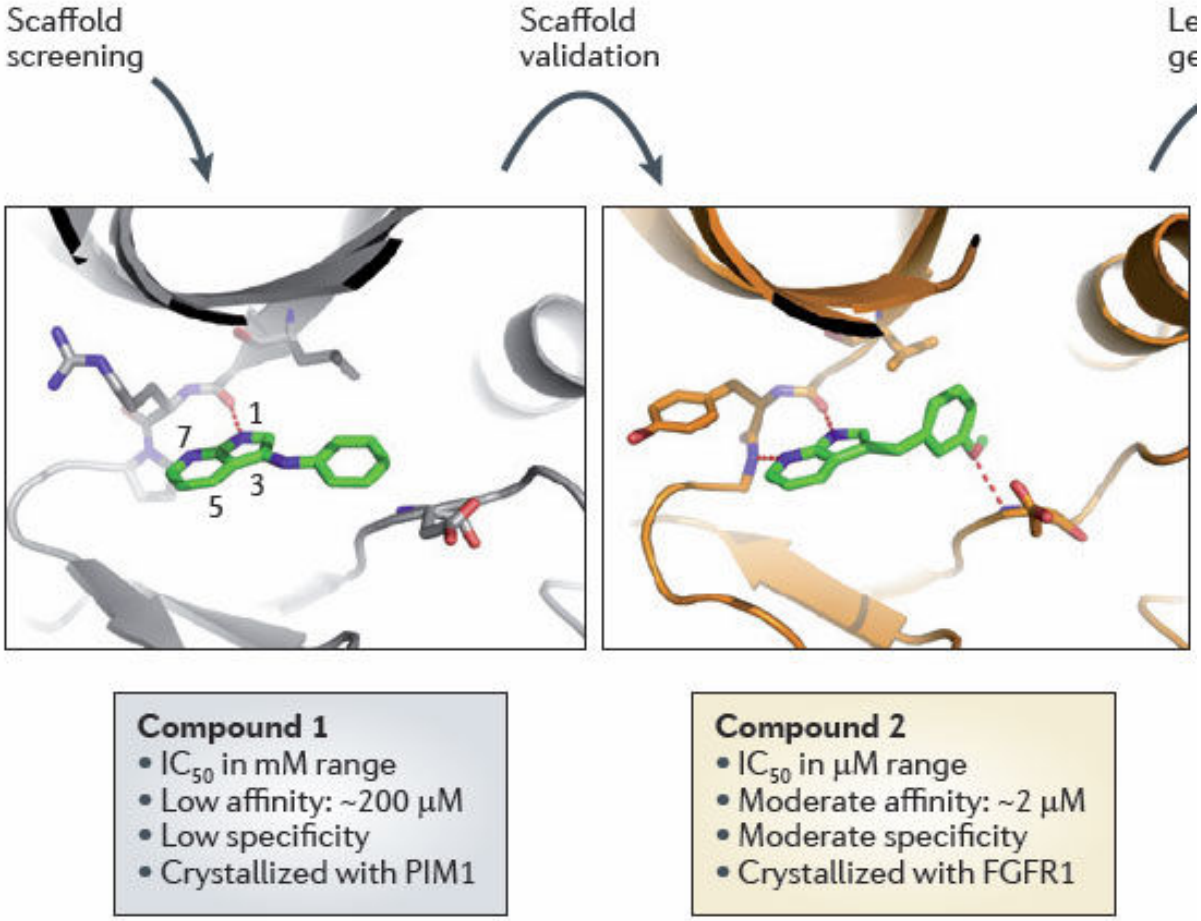
Vemurafenib (**V**600**E** **mu**tated B-**raf** **inhib**itor



Compound 1
- IC_{50} in mM range
- Low affinity: ~200 μM
- Low specificity
- Crystallized with PIM1

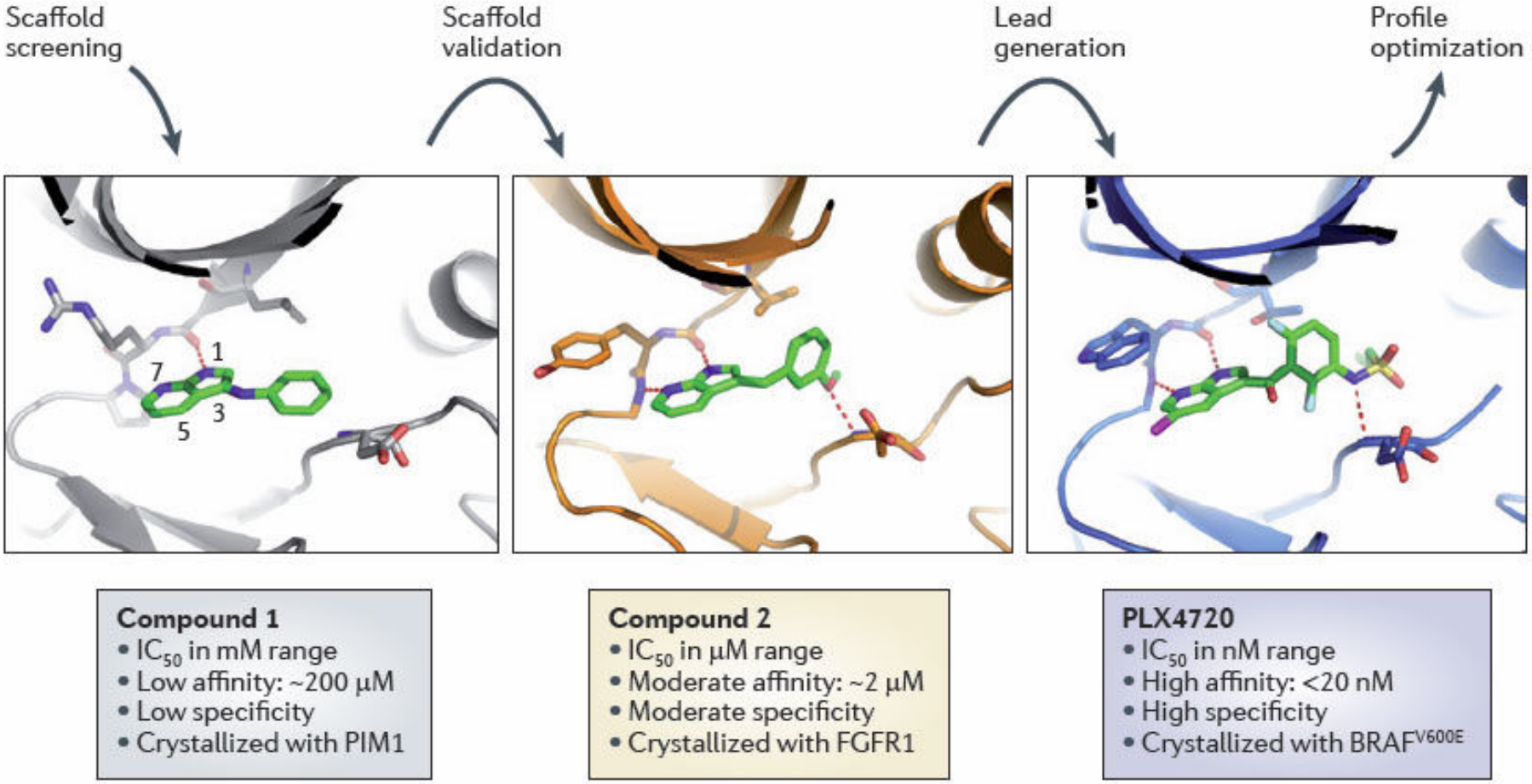
Bollag G *et al. Nature Reviews Drug Discovery* 11, 873-886 (2012)

Vemurafenib (**V**600**E** **mu**tated B-**raf** **inhib**itor



Bollag G *et al*. *Nature Reviews Drug Discovery* 11, 873-886 (2012)

Vemurafenib (**V**600**E** **mu**tated B-**raf** **inhib**itor



Bollag G *et al. Nature Reviews Drug Discovery* 11, 873-886 (2012)

And it works!

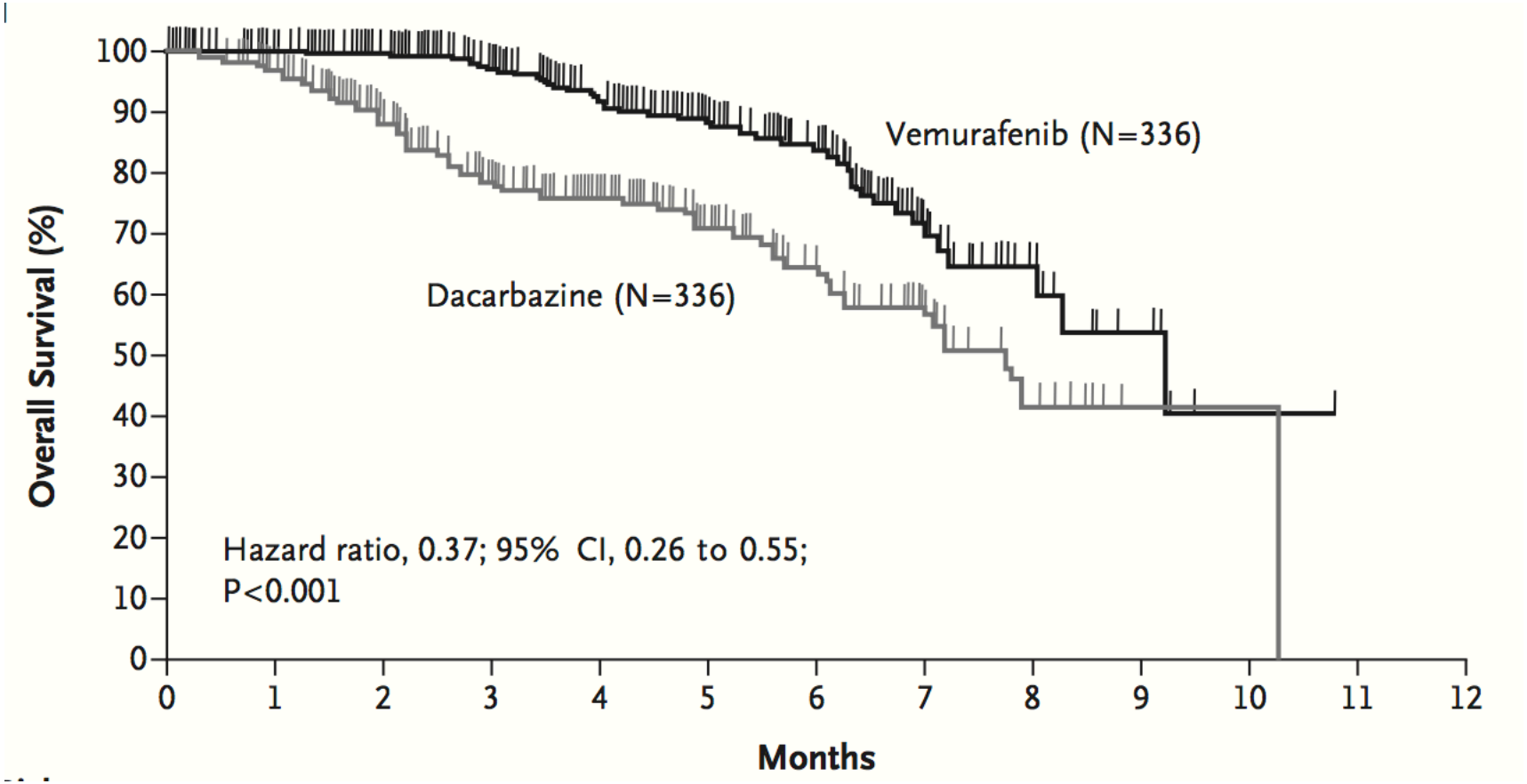
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

