

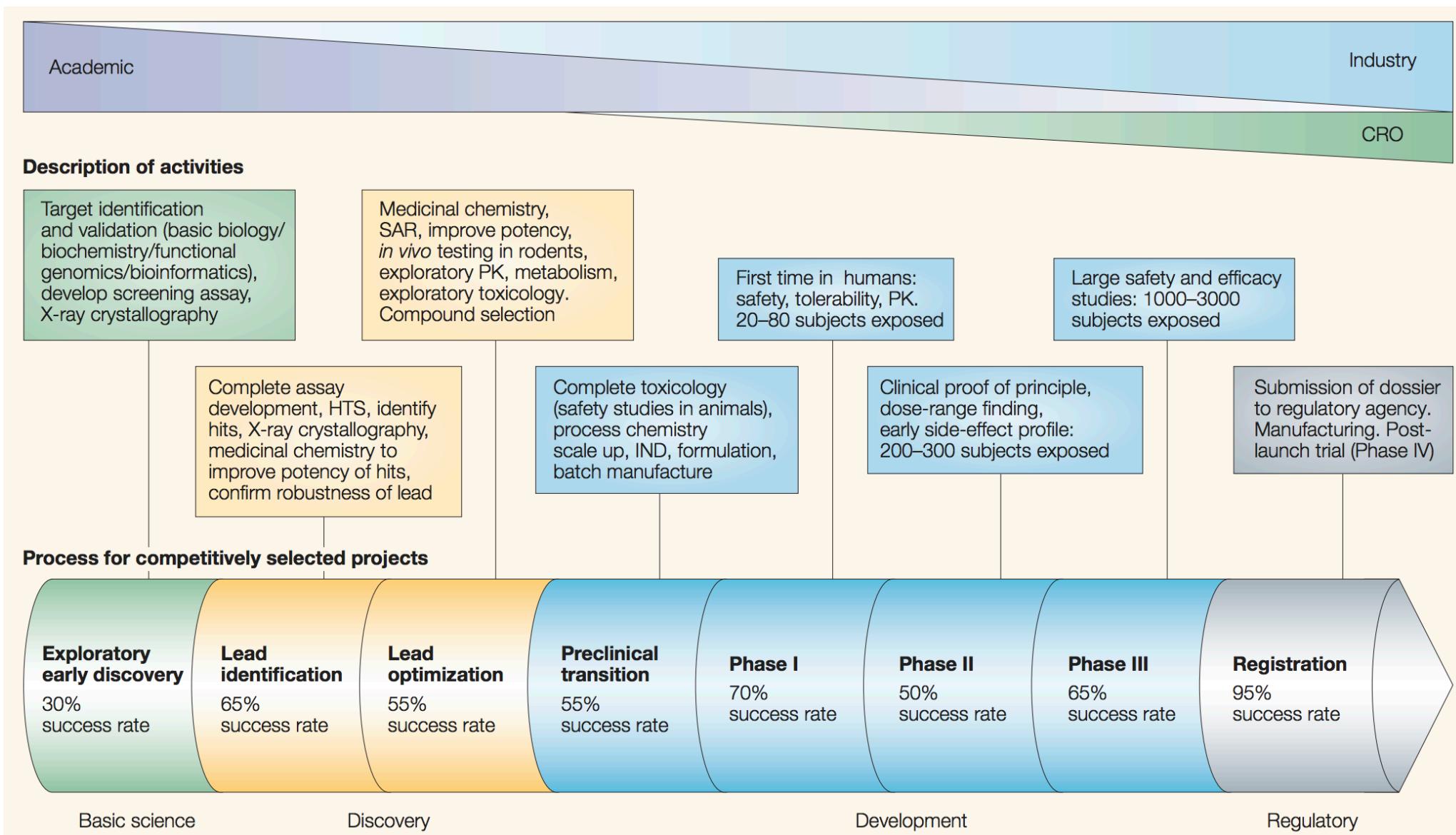
# Integrative biophysics for drug discovery

Aashish Manglik  
[Aashish.Manglik@ucsf.edu](mailto:Aashish.Manglik@ucsf.edu)

# Outline

- Drug discovery – finding needles in haystacks
- Hitchhiker's guide to chemical space
- Theory of fragment-based drug discovery
- Integrative biophysics enabling drug discovery

# Drug discovery



# Drug discovery

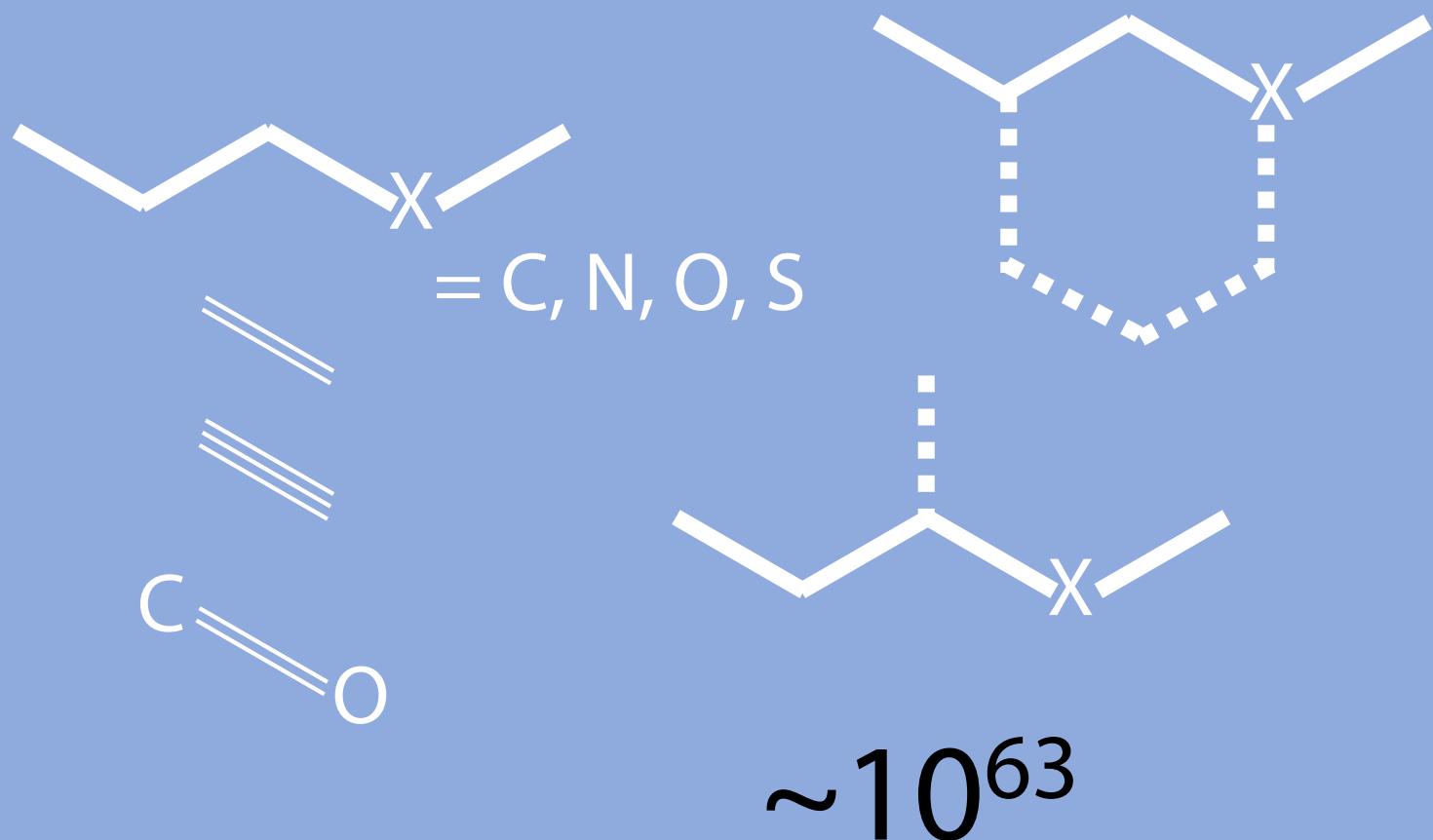


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# Chemical space

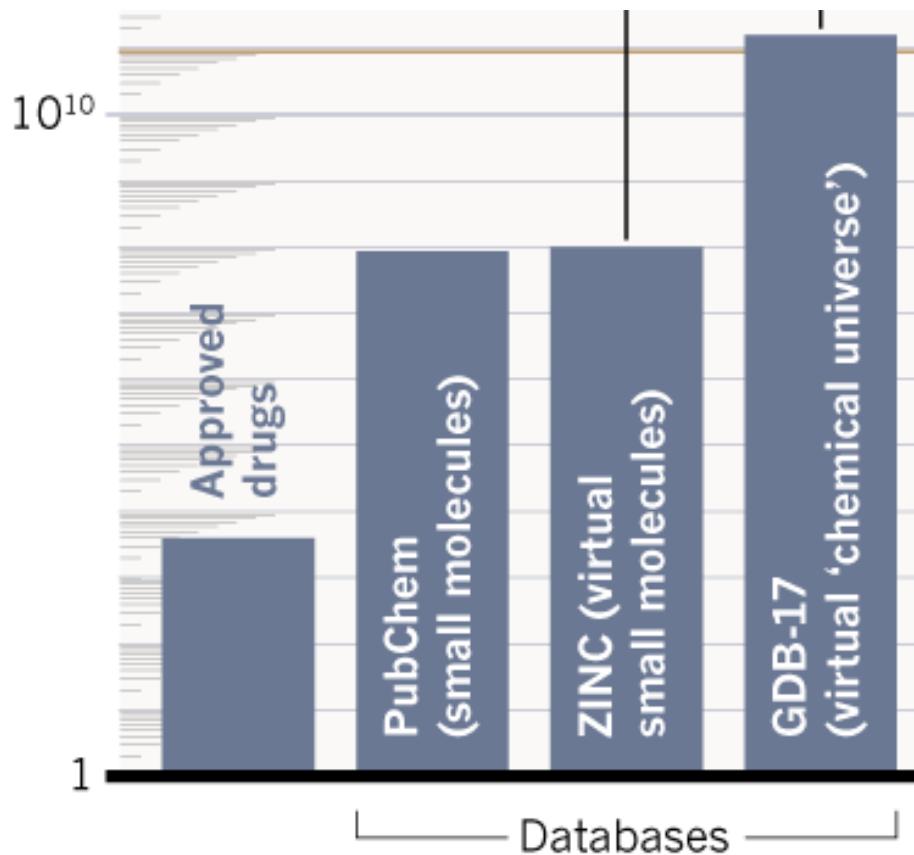
Possible compounds with <600 Da



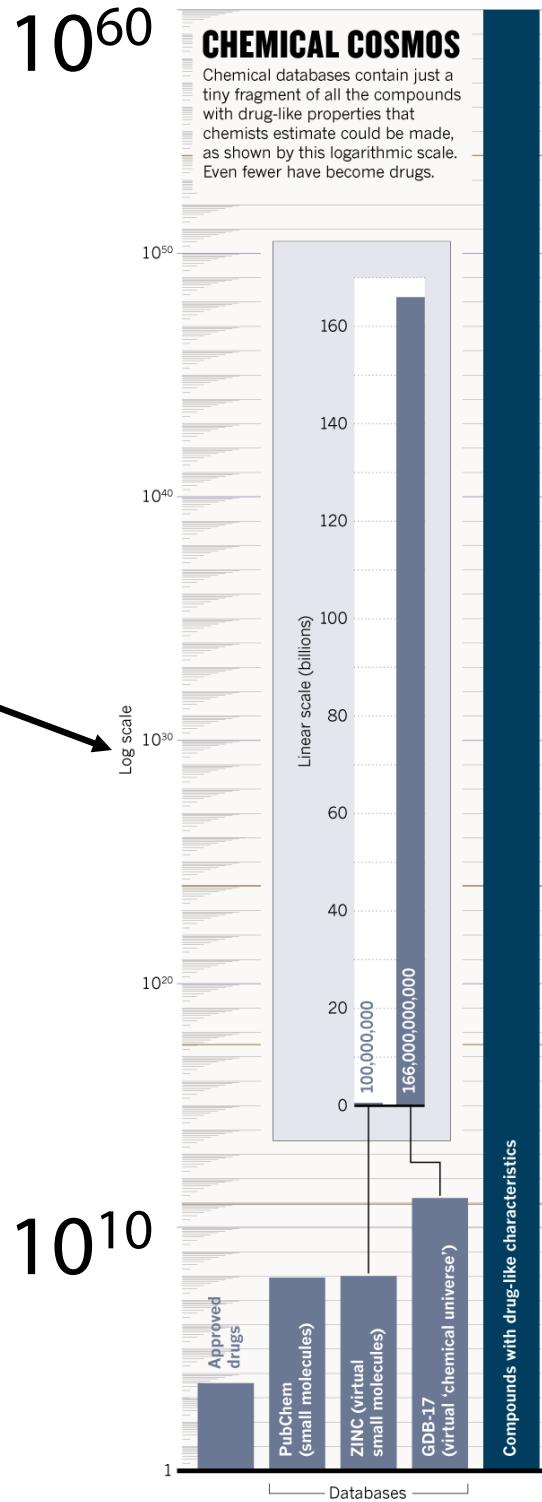
# Chemical space is Y(h)uge!



# Chemical space is Y(h)uge!



Log scale!



