Innovations in Drug Discovery:
Fragment-Based Drug Discovery & Activity-Based Protein Profiling

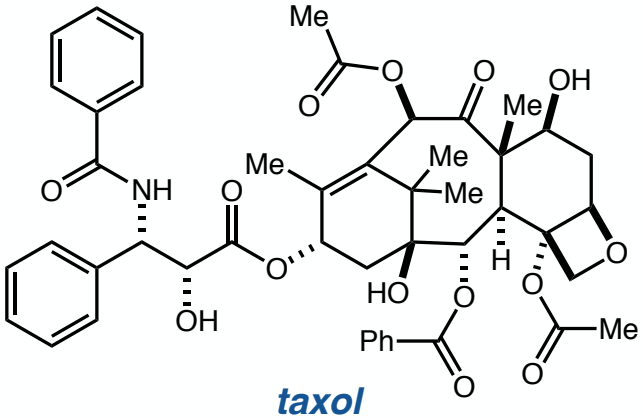
Literature presentation

Junyong Kim

April 21st, 2020

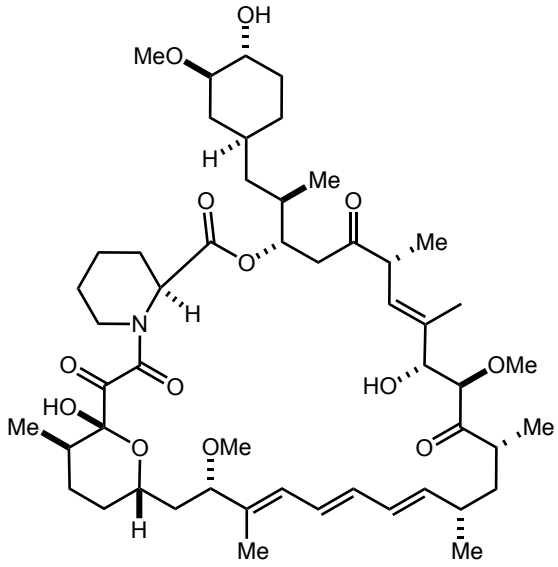
Chemical technologies impacted drug discovery

1960s: natural product-derived leads



taxol

pacific yew



rapamycin

S. hygroscopicus

Murray, C.W.; Rees, D.C. *Nat. Chem.* **2009**, *1*, 187.

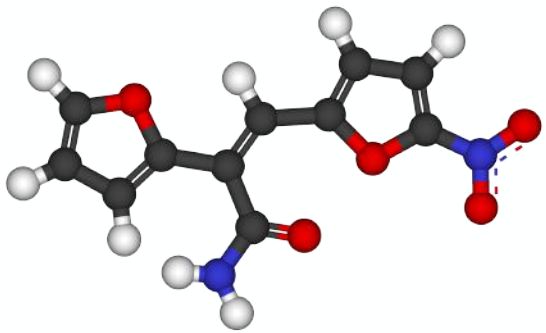
Contemporary Drug Discovery

Chemical technologies impacted drug discovery

1960s: natural product-derived leads

1970s: quantitative structure-activity relationships (QSAR)

Molecular structure



Molecular descriptor

Electronics

Sterics

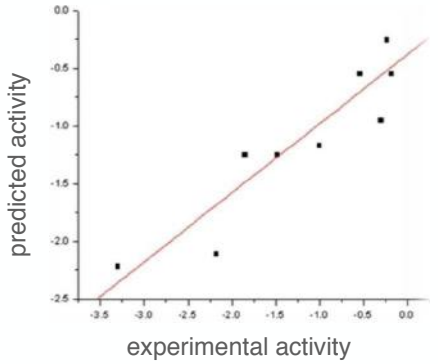
Lipophilicity

Hydrophobicity

Solubility

⋮

Multivariate analysis & activity prediction



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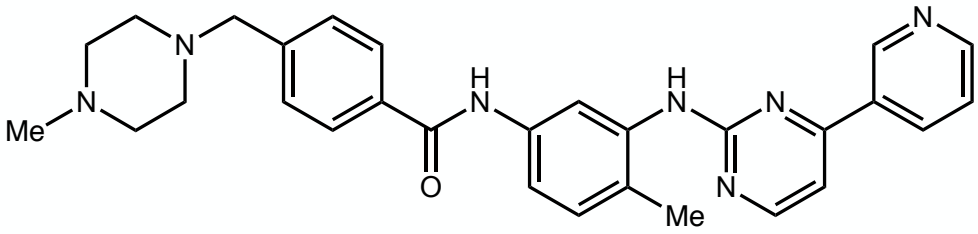
Contemporary Drug Discovery