N ENGL J MED 364;26 NEJM.ORG JUNE 30, 2011

Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation

Vemurafenib improves overall survival

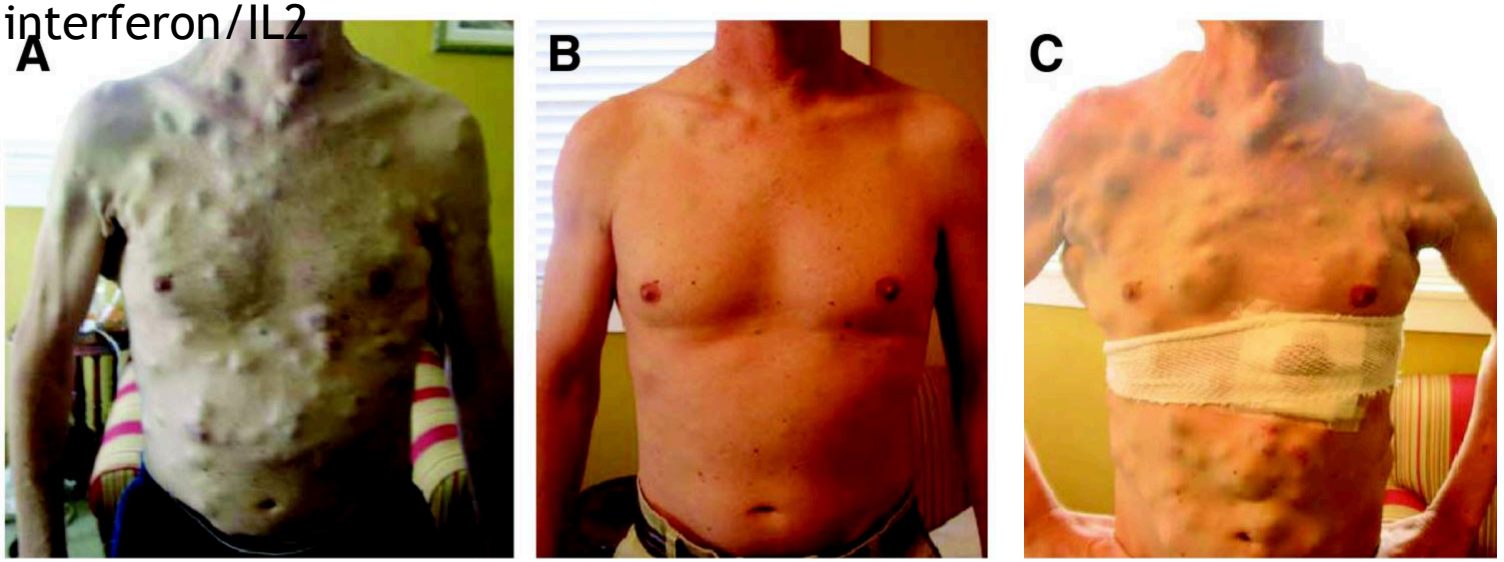


But nothing is ever easy…

After ipilimumab, dacarbazine, carboplatin/ paclitaxel/ interferon/IL2

+ 15 weeks of vemurafenib

+ 23 weeks of vemurafenib



Wagle N. *J Clin Oncol.* 29:3085-96 (2011)

But nothing is ever easy…

Vol 464|18 March 2010|doi:10.1038/nature08902 nature

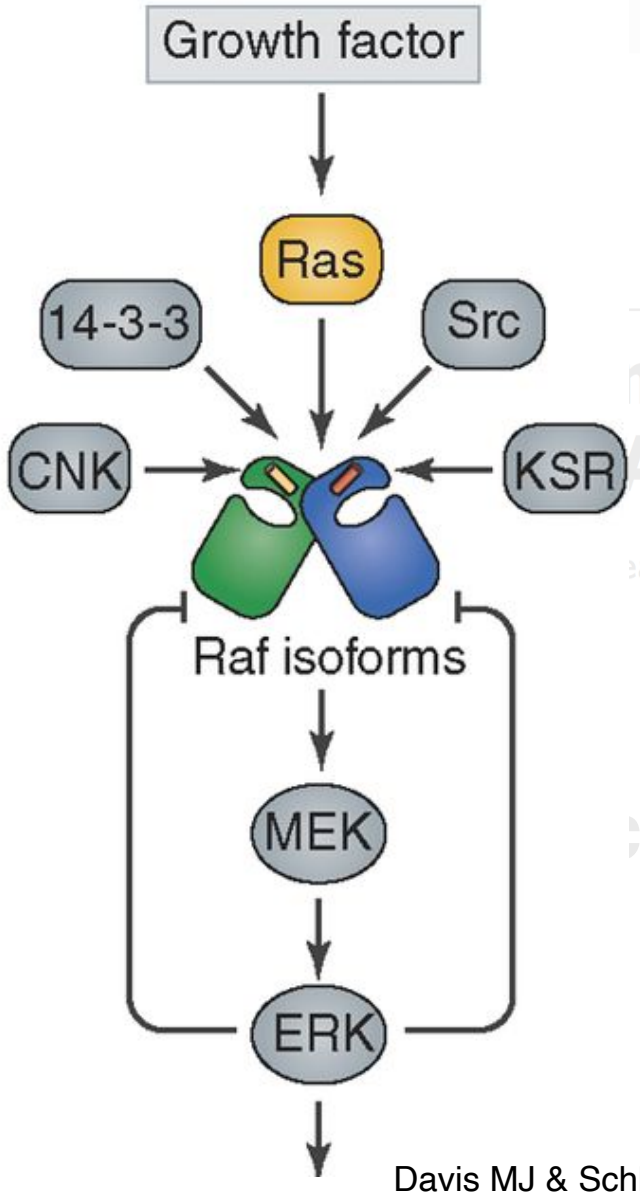
LETTERS

RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF

Poulikos I. Poulikakos[1], Chao Zhang[2], Gideon Bollag[3], Kevan M. Shokat[2] & Neal Rosen[1]

=> ~30% squamous cell-carcinomas

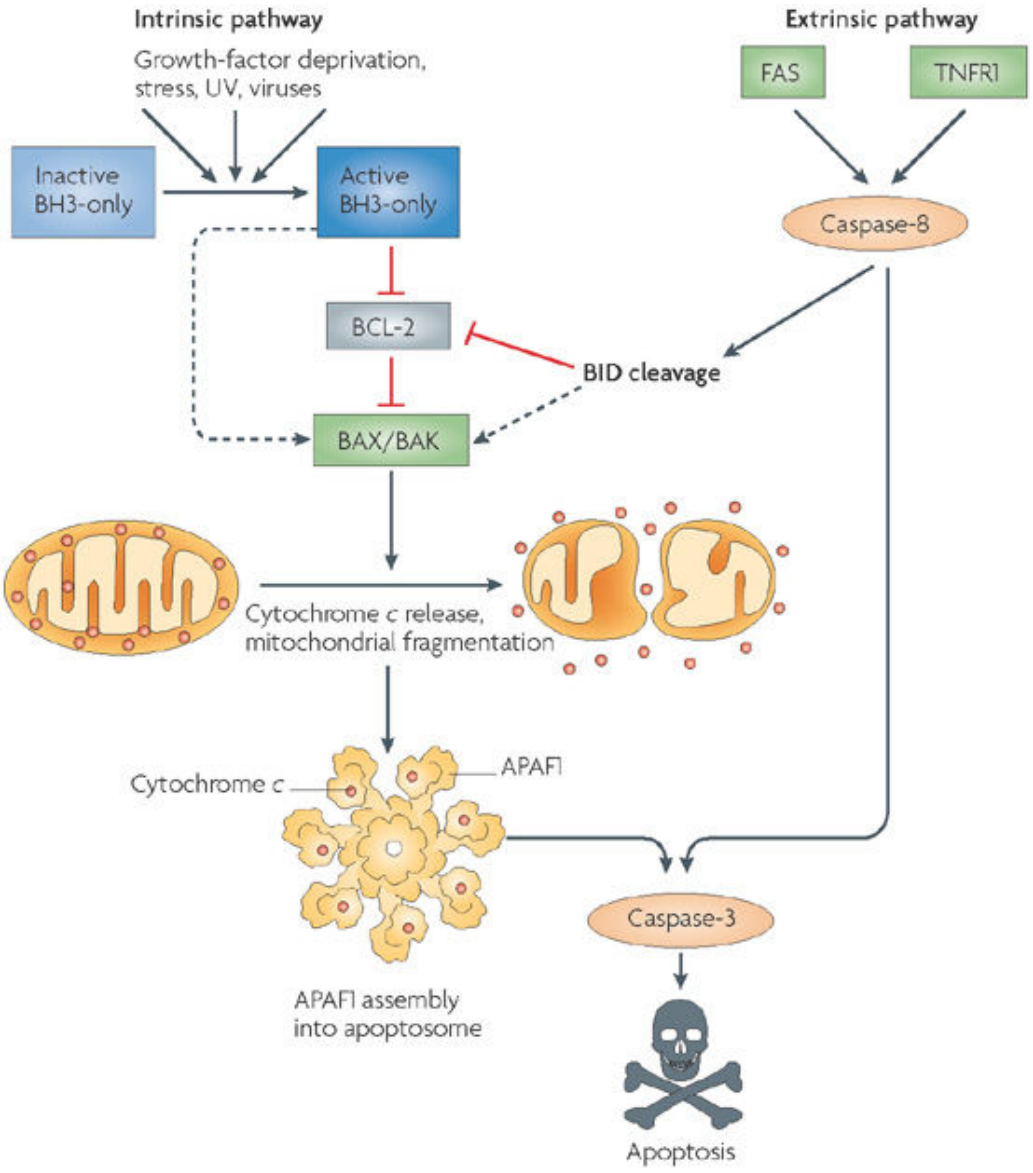
But nothing is ever easy…



Davis MJ & Schlessinger J. *J Cell Biol.* 199:15-19 (2012).

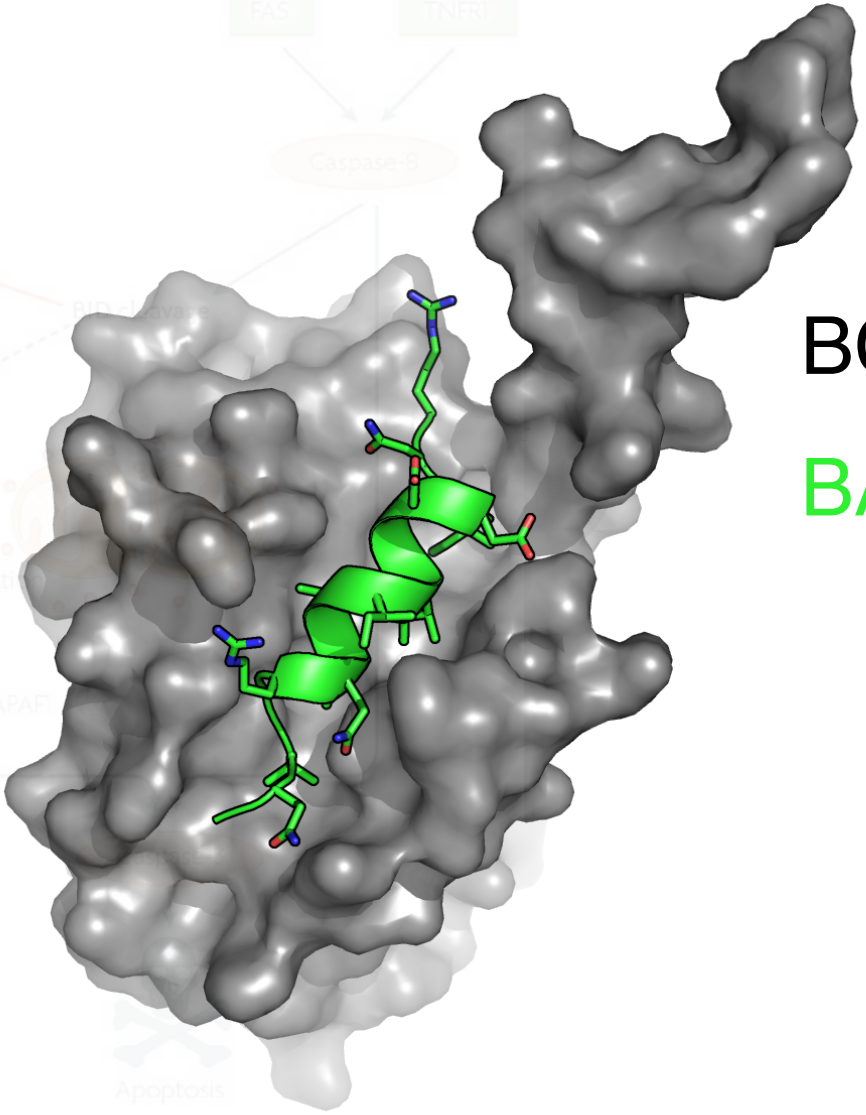
But what about ”challenging” targets?