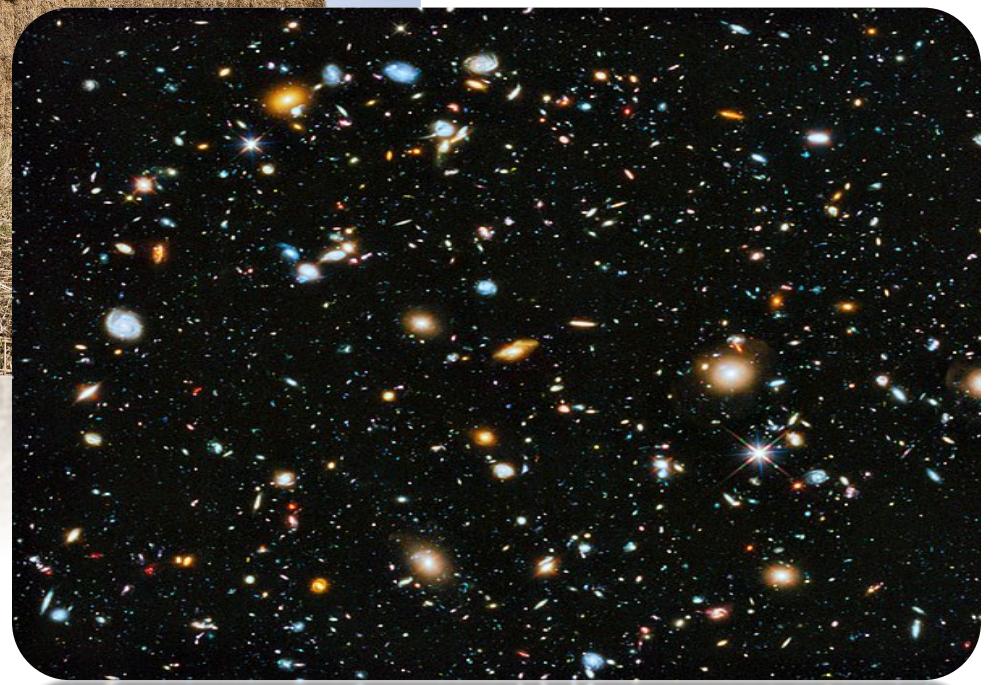
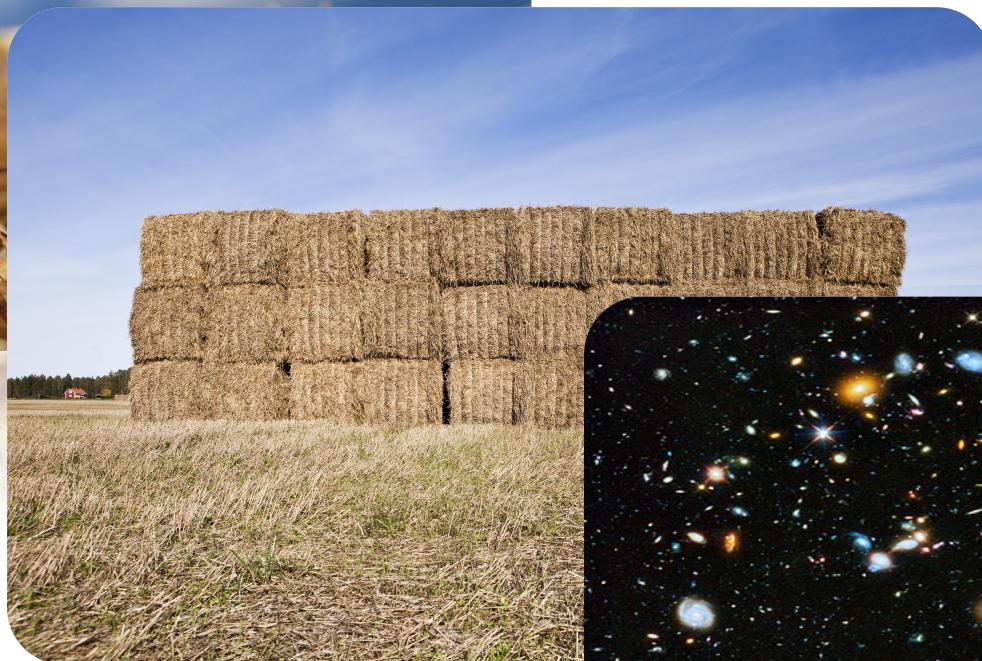
Atoms in the solar system

Stars in the universe

Neurons in the human brain

Mullard A *Nature* 549,445 (2017)

# Needles in enormous haystacks



Finding that rare needle...

# High throughput screening

**Library**

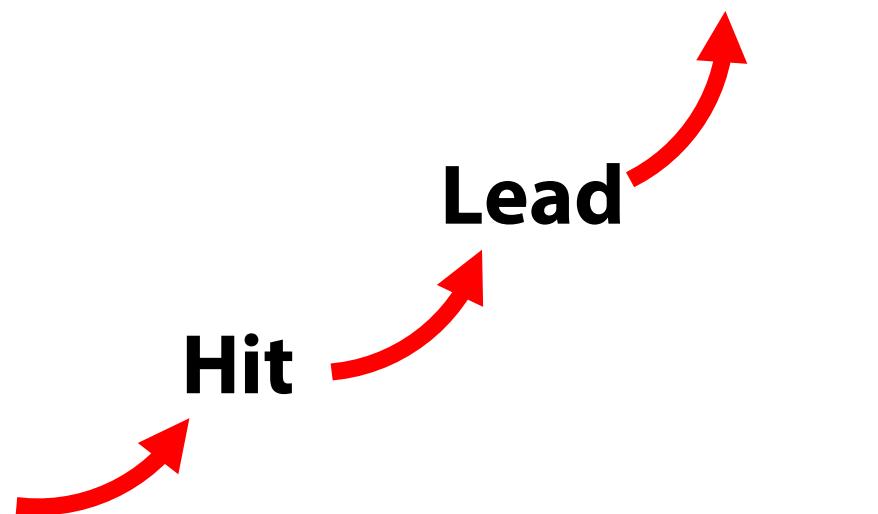
30 heavy atoms  
 $\sim 10^6\text{-}10^8$



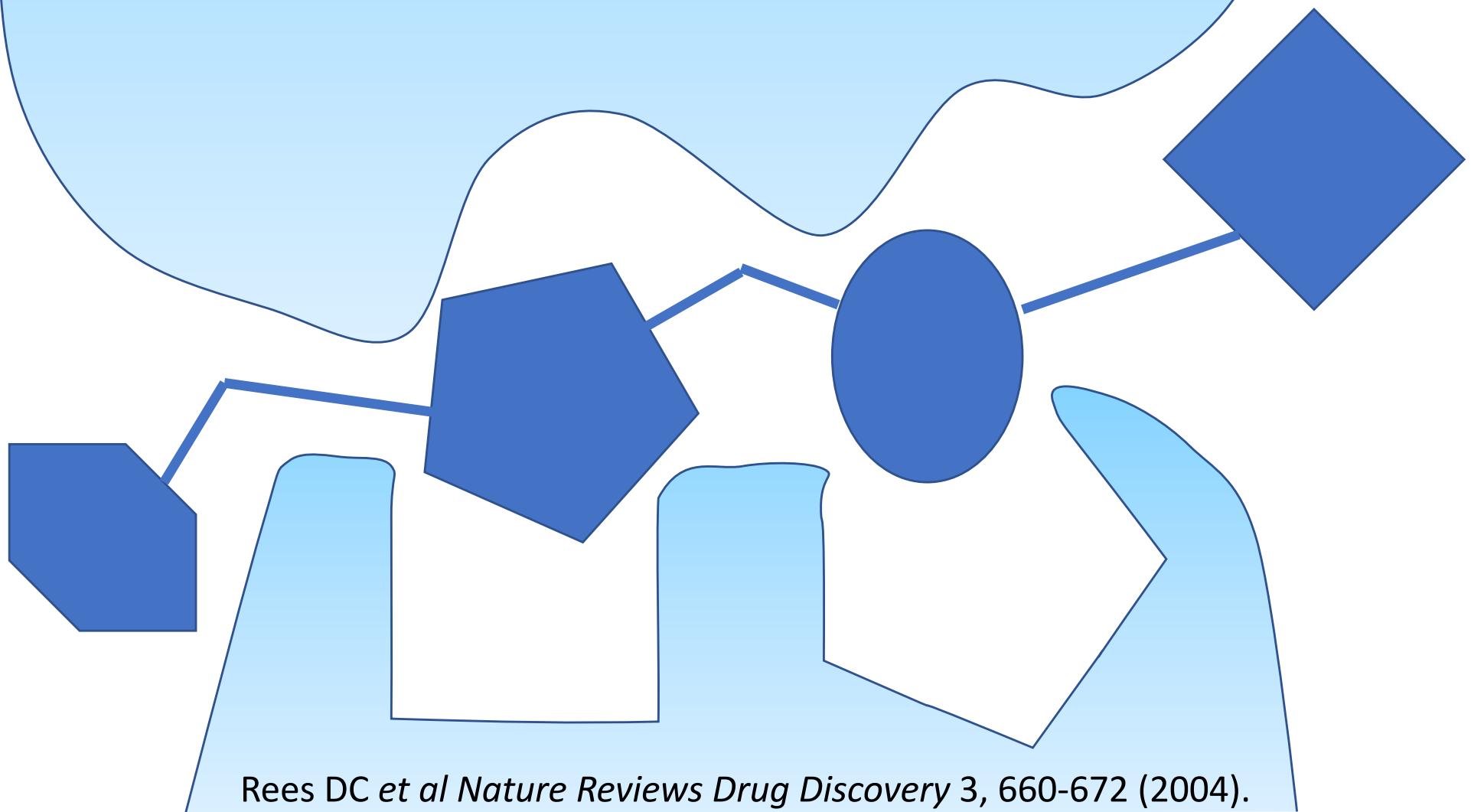
**Candidate**

**Lead**

**Hit**



# Drug discovery – HTS hit



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

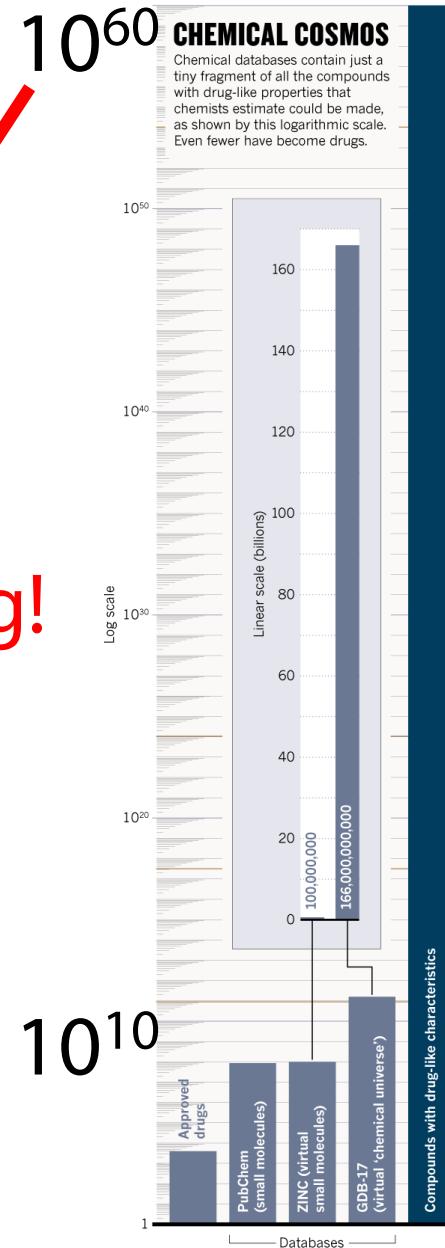
# High throughput screening

## Library

30 heavy atoms  
 $\sim 10^6\text{-}10^8$



Vast undersampling!

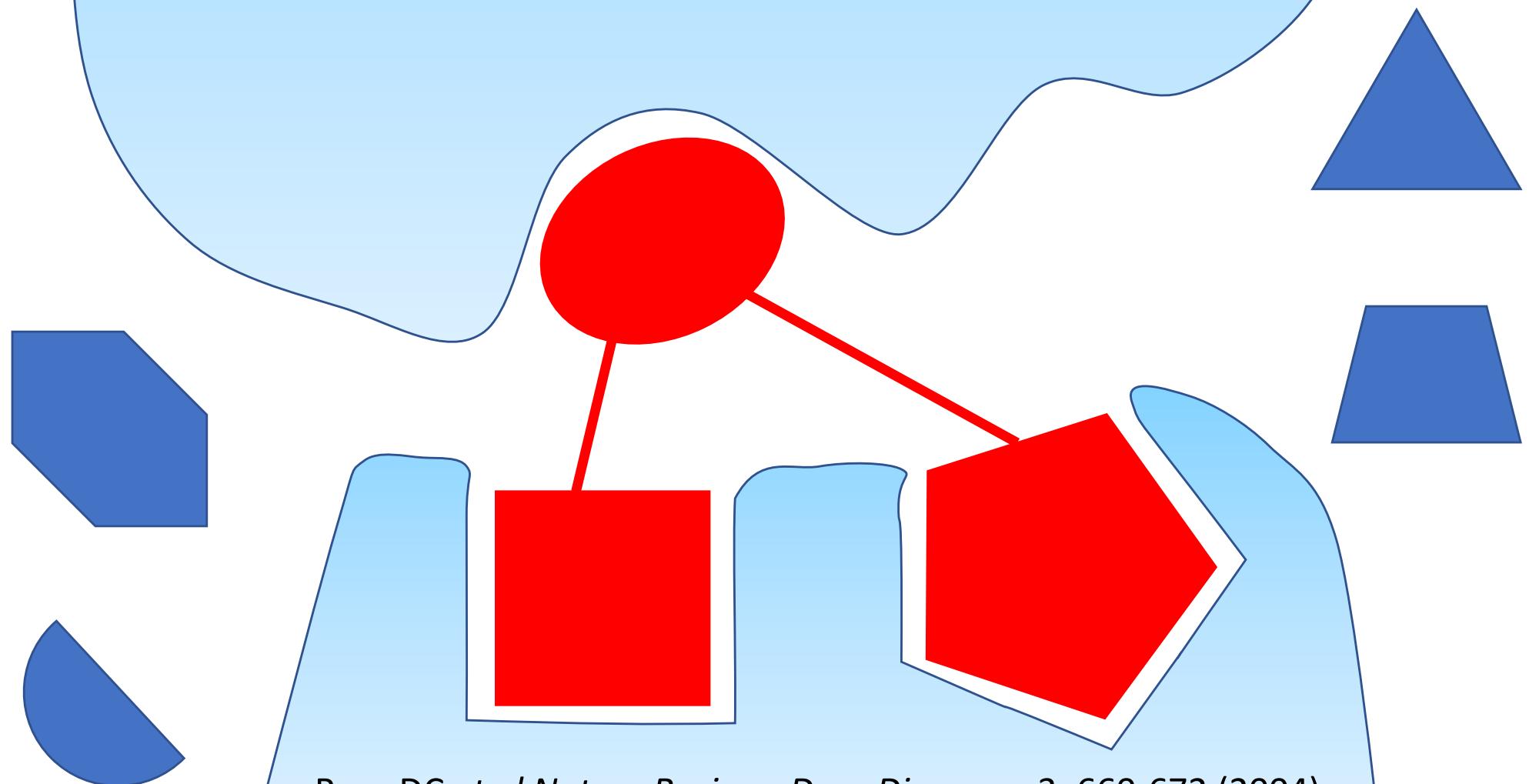


Candidate  
Lead

# Outline

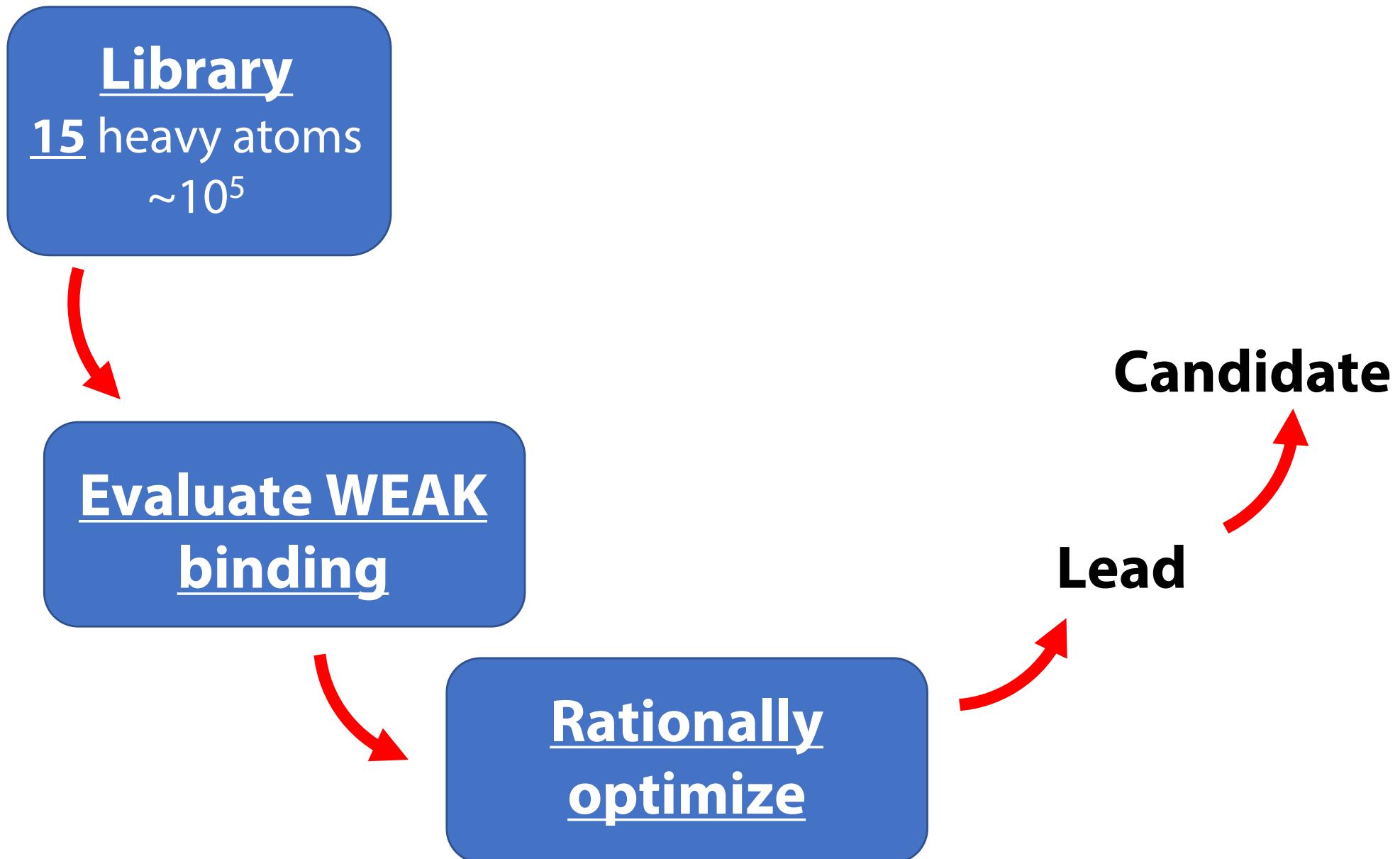
- Drug discovery – finding needles in haystacks
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# Fragment based drug discovery