Discovery of venetoclax



↑BCL-2 => ↓ apoptosis

BCL-xl is a classic “challenging” targe



BCL-X_L

BAD peptide

“SAR by NMR”

9373 fragments

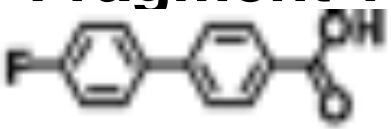
NMR to test sets of 10

660 cmpds

NMR to retest

49 w/ Kd < 5 mM

Fragment 1



+3472 fragments

NMR to test sets of 5

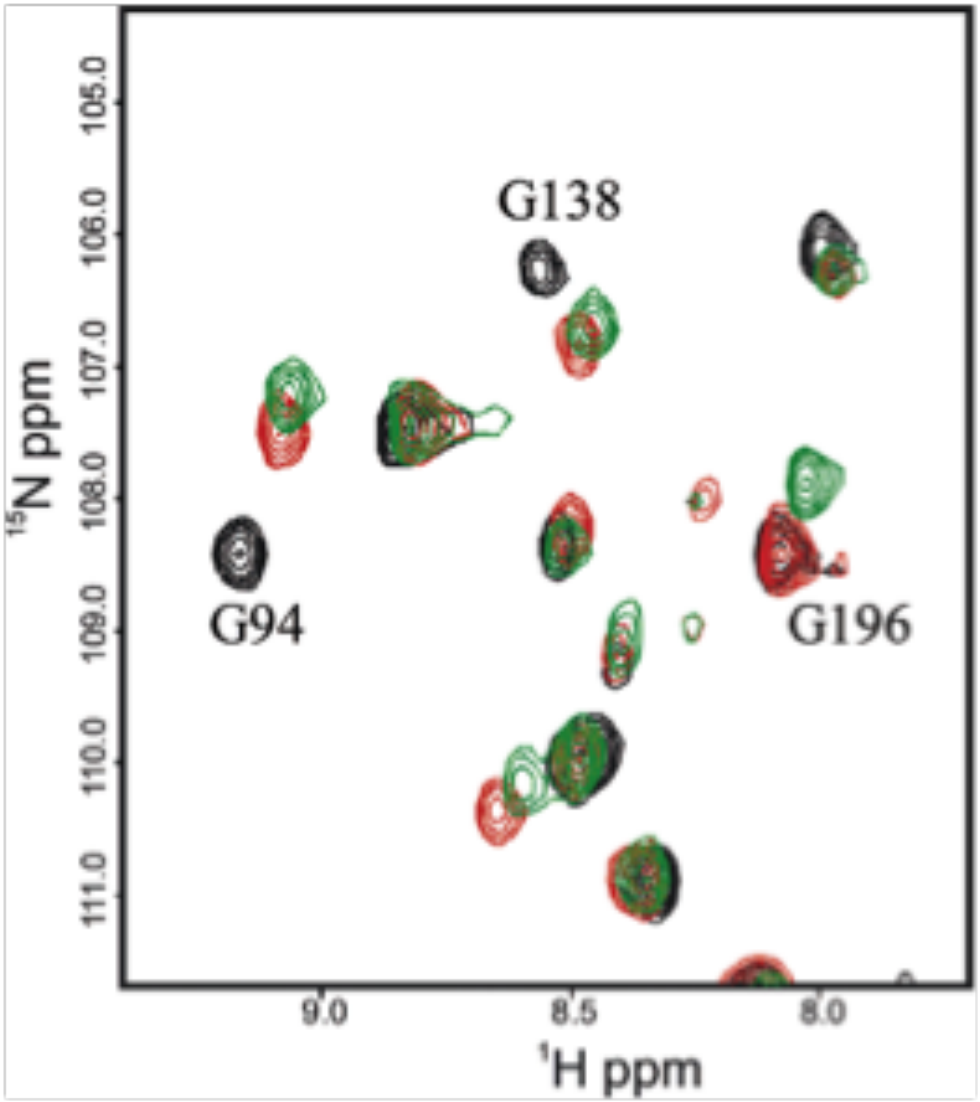
300 cmpds

NMR to retest

24 w/ Kd < 5 mM

Fragment 2

Fragment Based Discovery by NMR

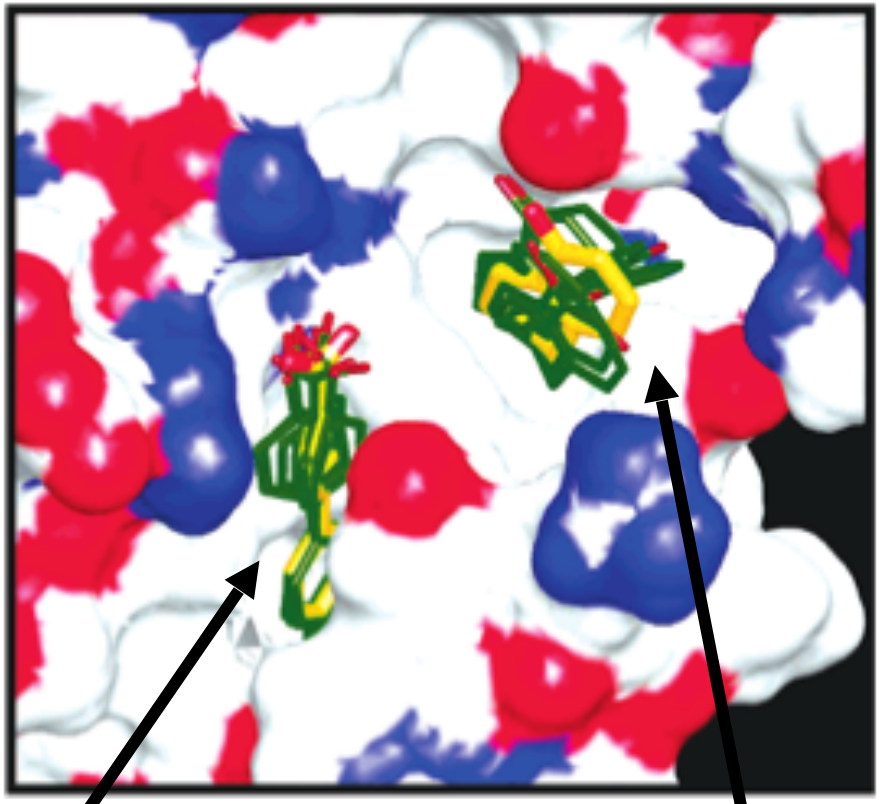


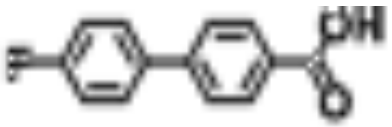
BCL-X_L protein alone

+ Fragment 1

+ Fragment 2

Fragment Based Discovery by NMR

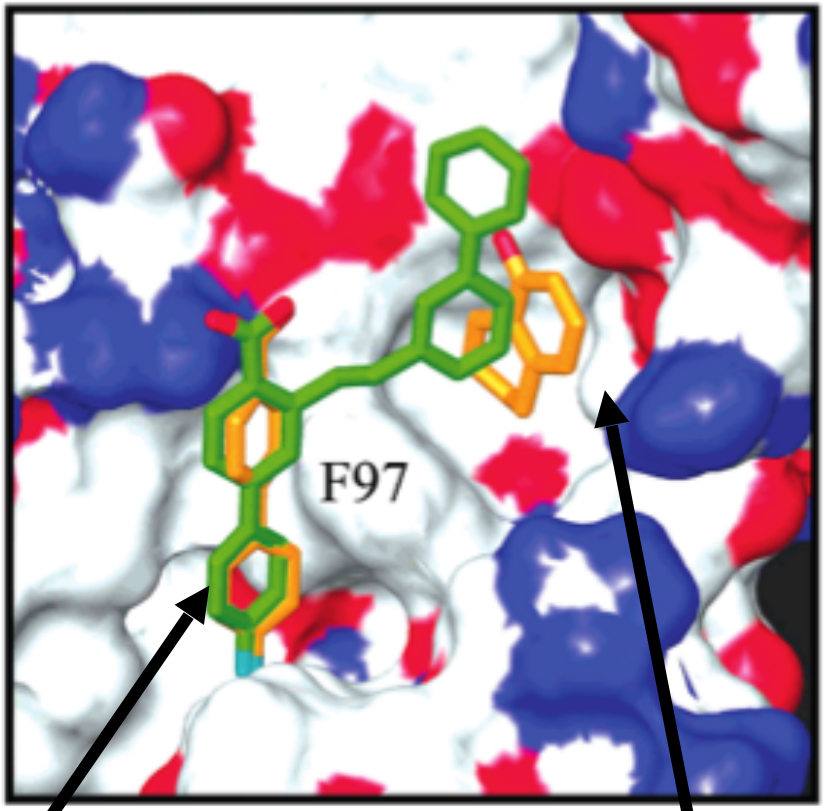




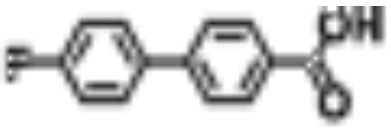
Fragment 1

Fragment 2

Fragment Based Discovery by NMR



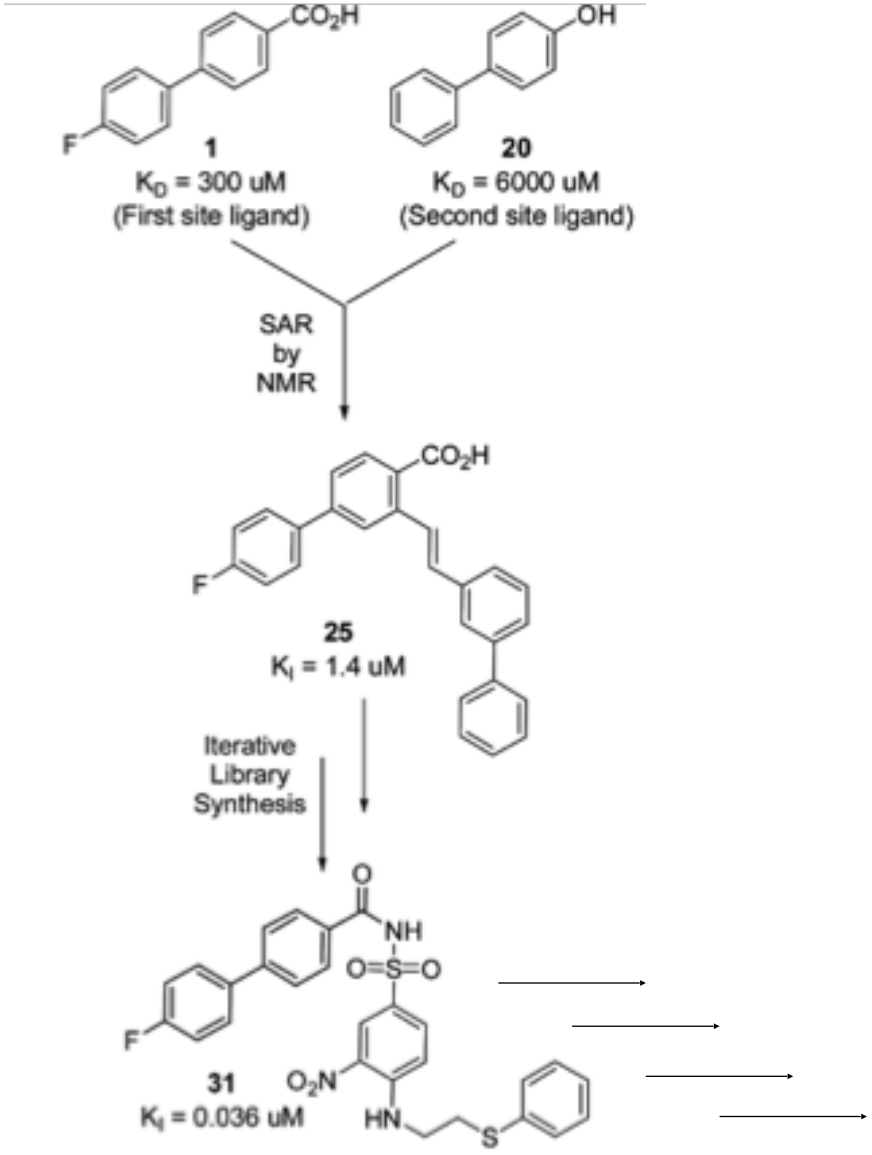
Cmpd 25
(1.4 μM)



(300 μM)
+ Fragment 1

(6000 μM)
+ Fragment 2

Fragment Based Discovery by NMR

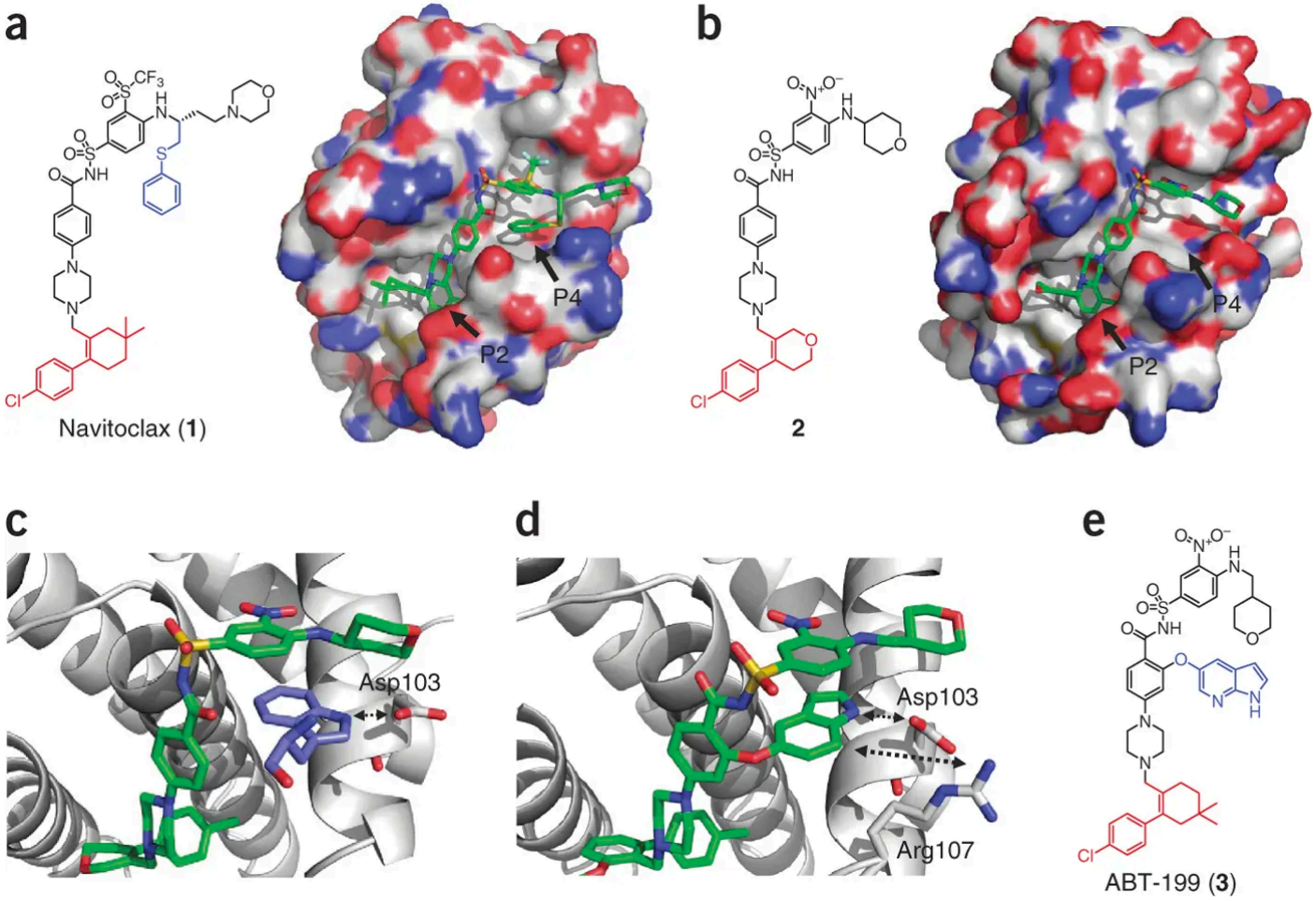


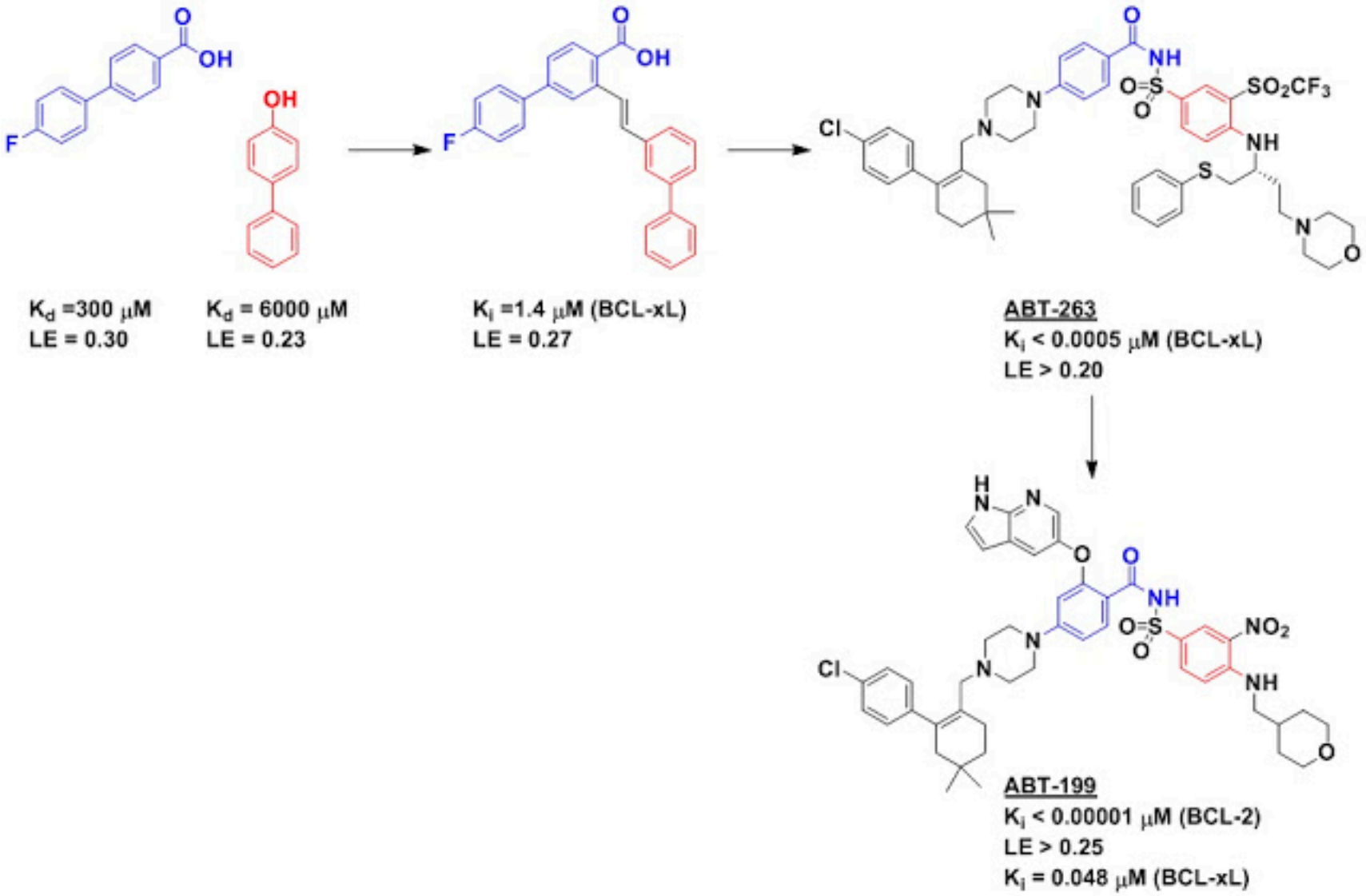
Crystallography lead to combining ideas

ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets

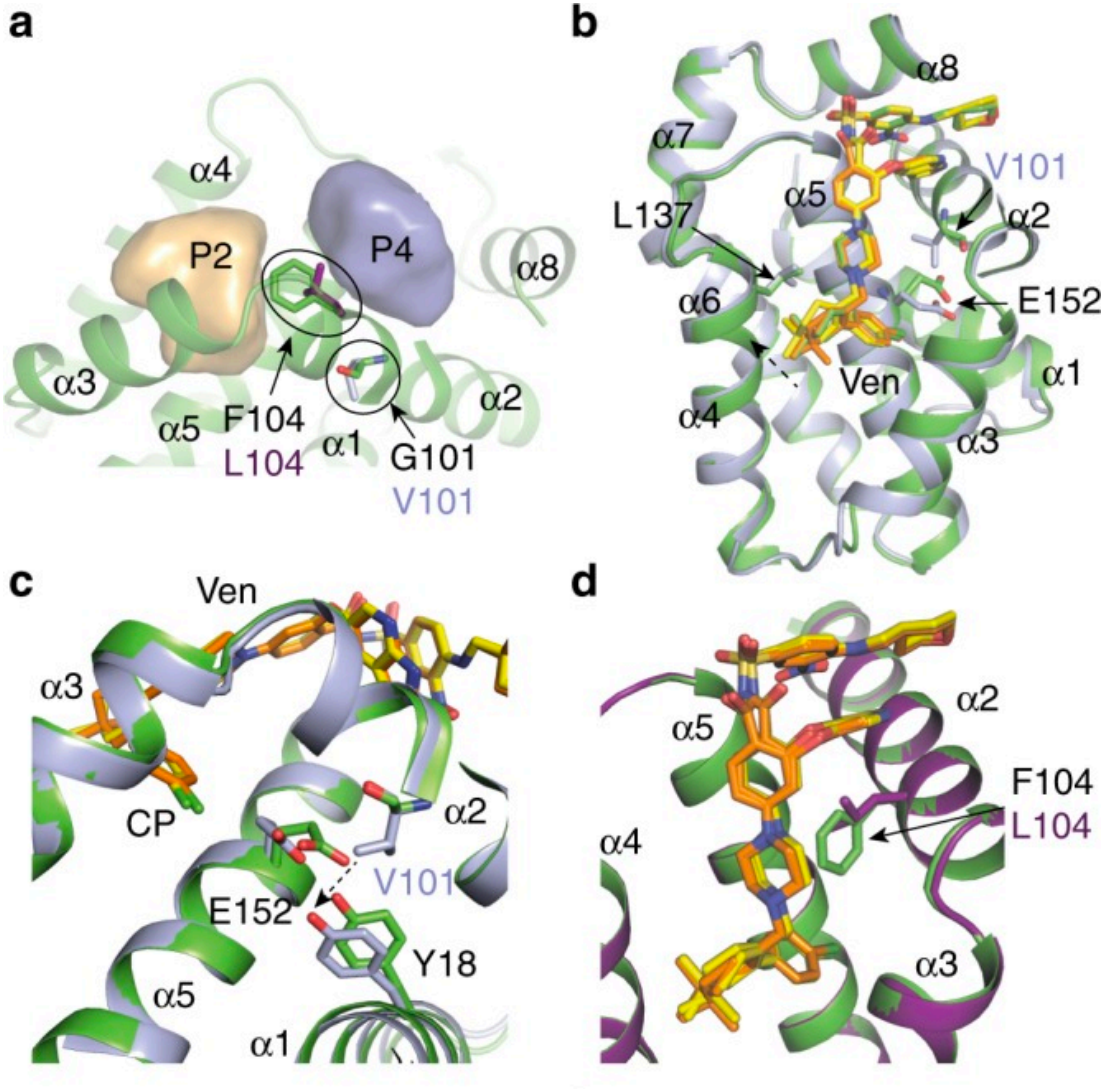
Andrew J Souers ✉, Joel D Leverson, [...] Steven W Elmore

Nature Medicine **19**, 202–208(2013) | Cite this article




O
OH
F
OH
K_d =300 μM
LE = 0.30
K_d = 6000 μM
LE = 0.23
K_i =1.4 μM (BCL-xL)
LE = 0.27
Cl
N
NH
SO_2CF_3
S
ABT-263
K_i < 0.0005 μM (BCL-xL)
LE > 0.20
H
NO_2
ABT-199
K_i < 0.00001 μM (BCL-2)
LE > 0.25
K_i = 0.048 μM (BCL-xL)

Resistance to venetoclax is already emerging

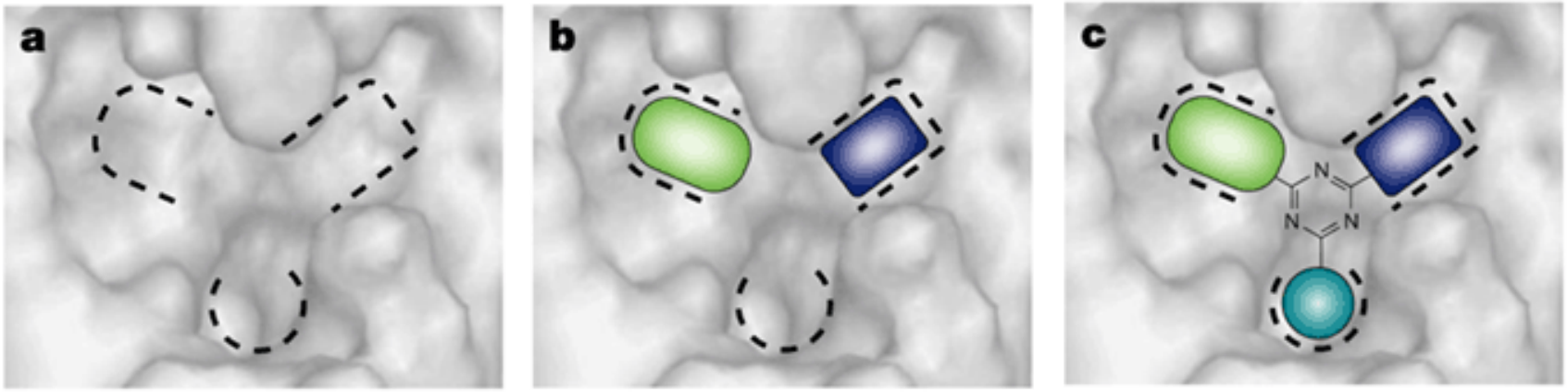


Nat Commun. 2019; 10: 2385. PMCID: PMC6547681
Published online 2019 Jun 3. doi: 10.1038/s41467-019-10363-1 PMID: 31160589