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

# Fragment based drug discovery



# Fragment based drug discovery

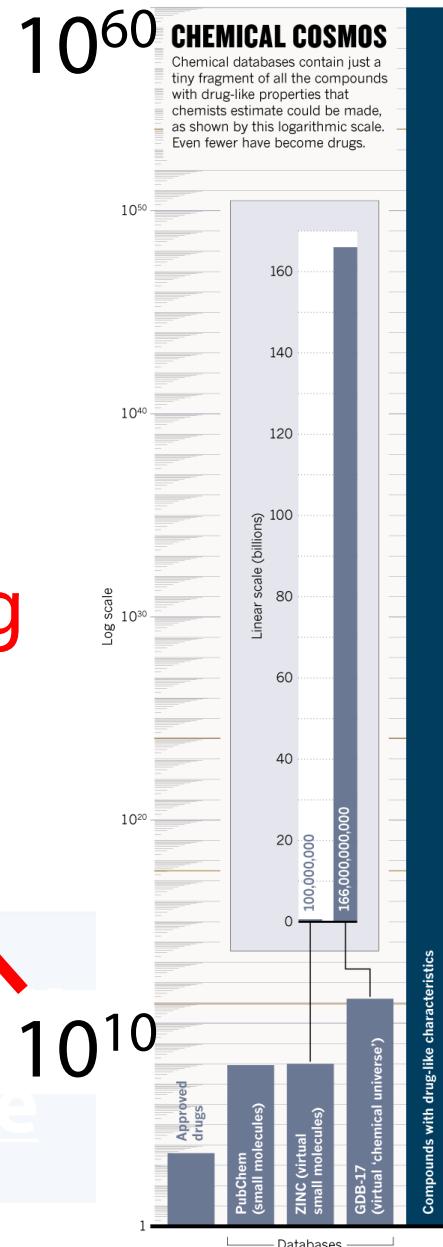
Library

15 heavy atoms  
 $\sim 10^5$

Less undersampling

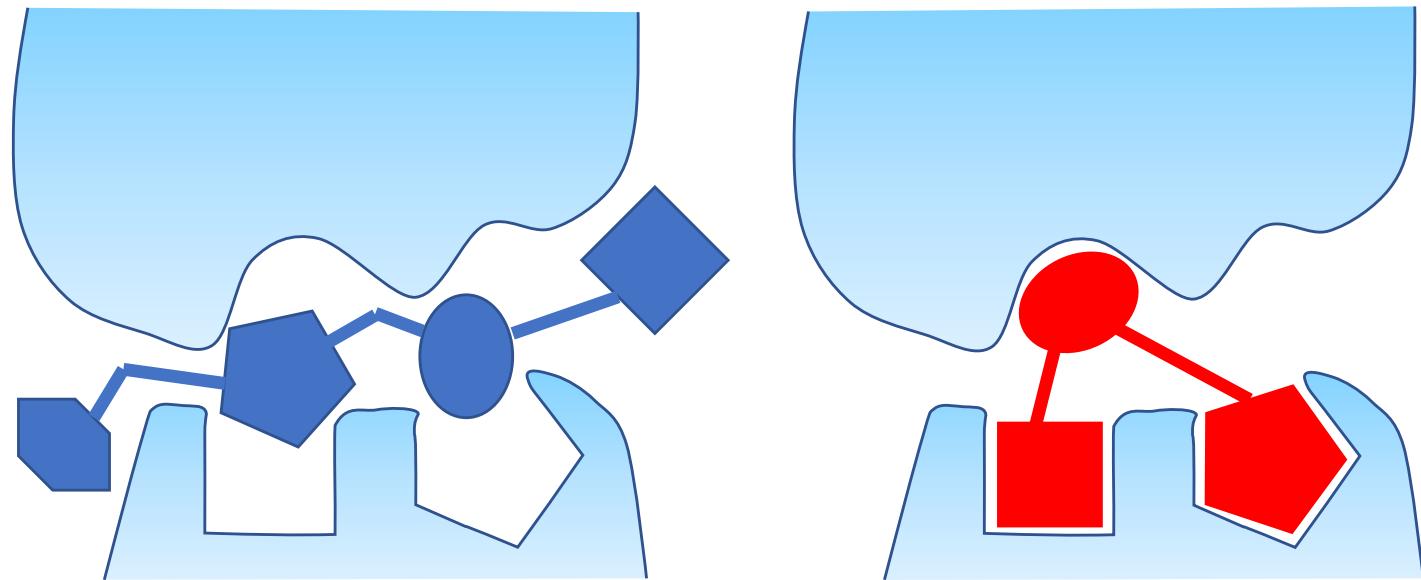
Evaluate WEAK  
binding

Rational  
optimize



Candidate lead

# 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	$\mu\text{M}$	$\text{mM}$
Optimization	Fixing problems, improving affinity	Iterative improvement
Main downside	Attrition, can't solve "challenging" targets	Biophysical methods are hard!