Structures of BCL-2 in complex with venetoclax reveal the molecular basis of resistance mutations

Richard W. Birkinshaw,[✉1,2] Jia-nan Gong,[1,2] Cindy S. Luo,[1,2] Daisy Lio,[1,2] Christine A. White,[1,2] Mary Ann Anderson,[1,2,3] Piers Blombery,[3,4,5] Guillaume Lessene,[1,2,6] Ian J. Majewski,[1,2] Rachel Thijssen,[1,2] Andrew W. Roberts,[1,2,3,7,8] David C. S. Huang,[1,2] Peter M. Colman,[1,2] and Peter E. Czabotar[✉1,2]

Crystallographic **fragment screening** allows us to cover the PTP1B surface and chemical space to find ligands for these (and undiscovered!) cryptic sites



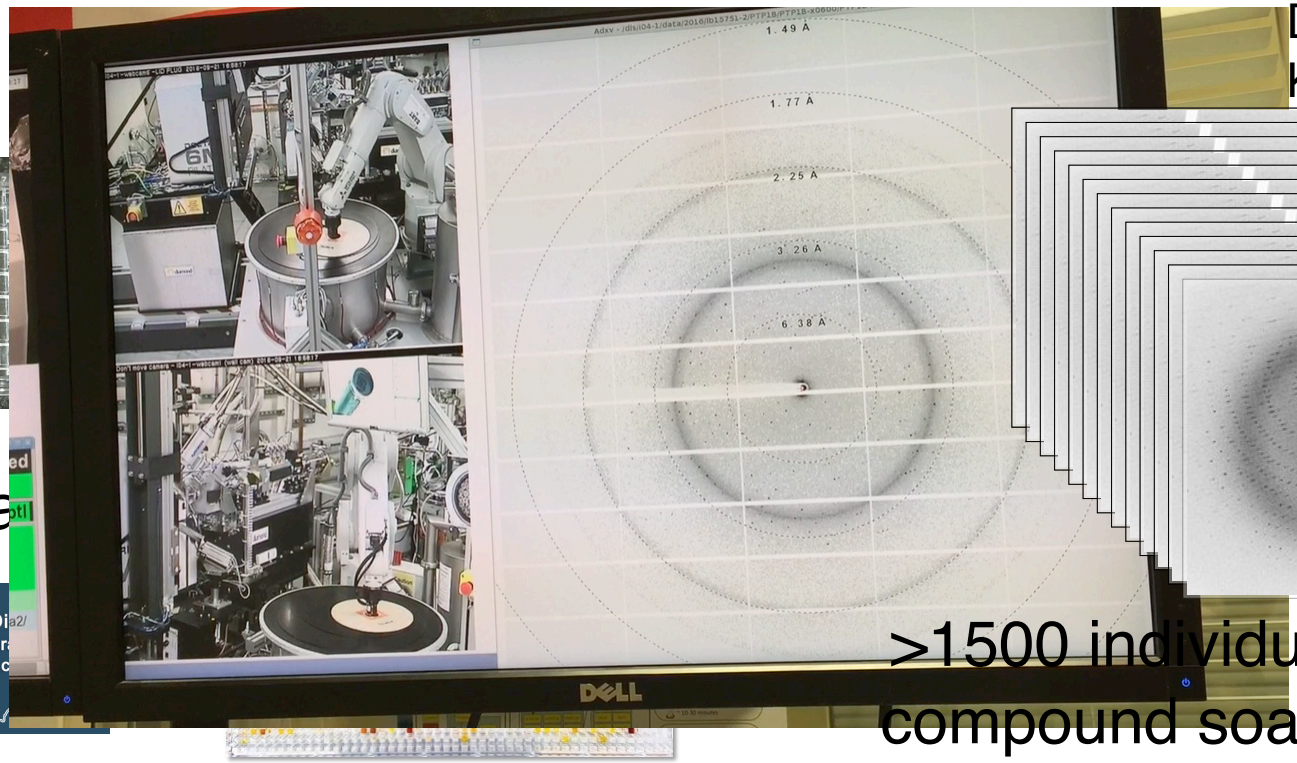
Blundell, Jhoti, Abell
Nat Rev Drug Disc, 2012

Subsequent fragment assembly can increase affinity

We beta-tested a new **fragment-soaking** pipeline at Diamond synchrotron


Daniel Keedy Justin Biel

collabora



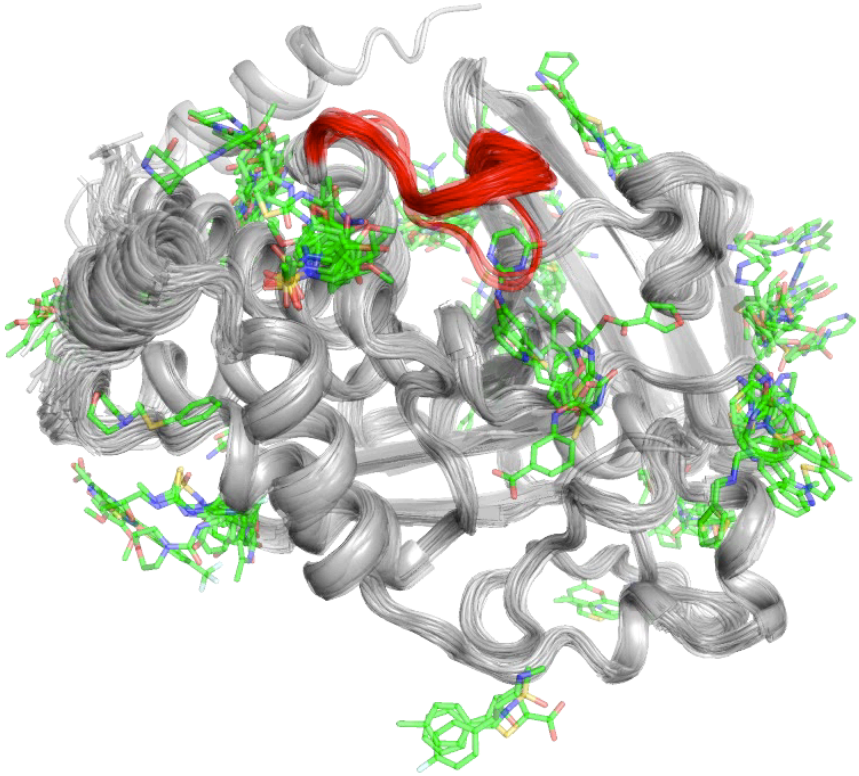
XCHEM

>1500 individual compound soaks and datasets!

Frank von Delft

Collins…von Delft, Acta Cryst D, 2016

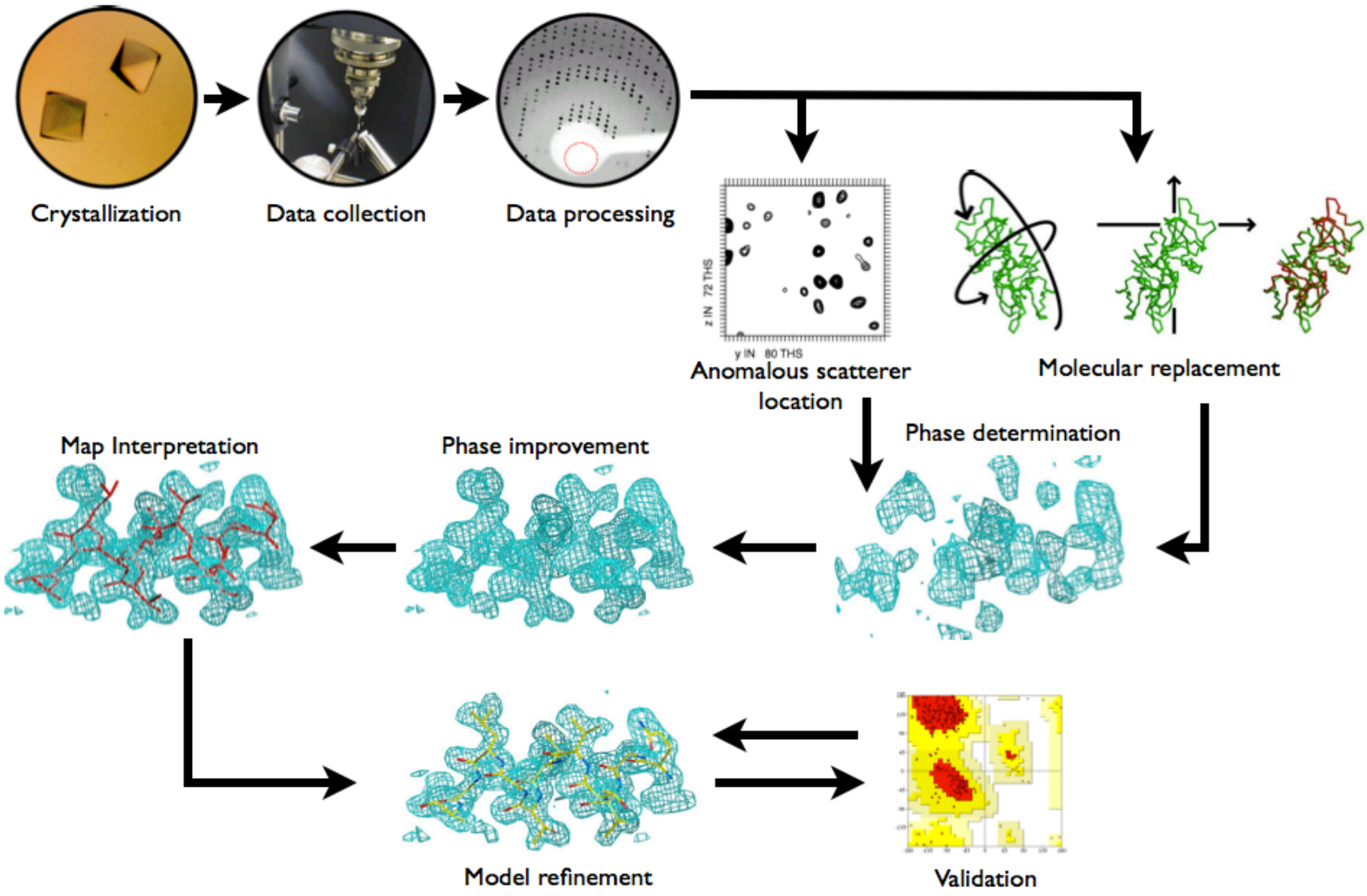
… **~150** datasets reveal well-justified all-atom **ligand binding poses** — and protein responses



Daniel Keedy

Justin Biel

Keedy…Fraser, eLife, 2018

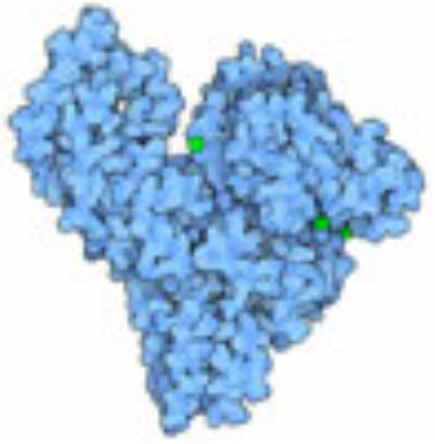

Crystallization
Data collection
Data processing
z IN 72 THS
y IN 80 THS
Anomalous scatterer location
Molecular replacement
Phase determination
Phase improvement
Map Interpretation
Model refinement
Validation

Fourier transforms 101

http://www.jezzamon.com/fourier/

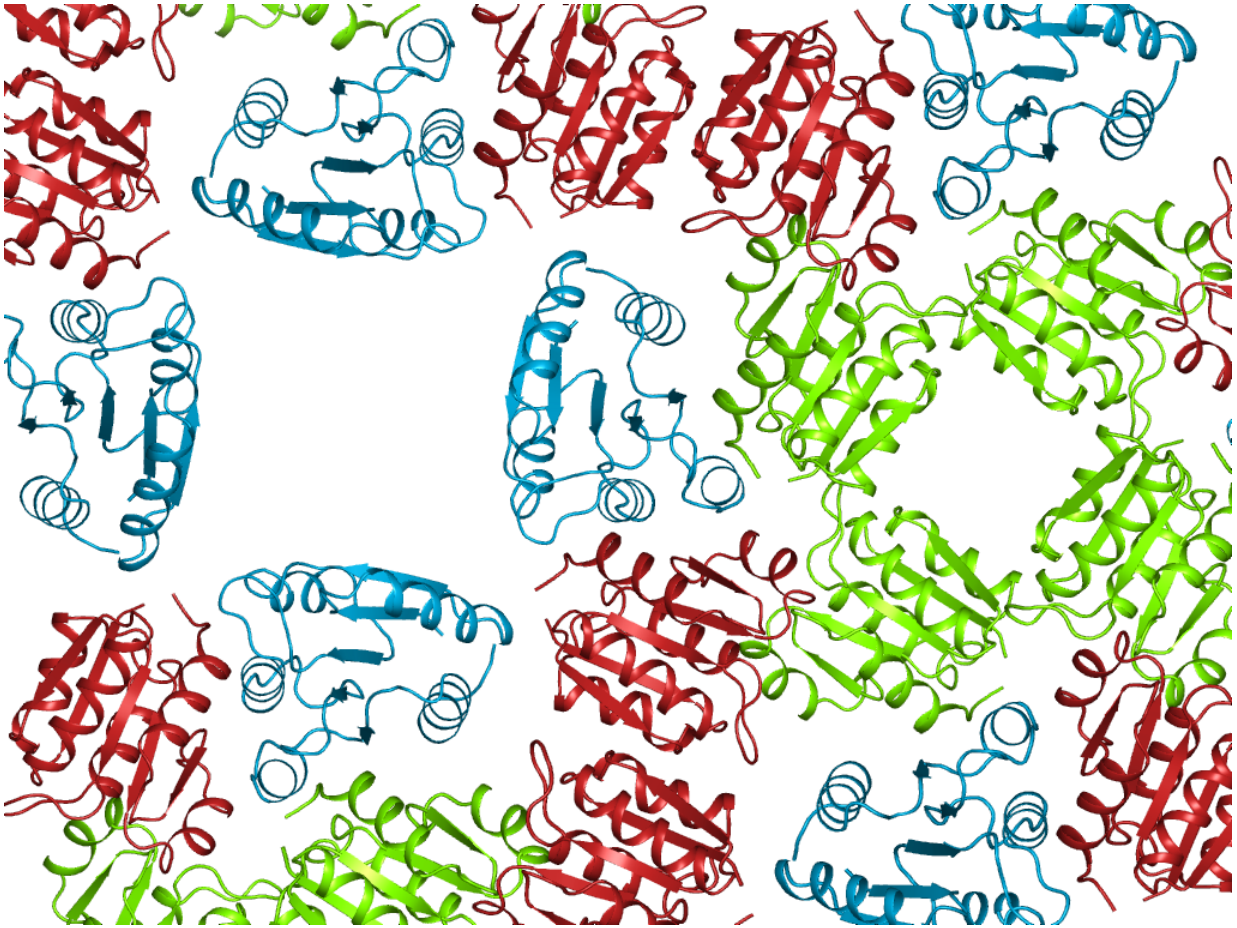
What is a protein structure?

What is a protein "structure"



- Is it a:
  - pretty cartoon...
  - space-filling set of spheres...
  - picture of the protein in the crystal...
  - computational picture of the protein...
  - representation of atoms that satisfies experimental constraints...
  - PDB formatted text file...
  - **model!!!**

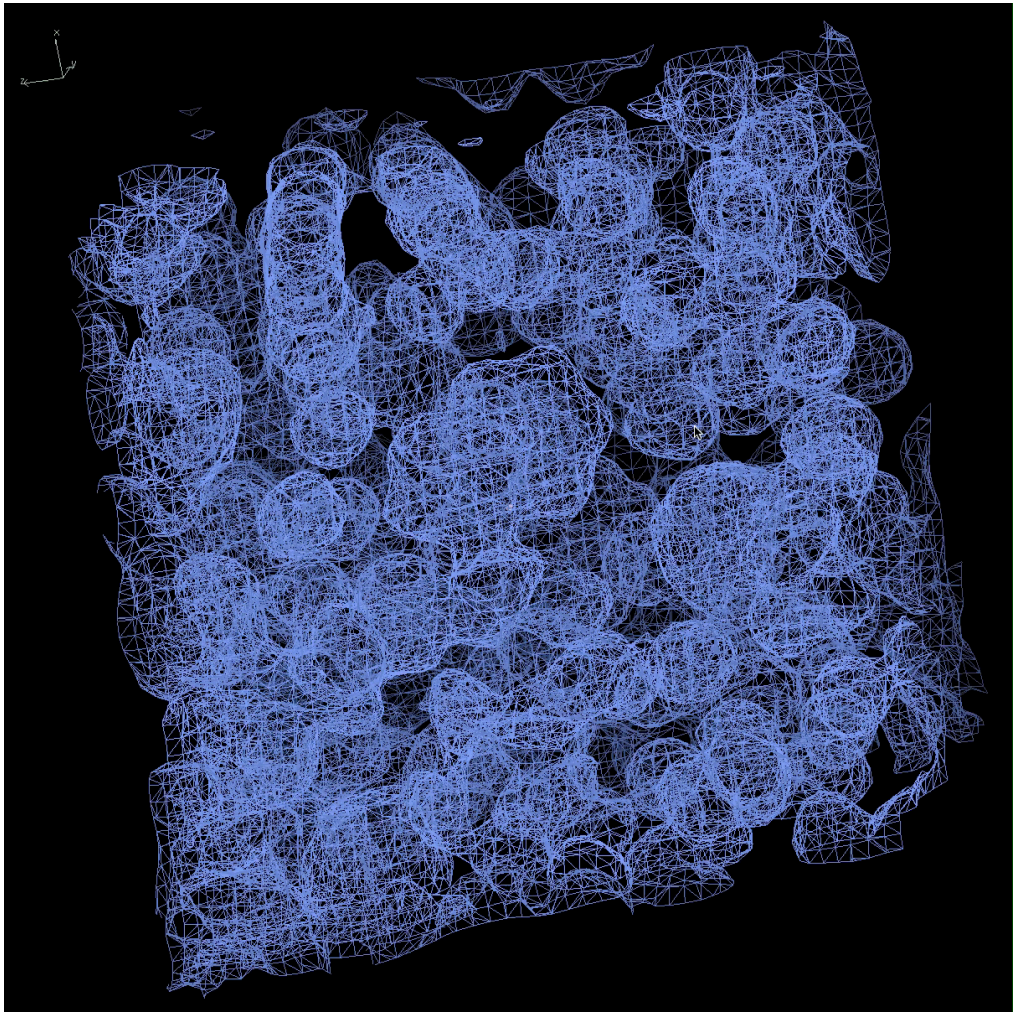
Moreover... a model of the crystal lattice...



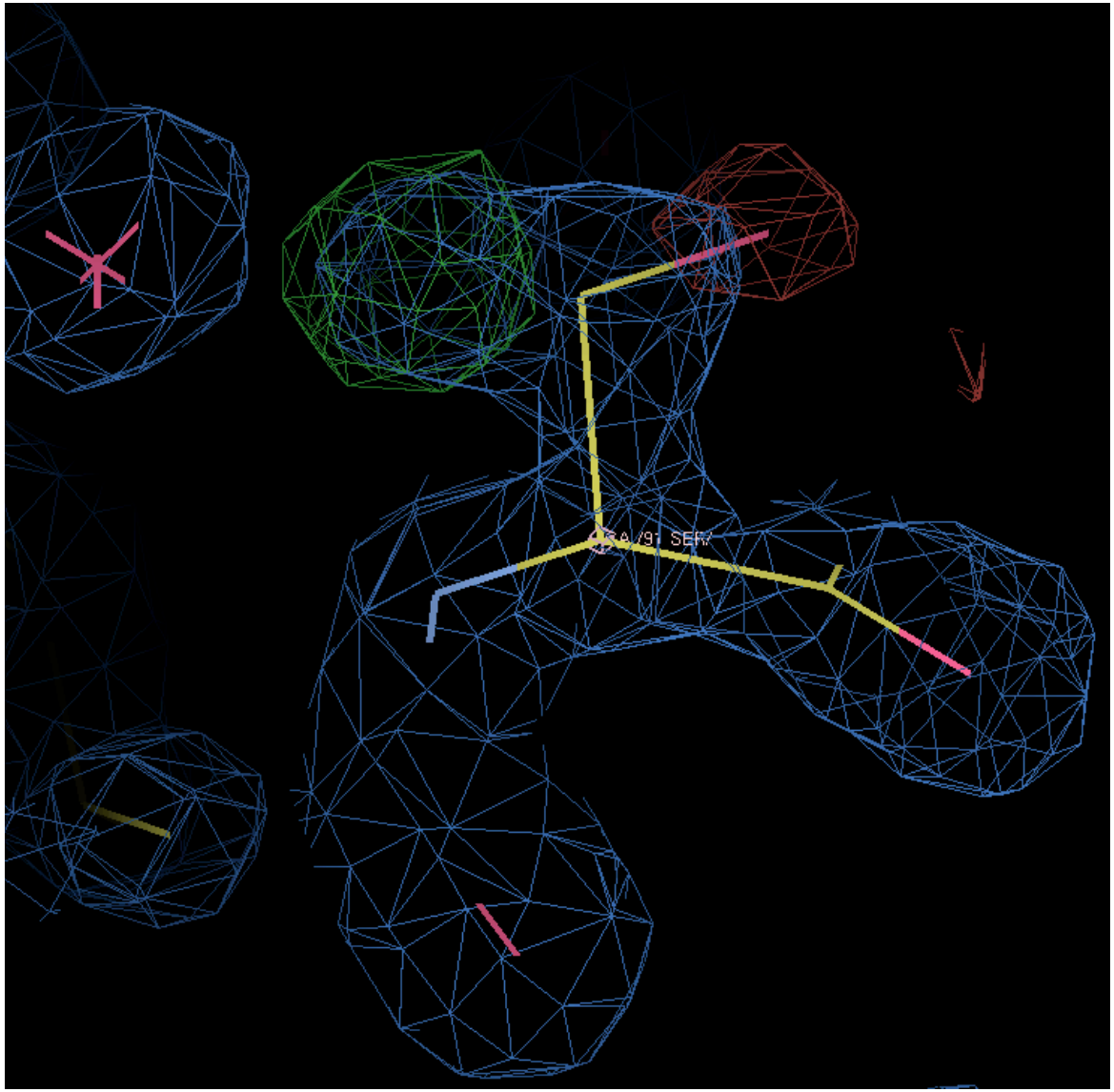
Protein Data Bank Files are text:

chemistry, sequence, position, certainty

```
HEADER    HYDROLASE                               10-DEC-06   2O7A
TITLE     T4 LYSOZYME C-TERMINAL FRAGMENT
COMPND    MOL_ID: 1;
COMPND   2 MOLECULE: LYSOZYME;
                                  ...

REMARK   3  FIT TO DATA USED IN REFINEMENT (NO CUTOFF).
REMARK   3   R VALUE    (WORKING + TEST SET, NO CUTOFF) : NULL
REMARK   3   R VALUE          (WORKING SET, NO CUTOFF) : 0.090
REMARK   3   FREE R VALUE                (NO CUTOFF) : 0.108
                                  ...

ATOM      1  N   VAL A   2     -19.742  -2.254 -19.976  1.00 54.44           N
ATOM      2  CA  VAL A   2     -19.867  -2.152 -18.529  1.00 54.48           C
ATOM      3  C   VAL A   2     -19.073  -0.927 -18.101  1.00 41.86           C
ATOM      4  O   VAL A   2     -19.367   0.178 -18.554  1.00 47.57           O
ATOM      5  CB  VAL A   2     -19.341  -3.411 -17.836  1.00 68.76           C
                                  ...

MASTER      287    0    3   10    0    0    0    6 1566    1   22   10
END
```


x
y
z

- **Ser residue needs a different rotamer**



Refinement is the process of minimizing Fo-Fc

...need to balance prior knowledge and data

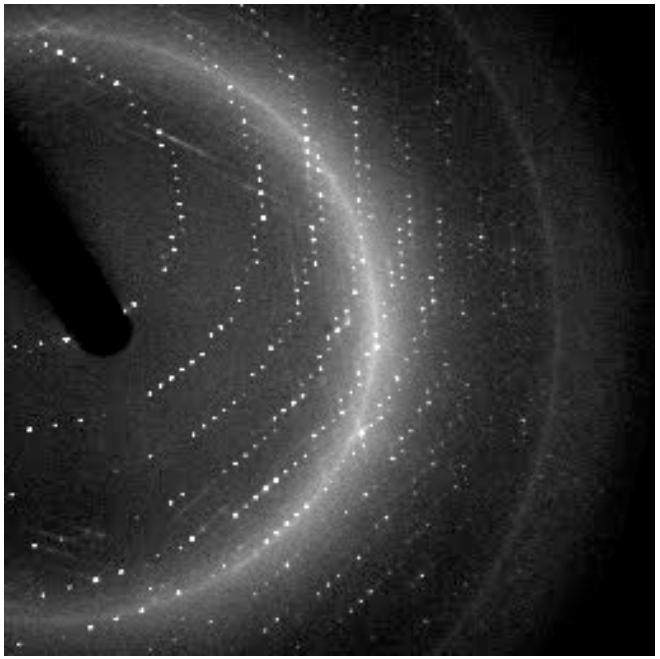
...an iterative process, difference maps minimized, and 2Fo-Fc maps improve (phases... we are coming to this)