# Fragment based drug discovery

Library

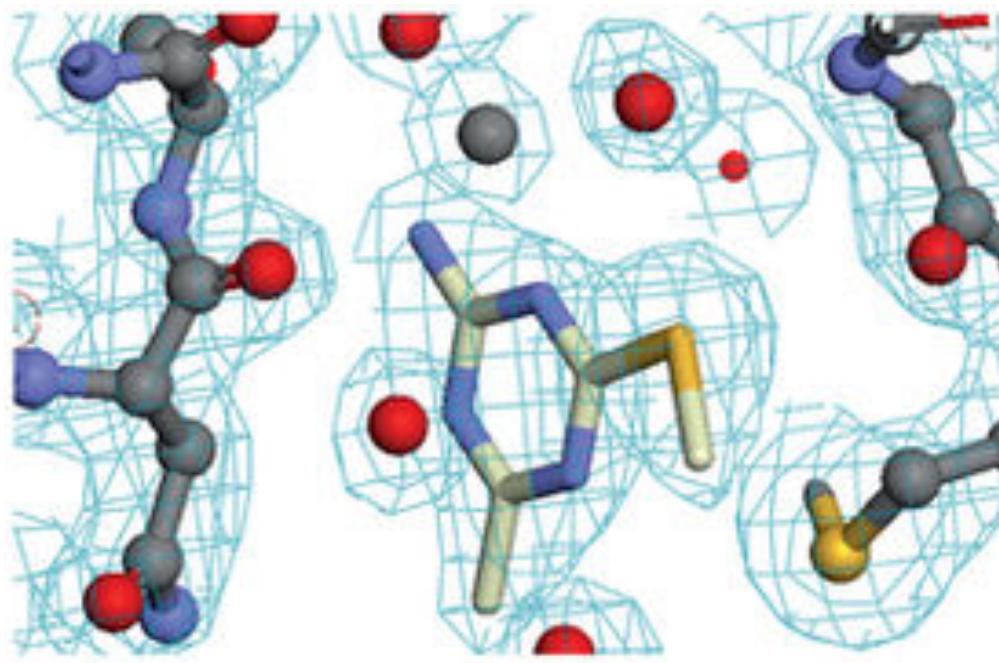
15 heavy atoms  
 $\sim 10^5$

Evaluate WEAK  
binding

Rationally  
optimize

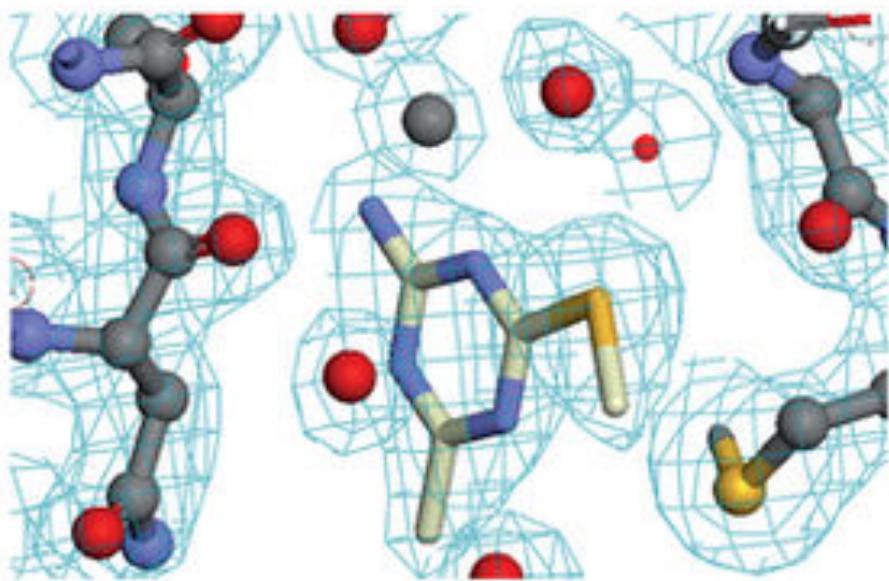
Lead

# Assessing drug-target interaction

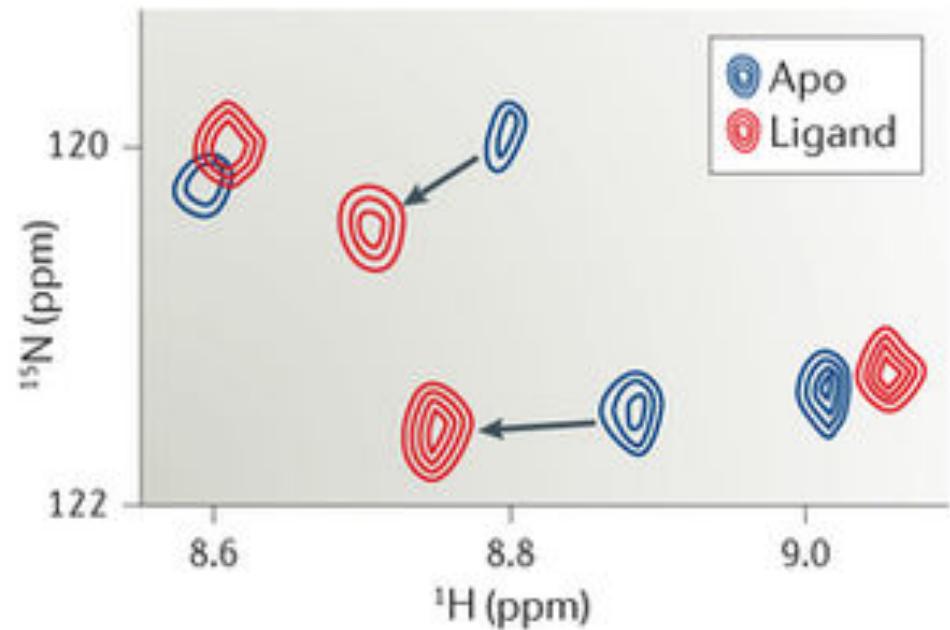


High resolution X-ray (or Cryo-EM) structure

# Assessing drug-target interaction

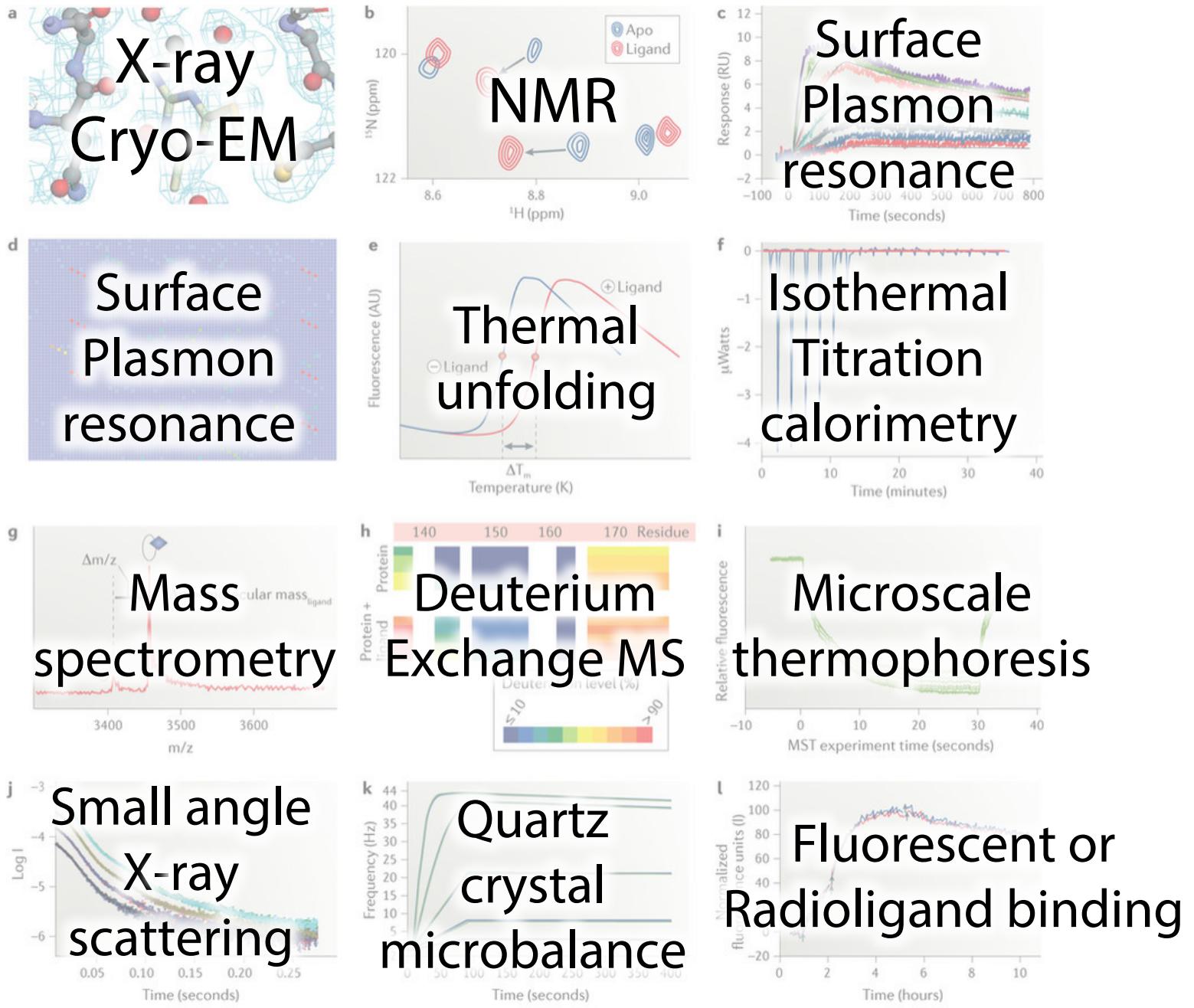


X-ray  
Cryo-EM



NMR

# Assessing drug-target interaction



# Fragment based drug discovery

Library

15 heavy atoms  
 $\sim 10^5$

Evaluate WEAK  
binding

Rationally  
optimize

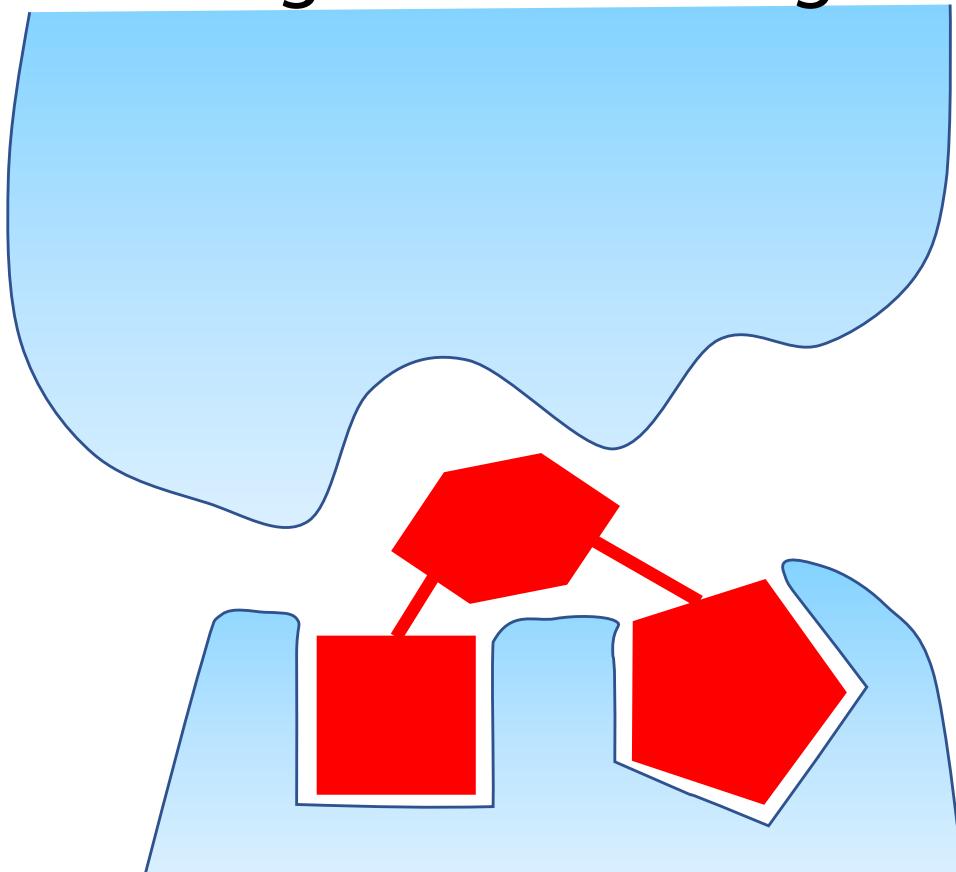
Candidate

Lead

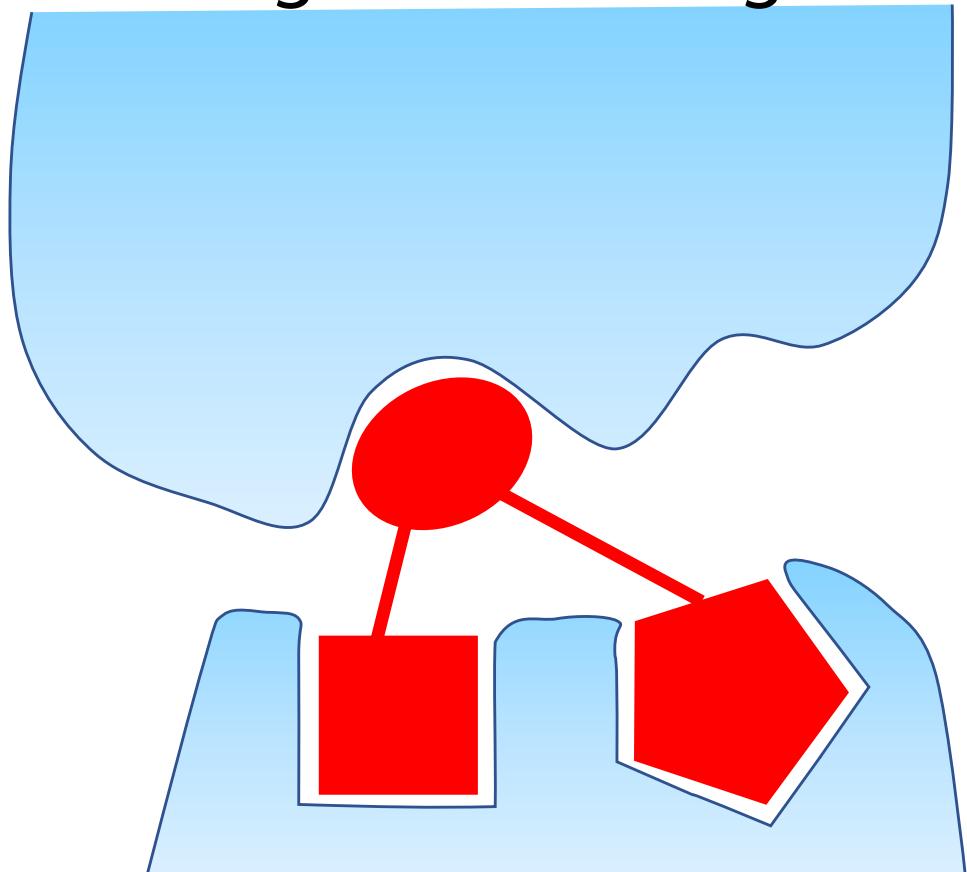


# Increasing fragment potency

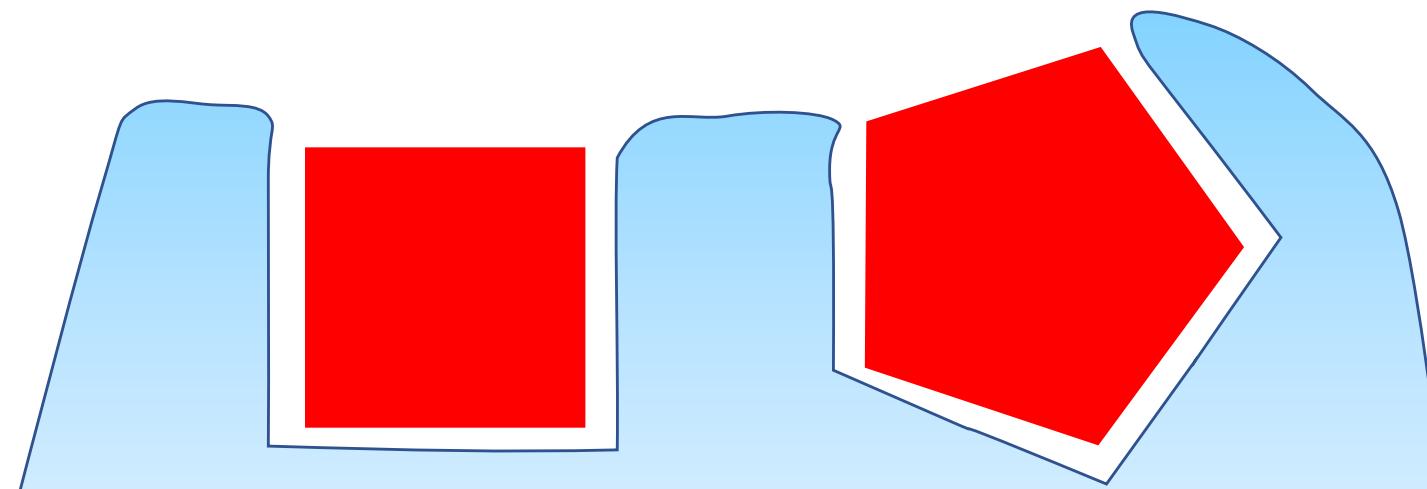
Fragment Growing



Fragment Linking



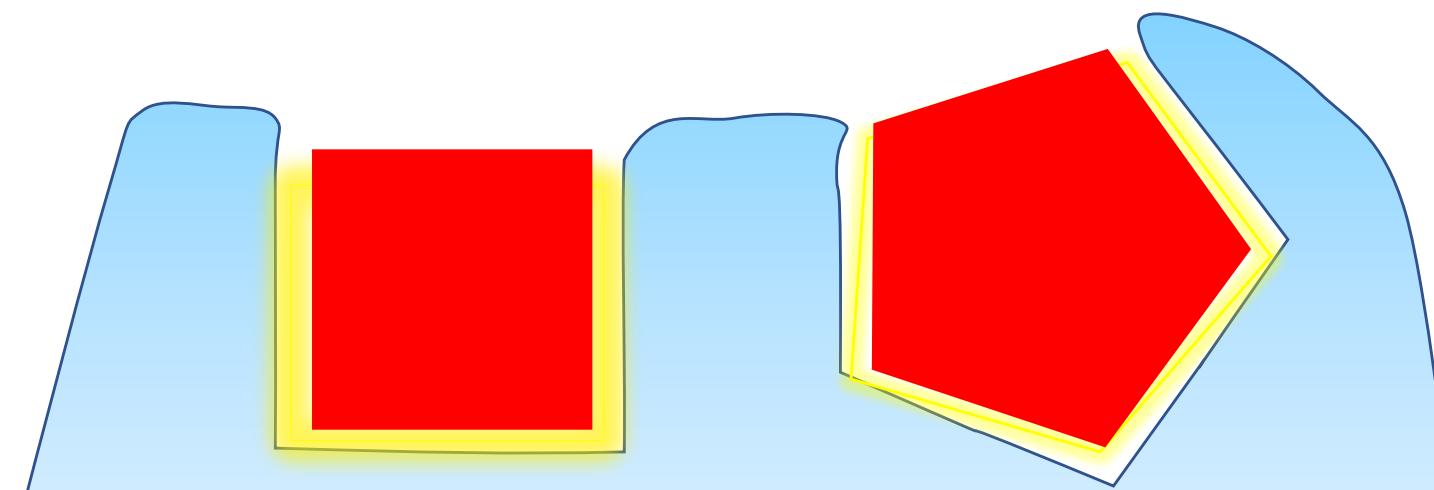
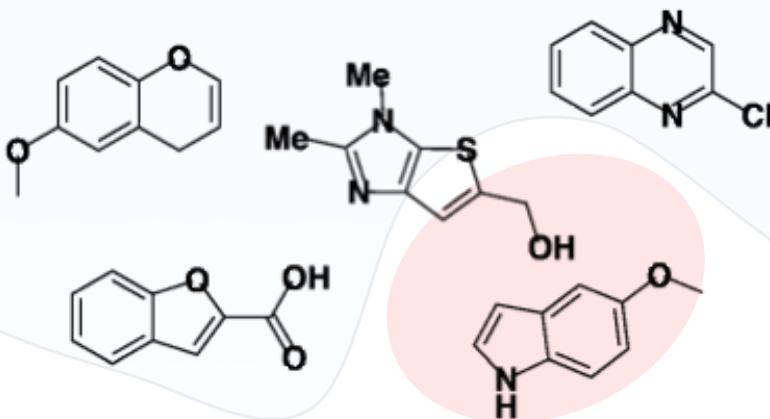
# Thermodynamics of binding



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

# Thermodynamics of binding

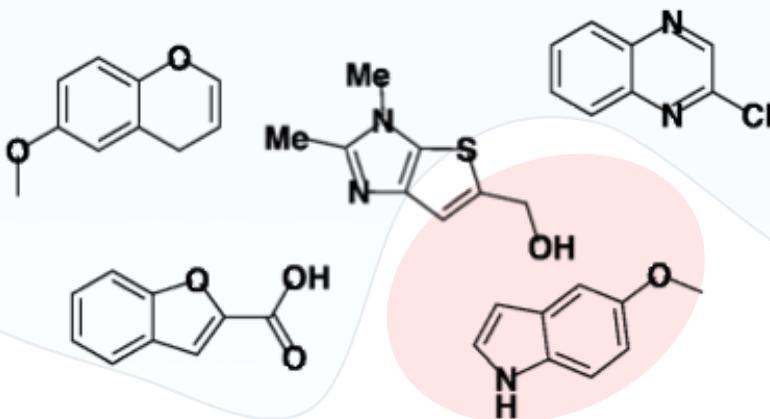
$$\Delta G = \Delta H - T\Delta S$$



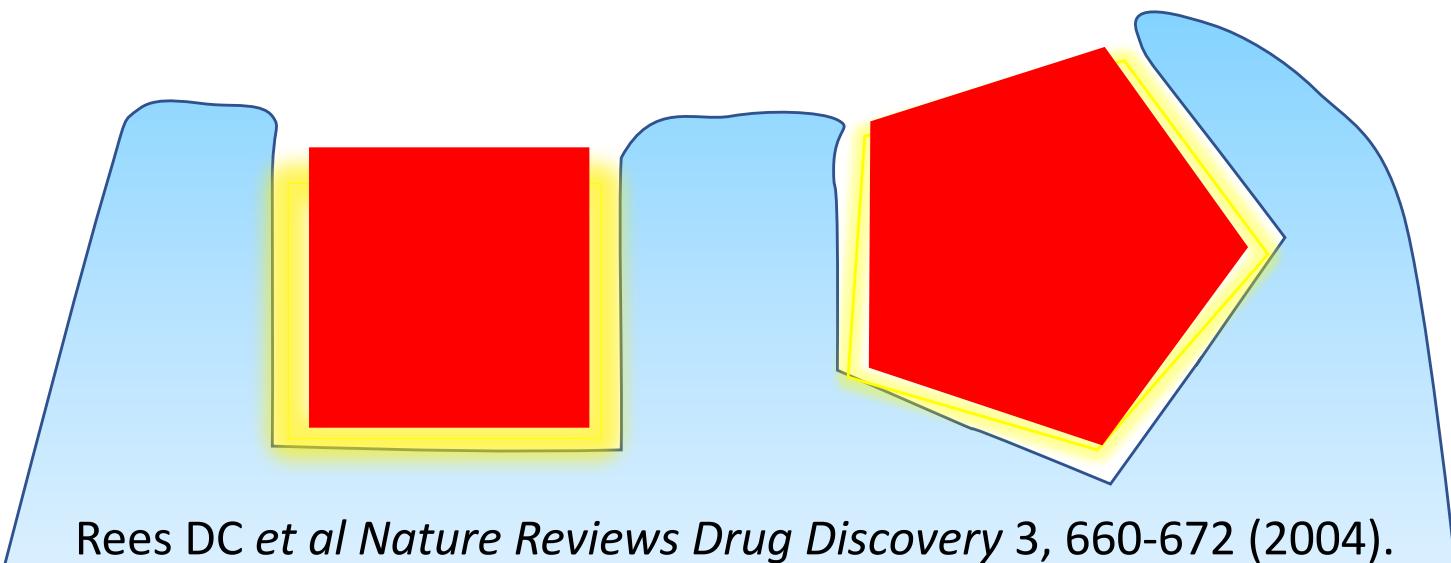
Rees DC et al *Nature Reviews Drug Discovery* 3, 660-672 (2004).