- **Structure refinement** is a process of changing a model parameters in order to optimize a goal (target) function:

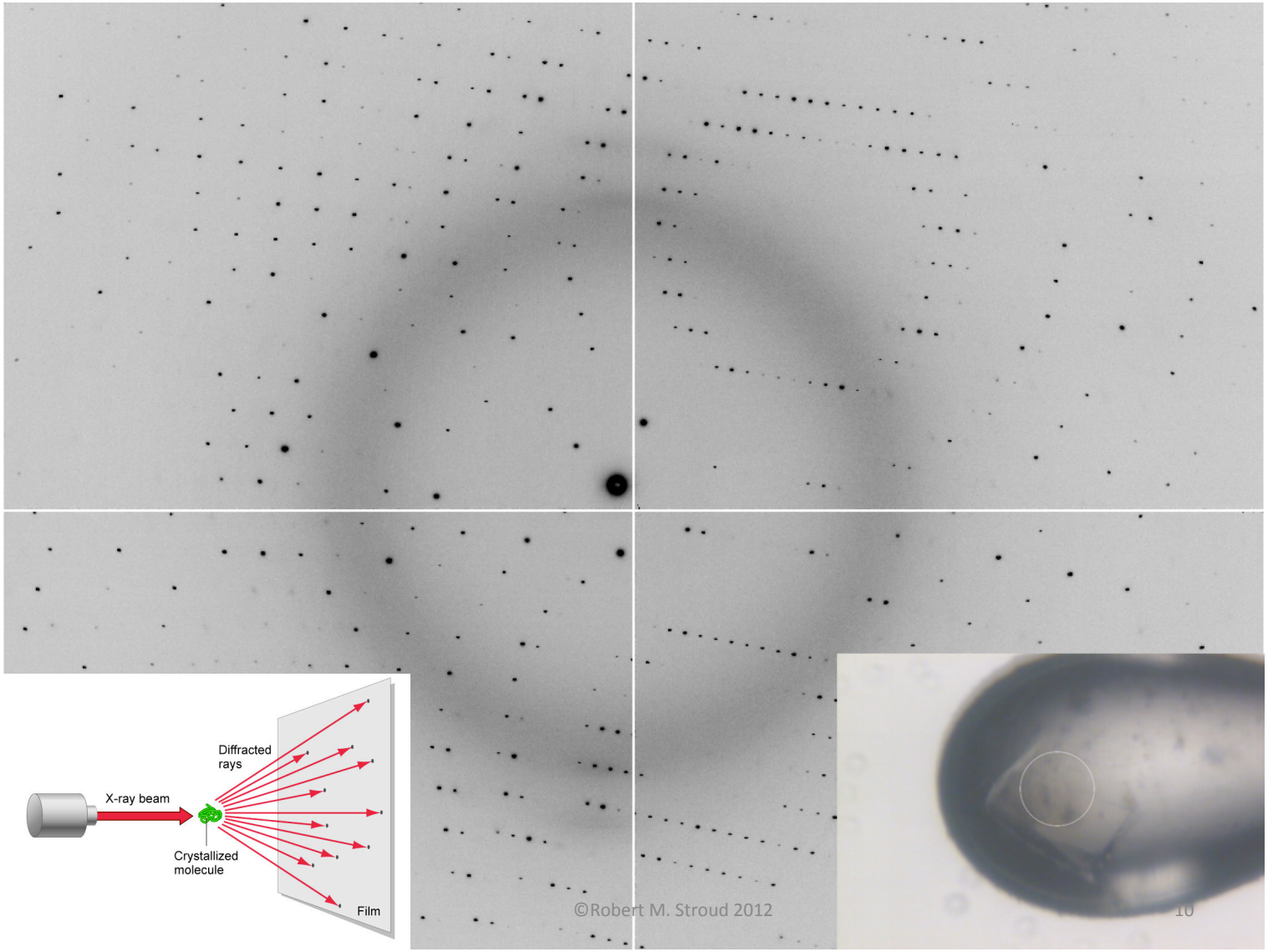
  $T = F$(Experimental data, Model parameters, *A priori* knowledge)

  - Experimental data – a set of diffraction amplitudes Fobs (and phases, if available).
  - Model parameters: coordinates, ADP, occupancies, bulk-solvent, …
  - *A priori* knowledge (restraints or constraints) – additional information that may be introduced to compensate for the insufficiency of experimental data (finite resolution, poor data-to-parameters ratio)

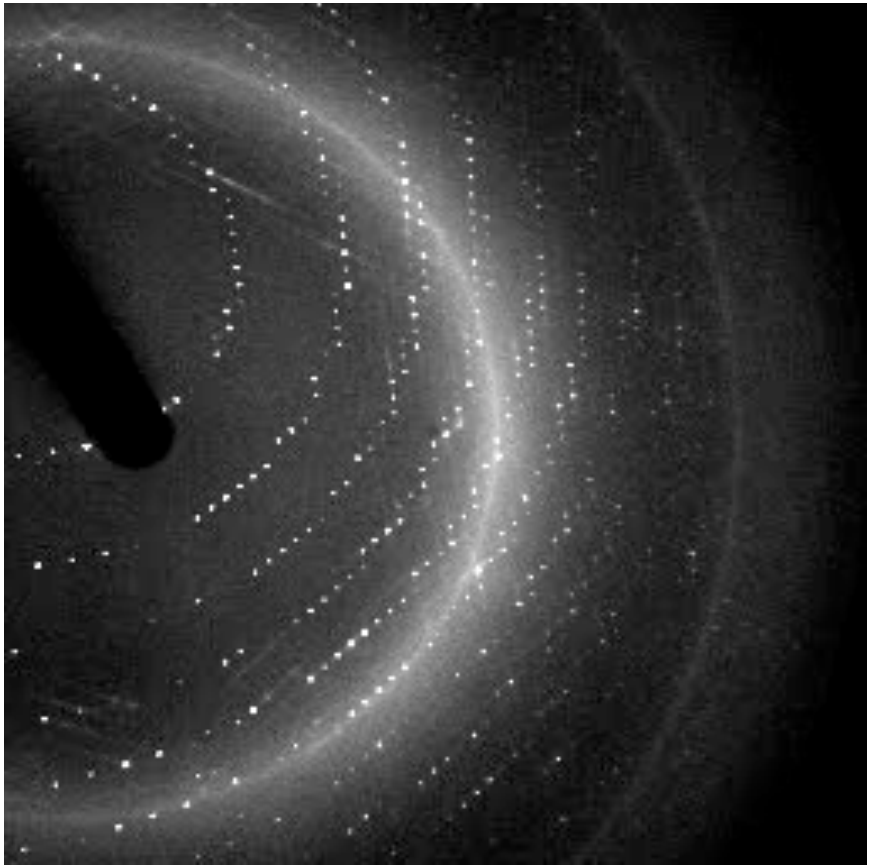
- Typically: $T = T_{DATA} + w * T_{RESTRAINTS}$
  - E_{DATA} relates model to experimental data
  - $E_{RESTRAINTS}$ represents *a priori* knowledge
  - w is a weight to balance the relative contribution of E_{DATA} and $E_{RESTRAINTS}$



We rotate the crystal to place a different set of reflections on the detector



Ewald sphere construction



given:

wavelength

angle

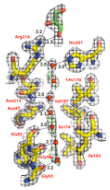
lattice

distance from detector

orientation of lattice relative to detector

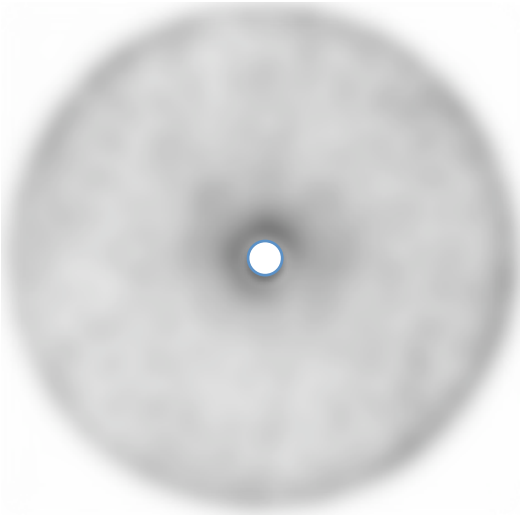
predicts:

which diffracted waves satisfy Bragg's law



FT

FT^{-1}



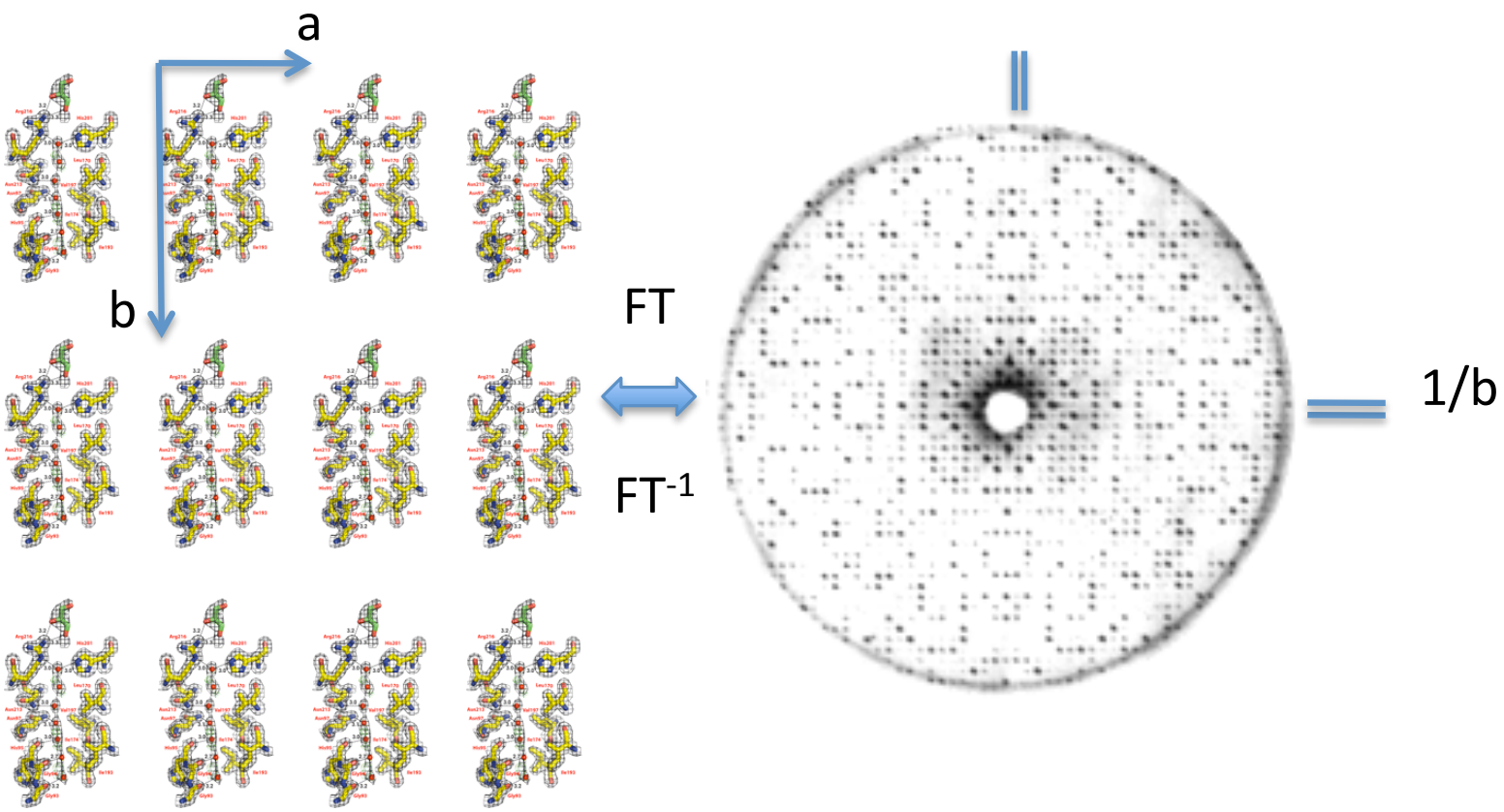
Scattering pattern is the Fourier transform of the structure

$$\mathbf{F(\underline{S})} = \Sigma_j f_j e^{(2\pi i \mathbf{rj.S})}$$

Structure is the 'inverse' Fourier transform of the Scattering pattern

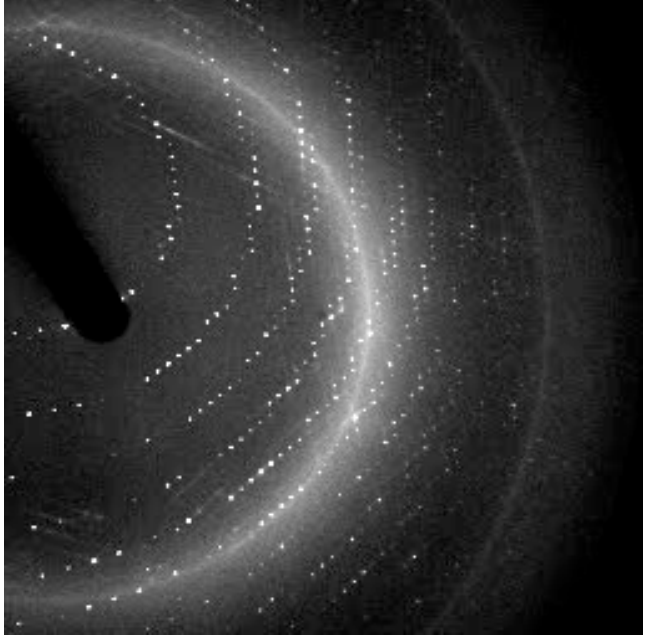
$$\rho(\underline{\mathbf{r}}) = \Sigma \mathbf{F(\underline{S})} e^{(-2\pi i \mathbf{r.S})}$$

A crystal only samples the parts of the transform that satisfy Bragg's Law



$$\underline{F}_{(h,k,l)} = \Sigma_j f_j e^{(2\pi i\ (hx+ky+lz))}$$

Every X-ray reflection (h,k,l) has a contributing wave from all atoms .