# Thermodynamics of binding

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



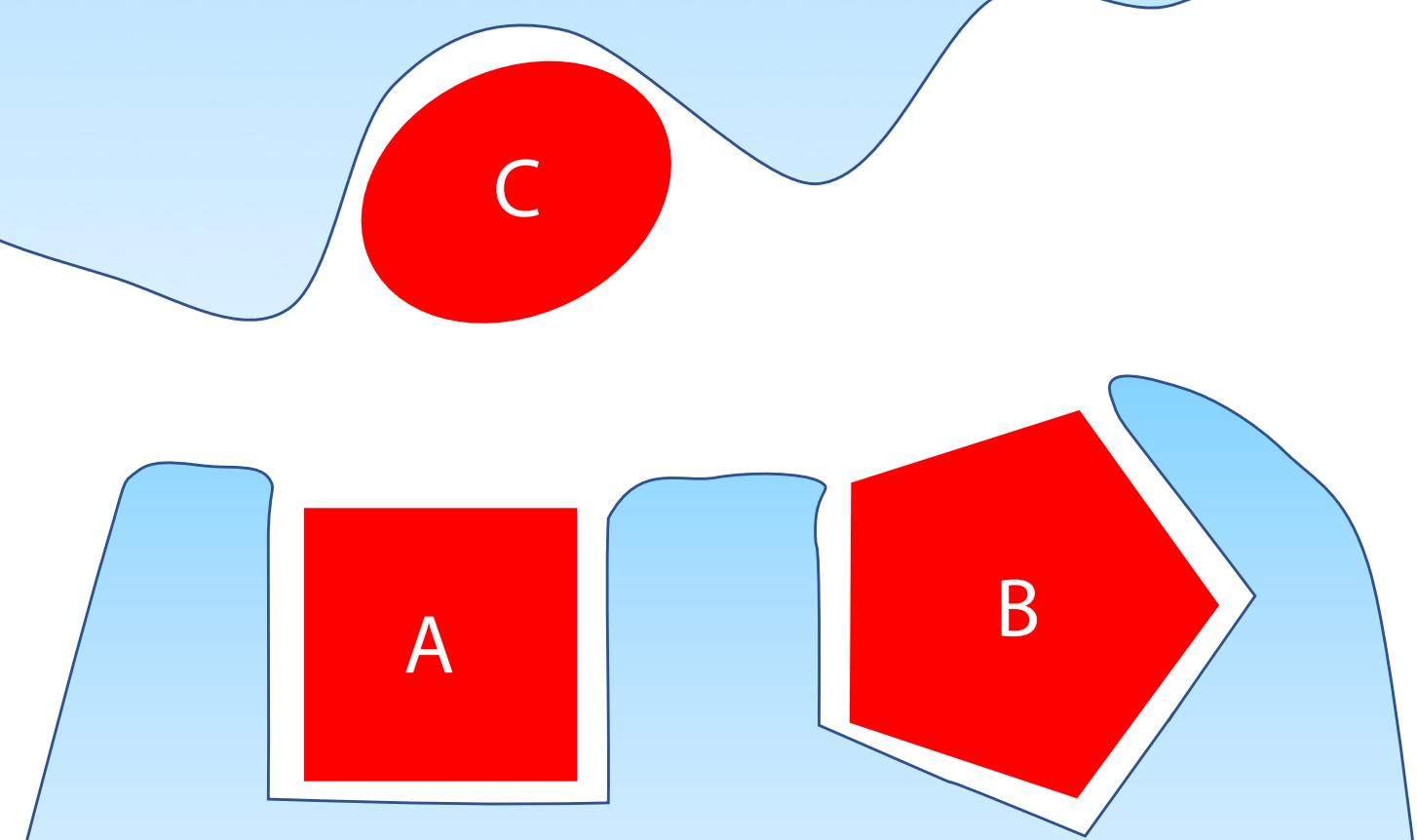
Fragments primarily  
exploit enthalpy



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

# Thermodynamics of linking

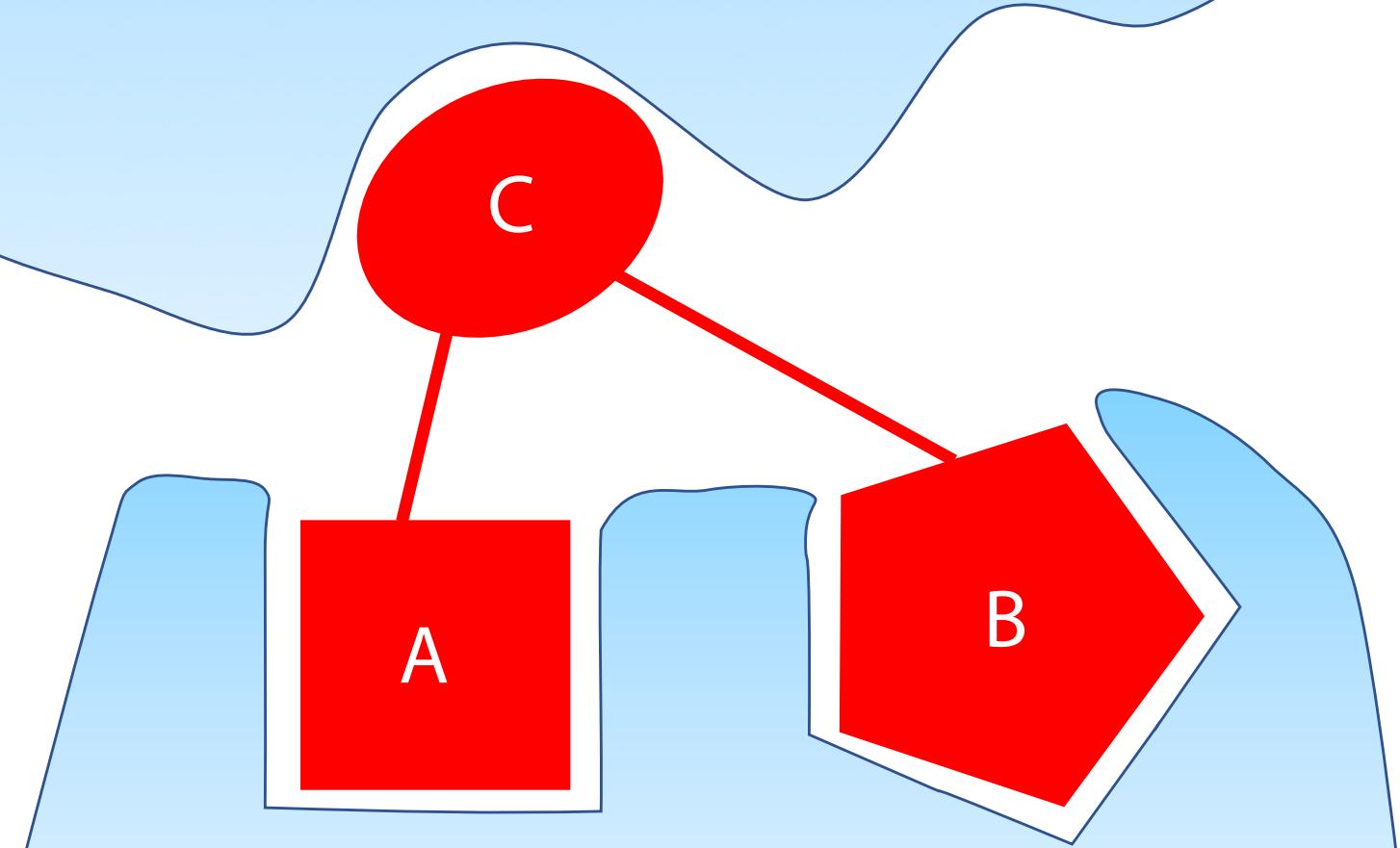
$$\Delta G_A \quad \Delta G_B \quad \Delta G_C$$



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

# Thermodynamics of linking

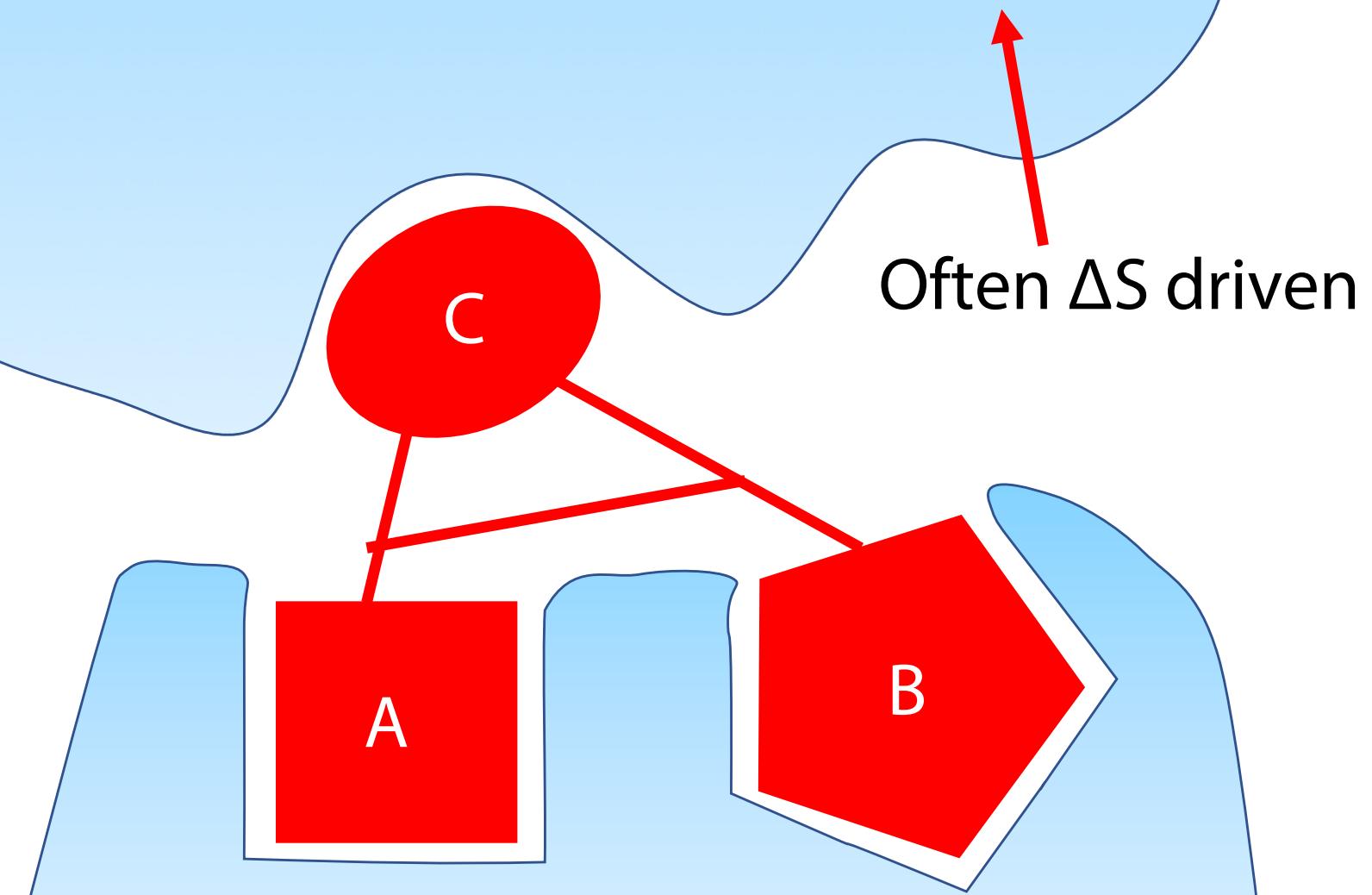
$$\Delta G_{\text{sum}} = \Delta G_A + \Delta G_B + \Delta G_C$$



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

# Thermodynamics of linking

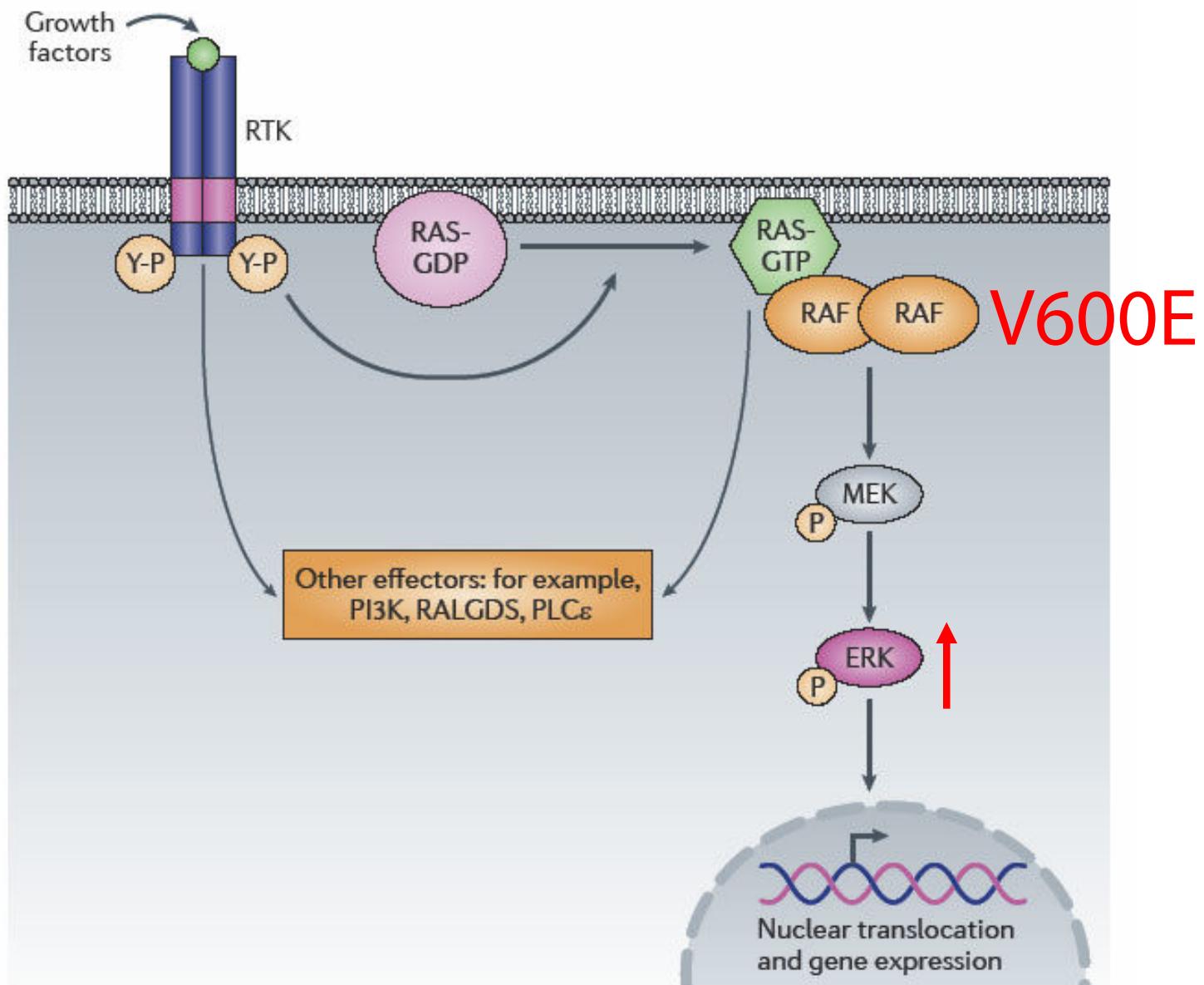
$$\Delta G_{\text{sum}} = \Delta G_A + \Delta G_B + \Delta G_C + \Delta G_{\text{other}}$$



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# Discovery of vemurafenib



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

# Vemurafenib (V600E mutated B-raf inhibitor)

**20,000 fragments**

150-350 kDa

<8 H-bonds

Soluble

Few rotatable bonds

200  $\mu\text{M}$  @ 5 kinases

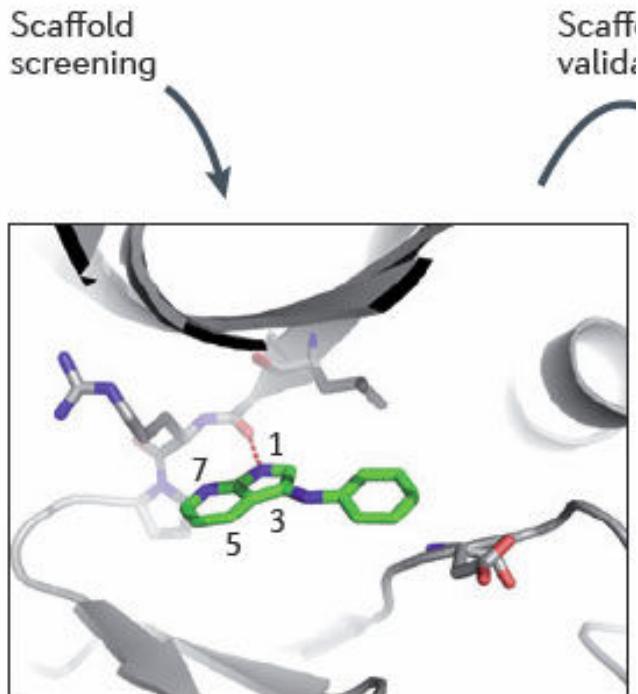
**238 hits**

**Fragment growth**

**1 scaffold**

**100 cocrystal structures**

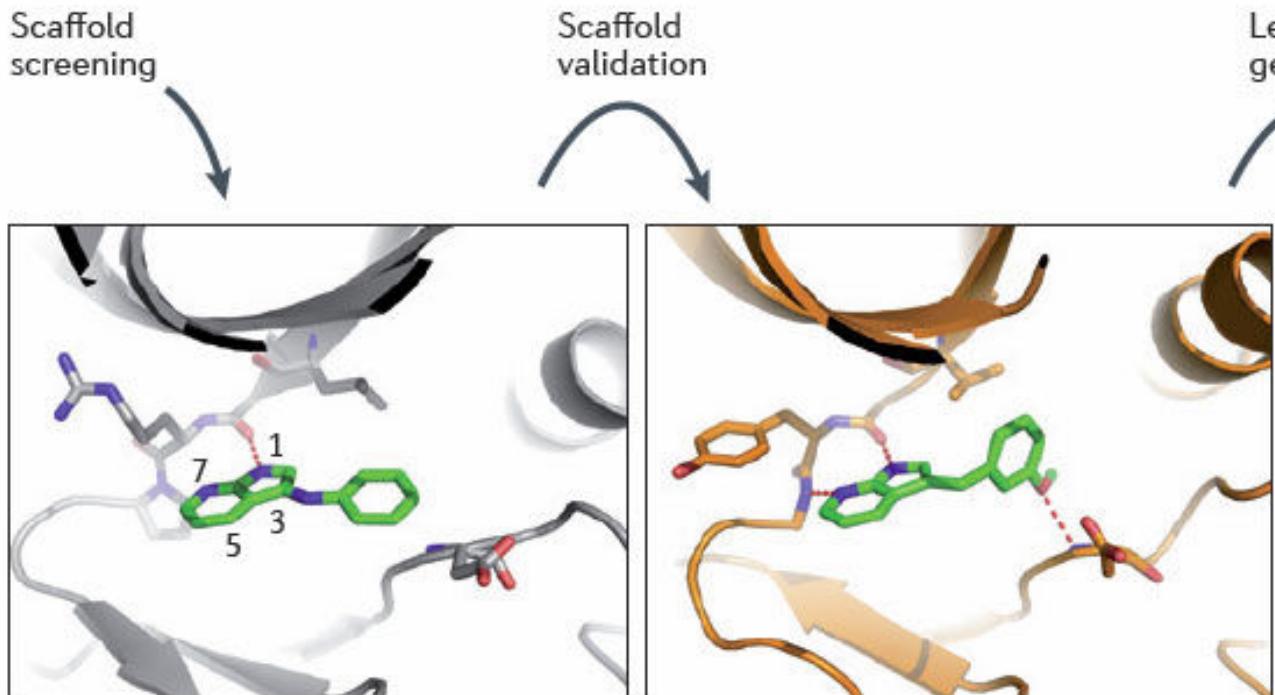
# Vemurafenib (V600E mutated B-raf inhibitor)



## Compound 1

- IC<sub>50</sub> in mM range
- Low affinity: ~200 μM
- Low specificity
- Crystallized with PIM1

# Vemurafenib (V600E mutated B-raf inhibitor)



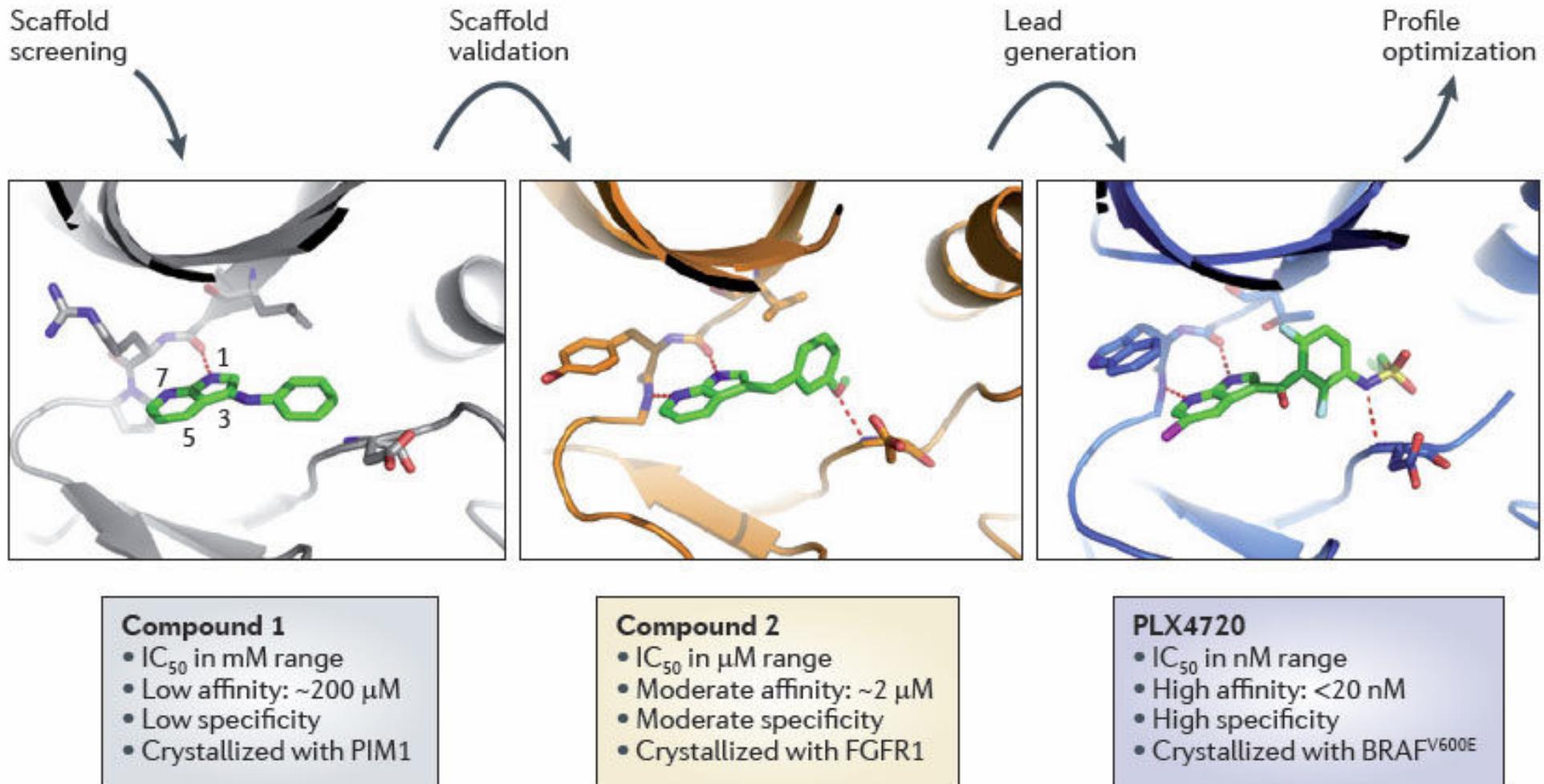
## Compound 1

- IC<sub>50</sub> in mM range
- Low affinity: ~200 μM
- Low specificity
- Crystallized with PIM1

## Compound 2

- IC<sub>50</sub> in μM range
- Moderate affinity: ~2 μM
- Moderate specificity
- Crystallized with FGFR1

# Vemurafenib (V600E mutated B-raf inhibitor)



# And it works!

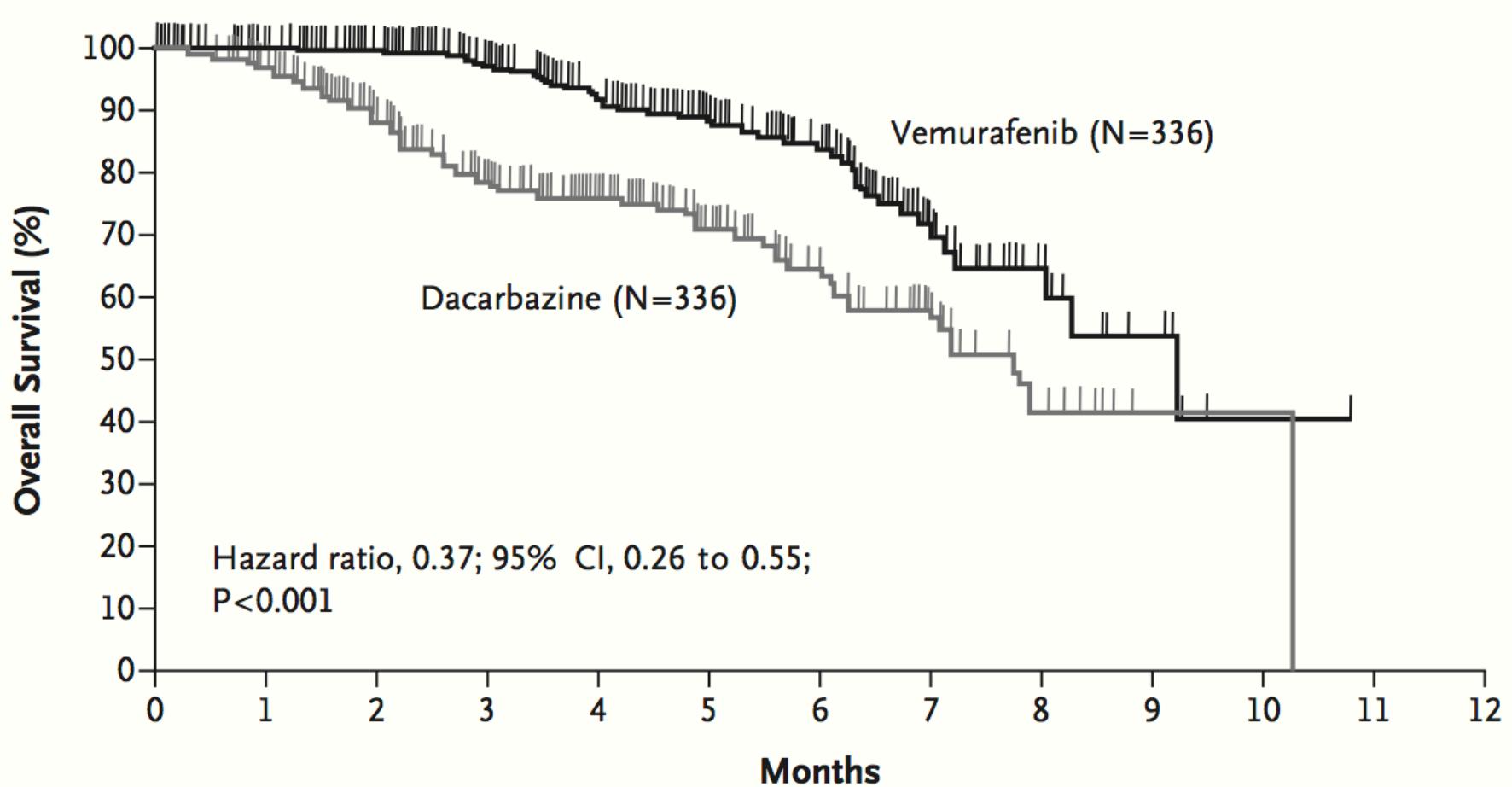
*The NEW ENGLAND JOURNAL of MEDICINE*

**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

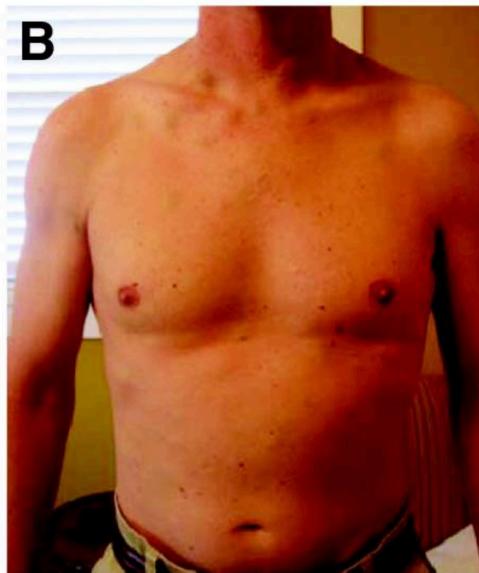


# But nothing is ever easy...

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

+ 15 weeks of  
vemurafenib

+ 23 weeks of  
vemurafenib



# But nothing is ever easy...

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

nature

## LETTERS

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

Poulikos I. Poulikakos<sup>1</sup>, Chao Zhang<sup>2</sup>, Gideon Bollag<sup>3</sup>, Kevan M. Shokat<sup>2</sup> & Neal Rosen<sup>1</sup>