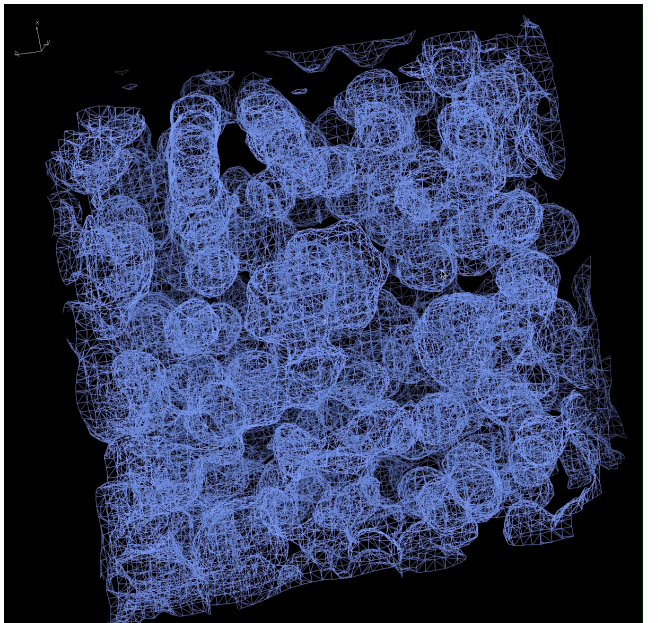


$$\rho(x,y,z) = \Sigma \underline{F}_{(h,k,l)}\ e^{(-2\pi i(hx+ky+lz))}$$

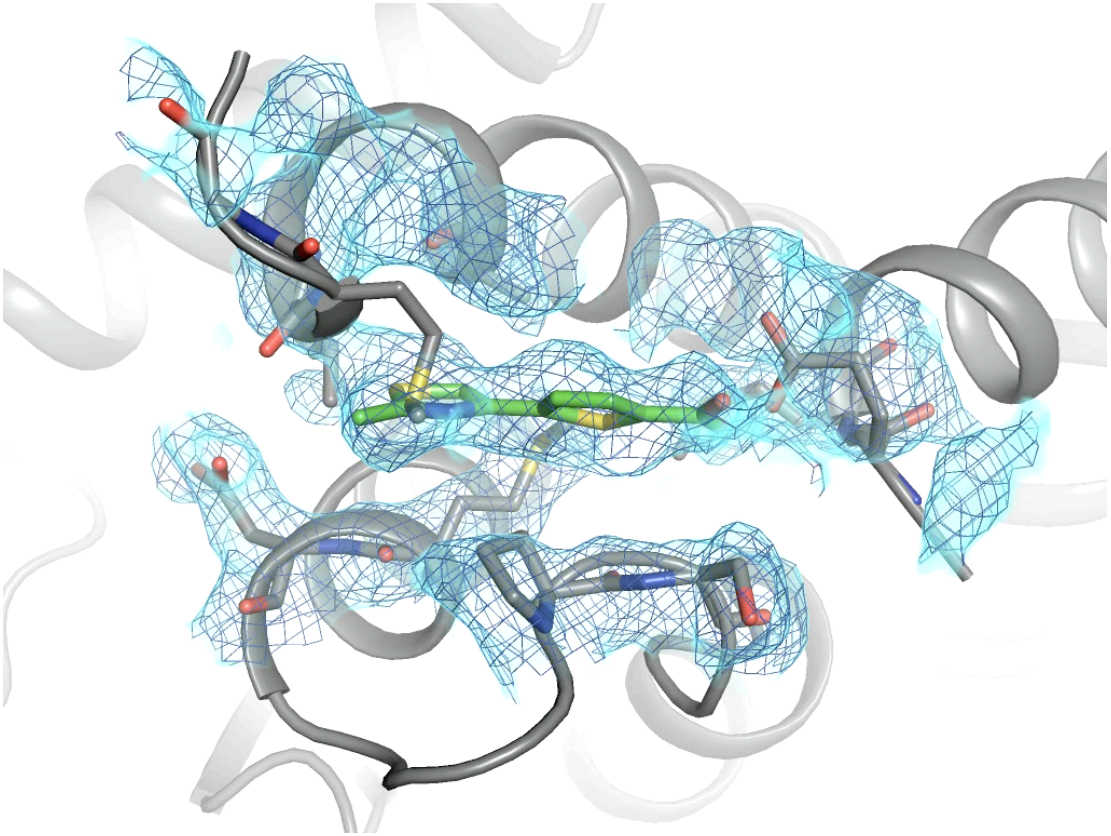
Fourier Transform

or $$\rho(x,y,z) = \Sigma |\underline{F}_{(h,k,l)}|\ e^{(-2\pi i(hx+ky+lz) + \phi_{hkl})}$$

Every point in the density map has contributions from every reflection



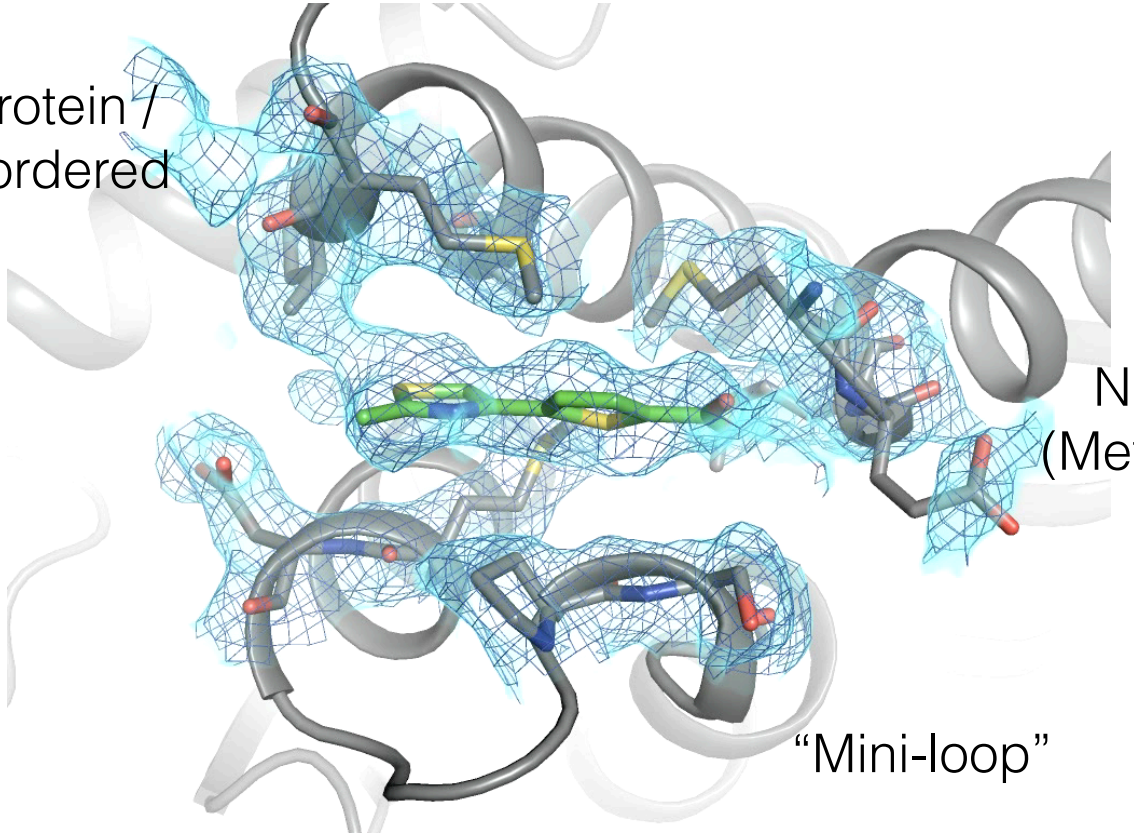
Crystallography reveals binding mode and conformational changes



Event
72% bg sub
1.25σ

Fragments at the “mini-loop” cryptic site induce movement of the α6-α7 transition and N-terminus

Shifted
end of ordered protein /
Start of quasi-disordered
α7 helix



N-terminus
(Met1 **ordered**)

“Mini-loop”

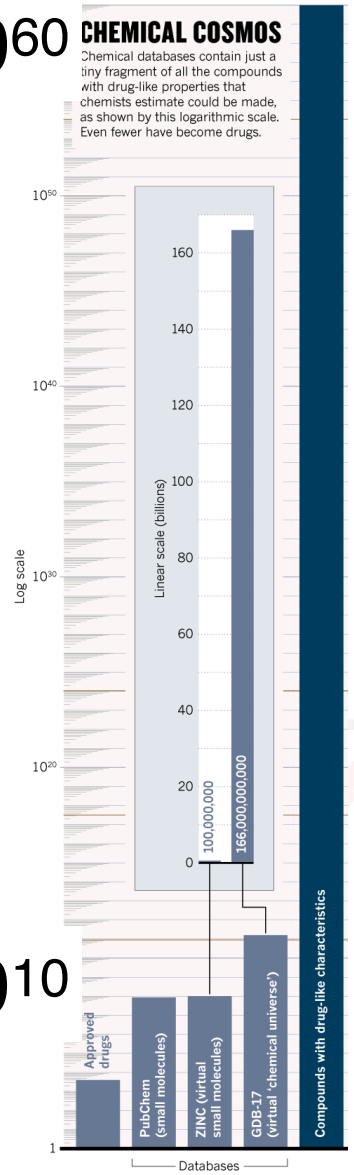
Event
72% bg sub
1.25σ

High throughput screening

Library
30 heavy atoms
~10^6

10^{60}

Vast undersampling!



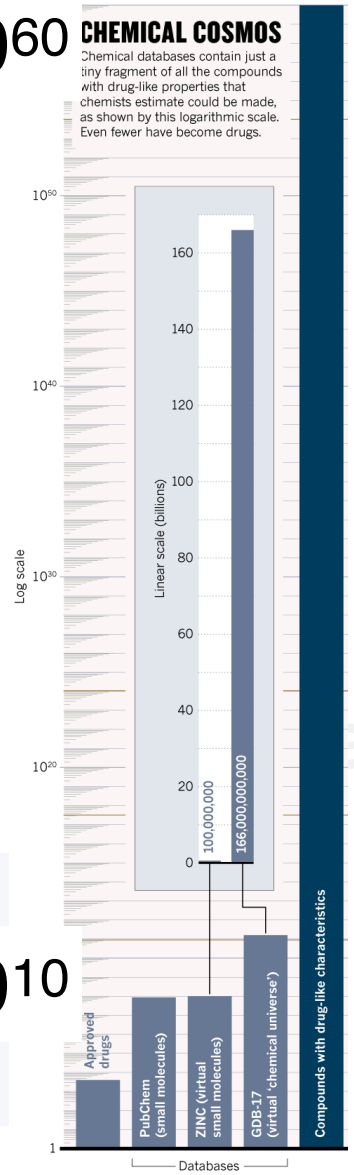
10^{10}

Fragment based drug discovery

Library
15 heavy atoms
~10^5

Less undersampling

10^{60}



10^{10}

Evaluate WEAK binding

Rational optimize

Candidate

ead