=> ~30% squamous cell-carcinomas

# But nothing is ever easy...

Vol 464 | 18 March 2010 | doi:10.1038/nature08

nature

## RAF inhibitors tip the balance in signalling in cell

Poulikos I, Poulikakos<sup>1</sup>, Chao Zhan

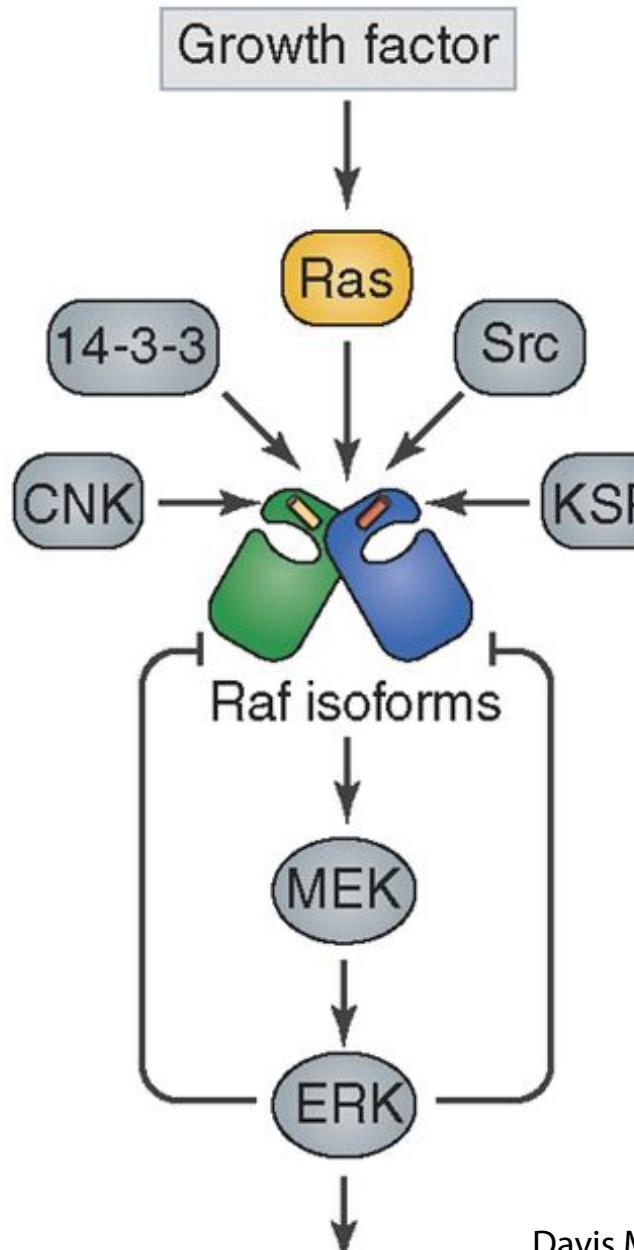
LETTERS

mers and ERK  
AF

real Rosen<sup>1</sup>

rcinomas

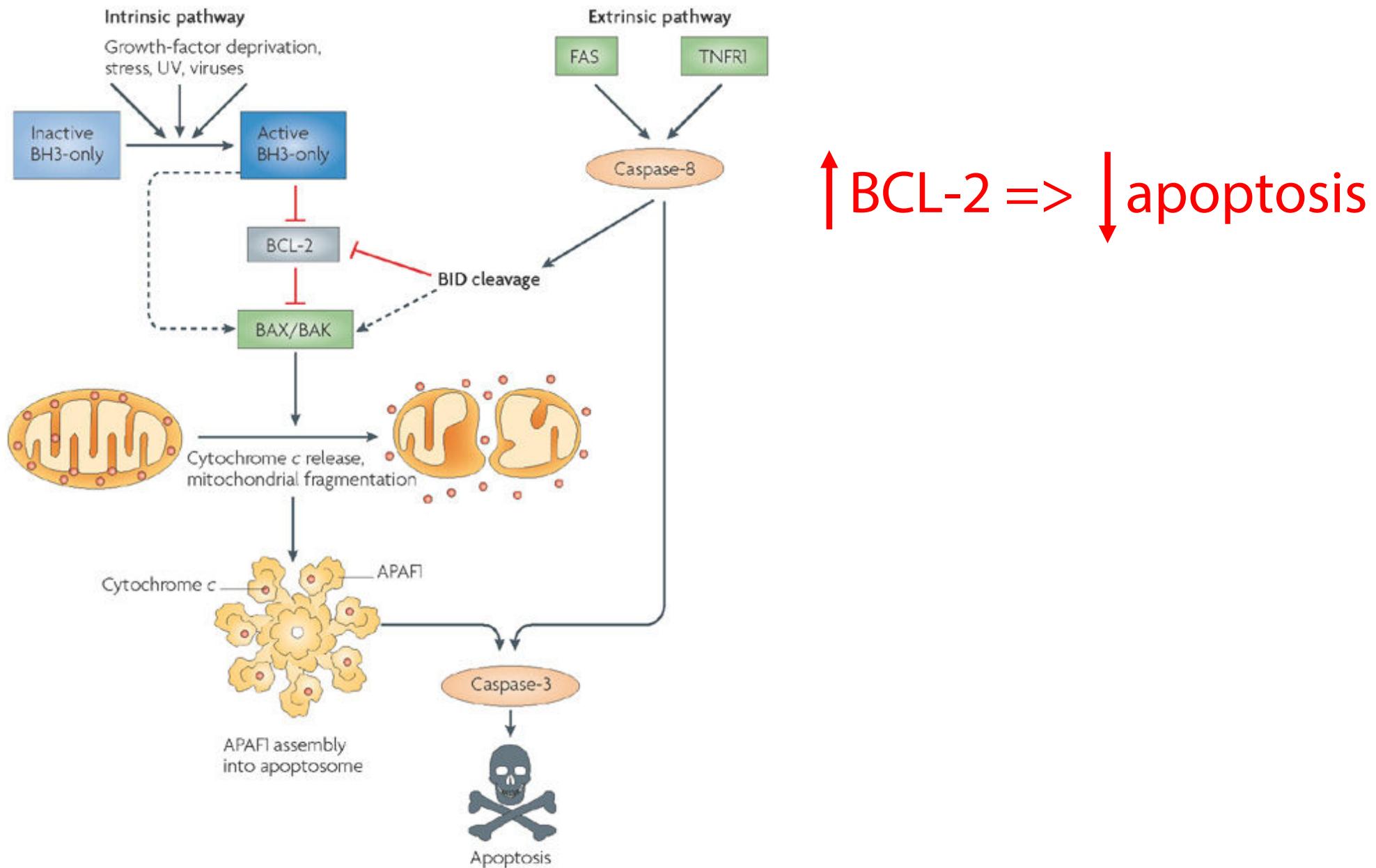
=> ~30%



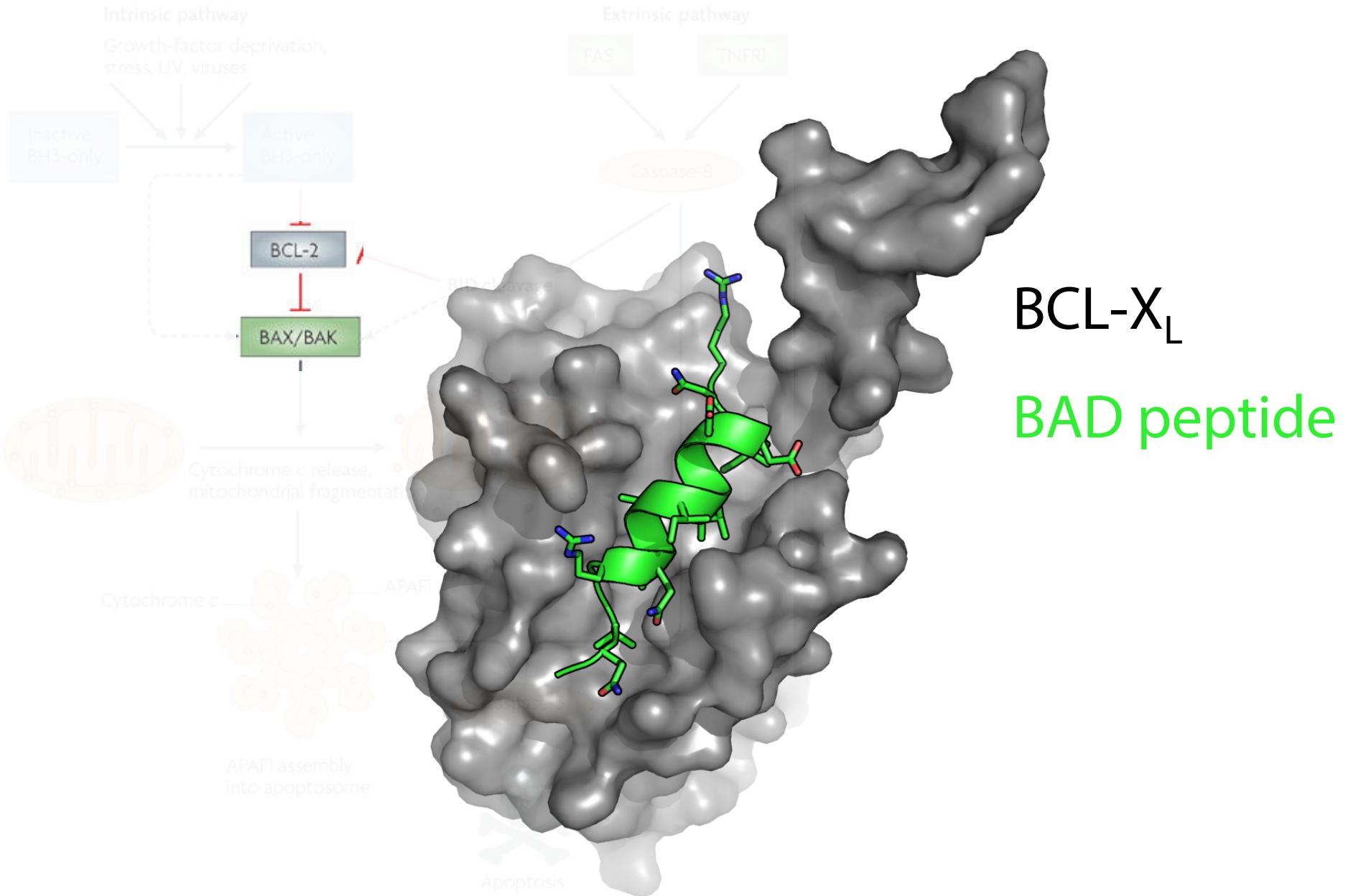
Davis MJ & Schlessinger J. *J Cell Biol.* 199:15-19 (2012).

But what about "challenging" targets?

# Discovery of venetoclax



# BCL-xL is a classic “challenging” target



# “SAR by NMR”

**9373 fragments**

**+3472 fragments**

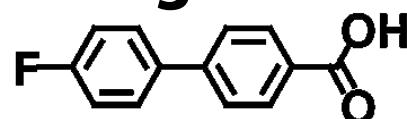
NMR to test sets of 10

**660 cmpds**

NMR to retest

**49 w/  
 $K_d < 5 \text{ mM}$**

**Fragment 1**



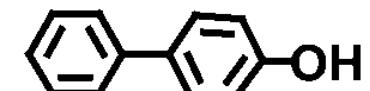
NMR to test sets of 5

**300 cmpds**

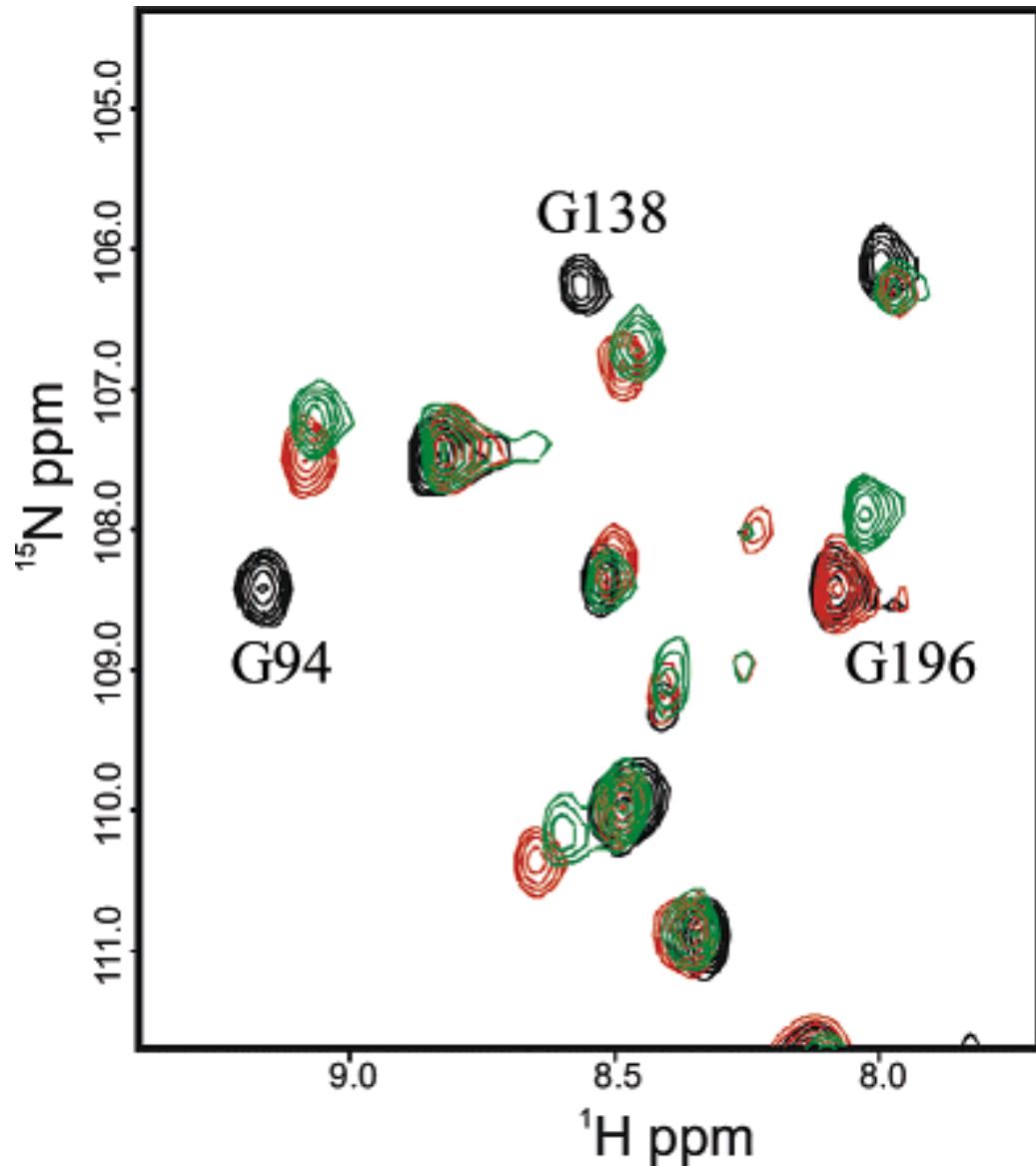
NMR to retest

**24 w/  
 $K_d < 5 \text{ mM}$**

**Fragment 2**

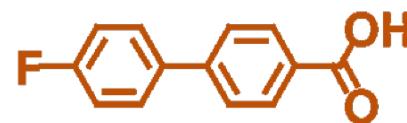


# Fragment Based Discovery by NMR



BCL-X<sub>L</sub> protein alone

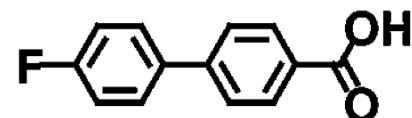
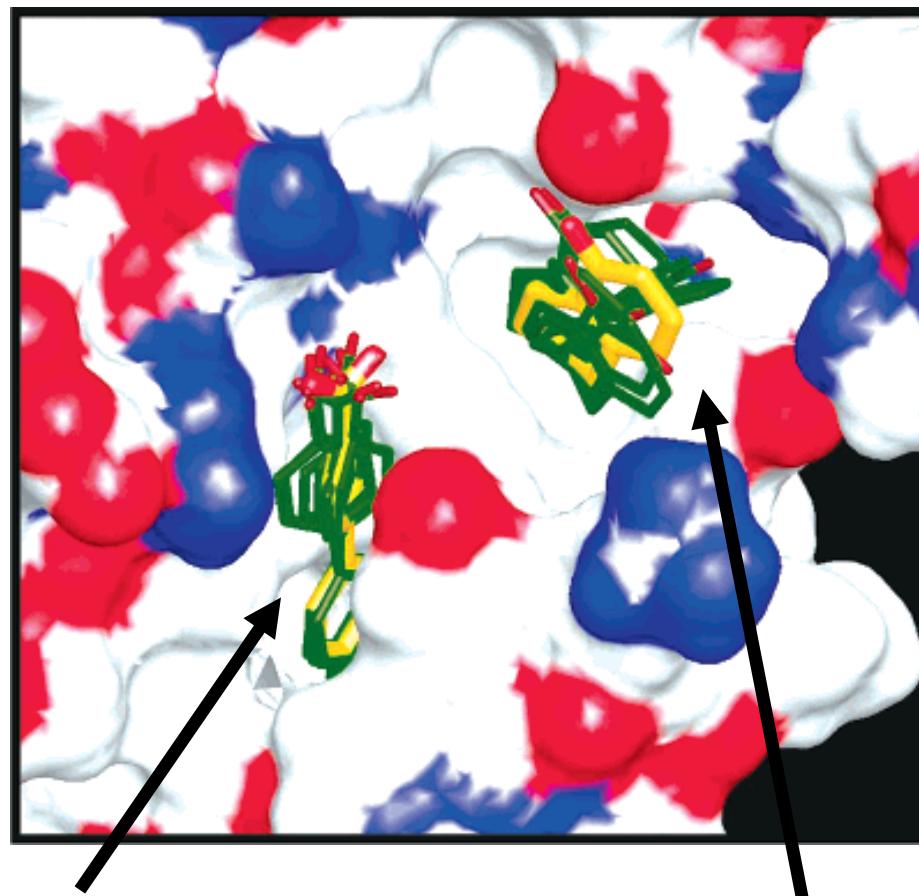
+ Fragment 1



+ Fragment 2



# Fragment Based Discovery by NMR

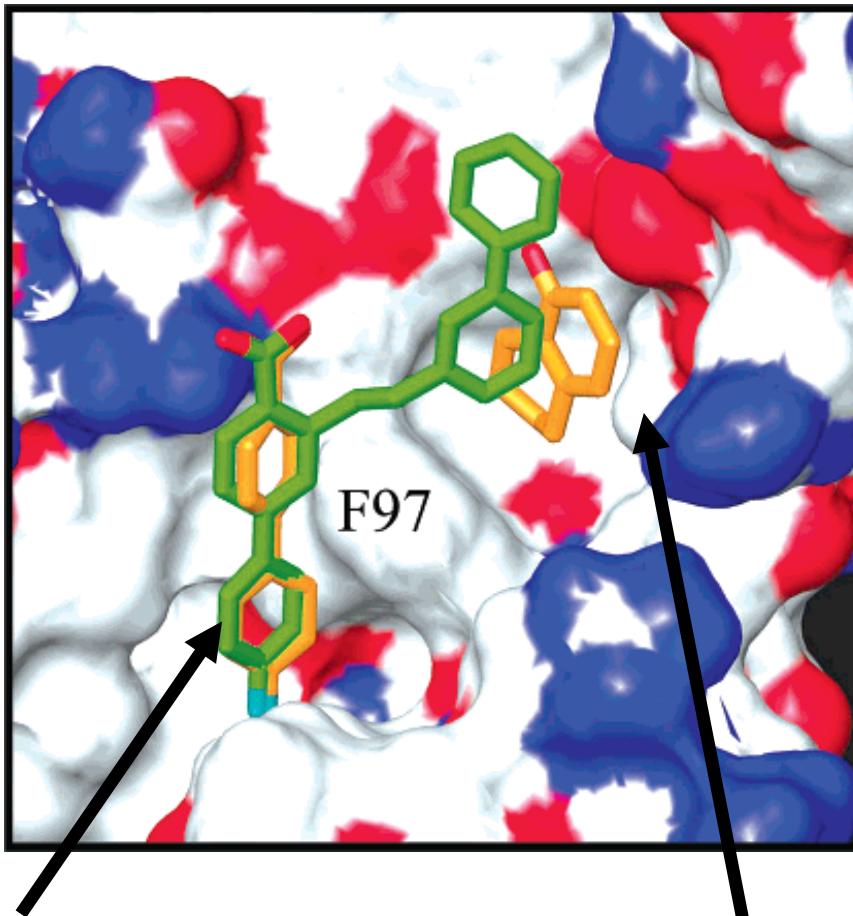


Fragment 1

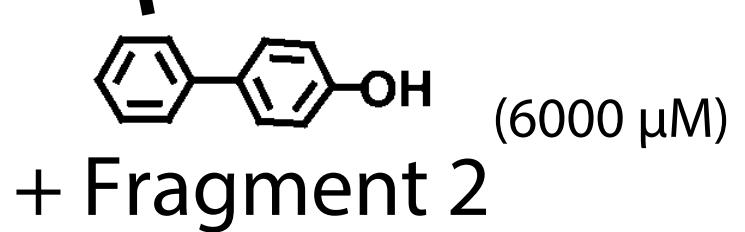
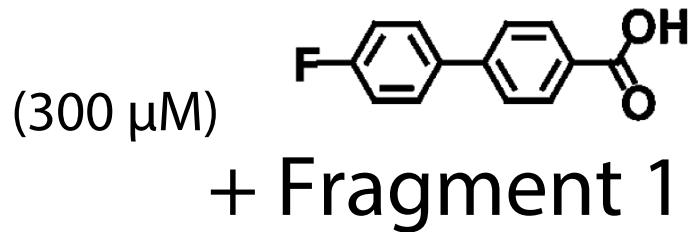


Fragment 2

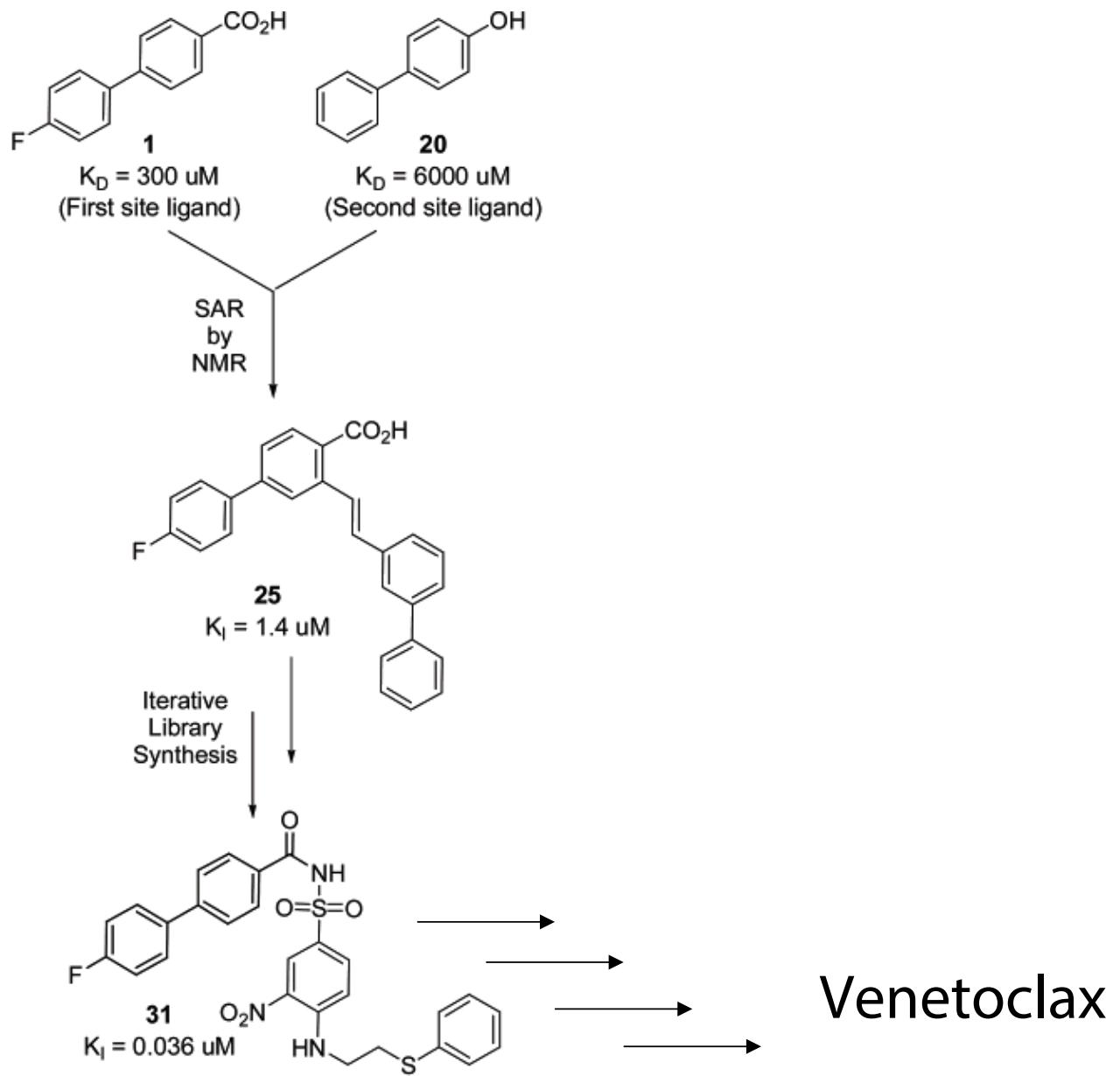
# Fragment Based Discovery by NMR



Cmpd 25  
(1.4  $\mu$ M)



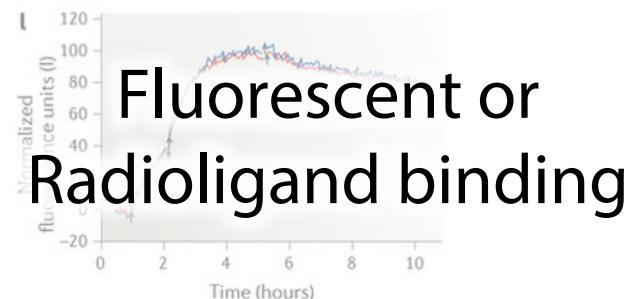
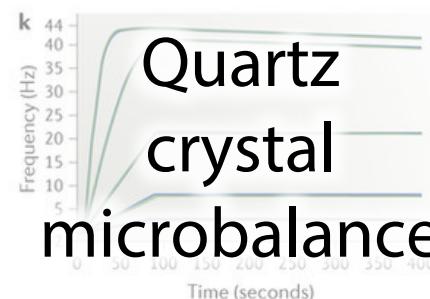
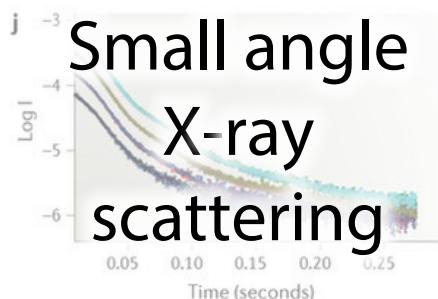
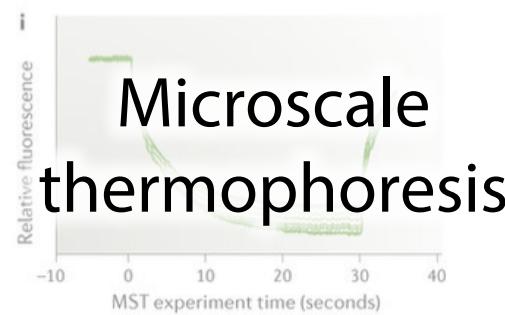
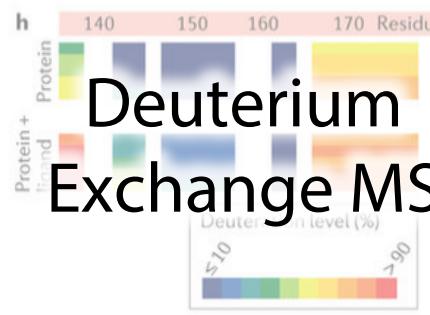
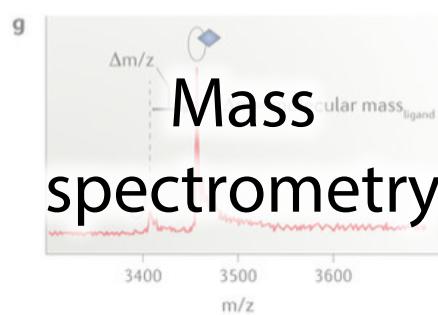
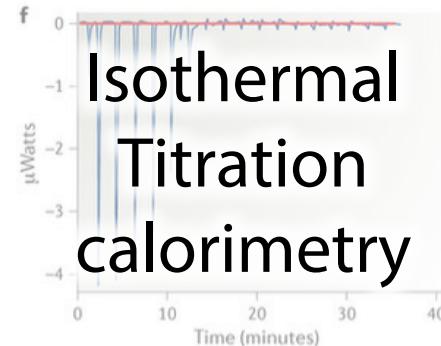
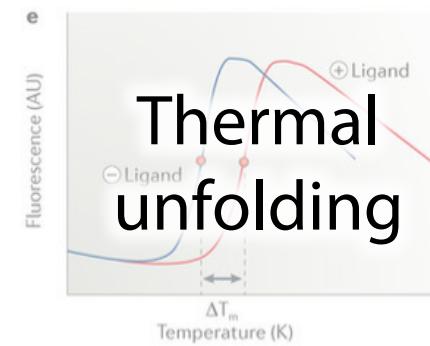
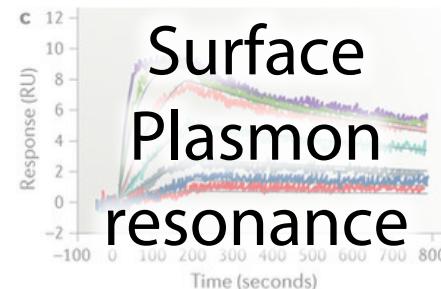
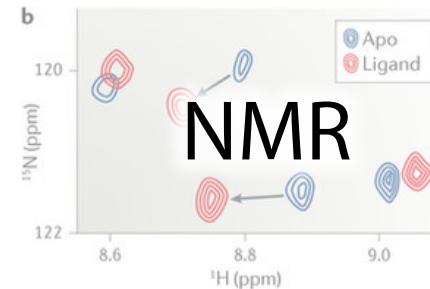
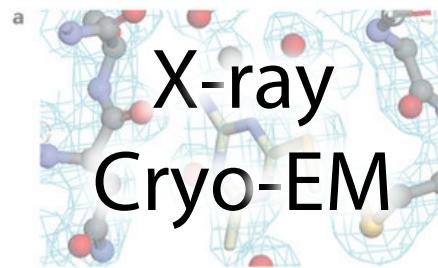
# Fragment Based Discovery by NMR



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# Integrative biophysics in drug discovery



# Questions?

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