Chemical technologies impacted drug discovery

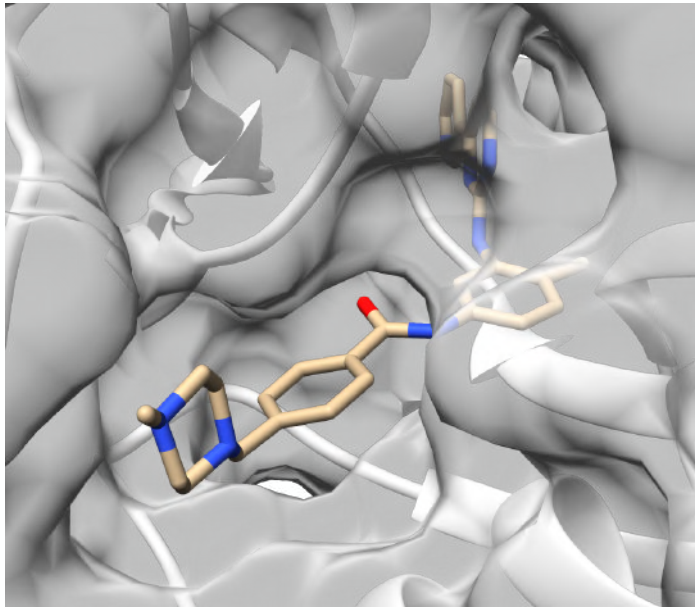
1960s: natural product-derived leads

1970s: quantitative structure-activity relationships (QSAR)

1980s: structure-based drug discovery (SBDD)



gleevec



Abl in complex with imatinib (PDB: 2HYY)

Murray, C.W.; Rees, D.C. *Nat. Chem.* **2009**, *1*, 187.

Contemporary Drug Discovery

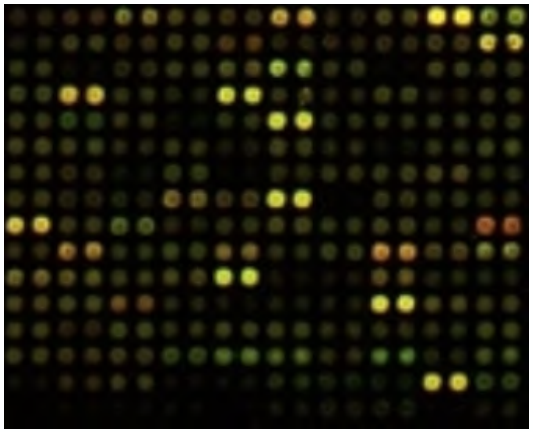
Chemical technologies impacted drug discovery

1960s: natural product-derived leads

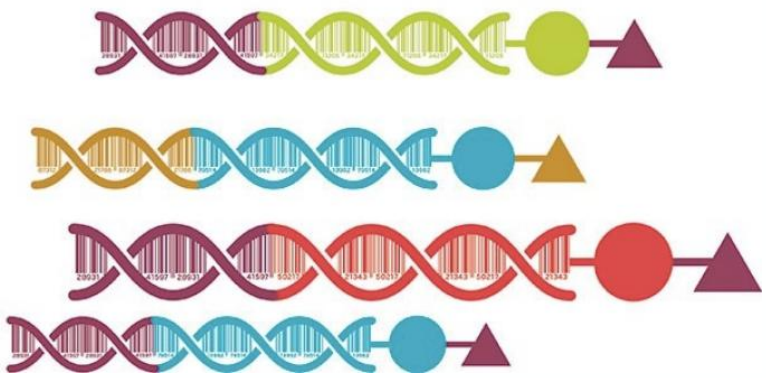
1970s: quantitative structure-activity relationships (QSAR)

1980s: structure-based drug discovery (SBDD)

1990s: combinatorial chemistry and high-throughput screening (HTS)



microarray



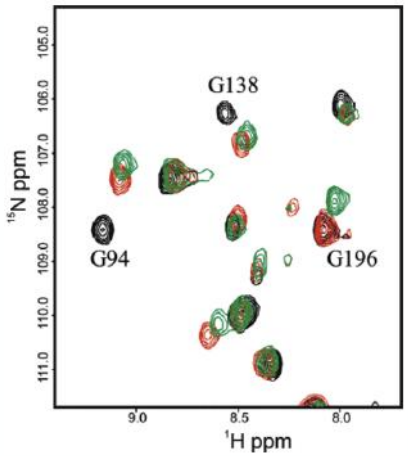
DNA-encoded library

https://cen.acs.org/articles/95/i25/DNA-encoded-libraries-revolutionizing-drug.html

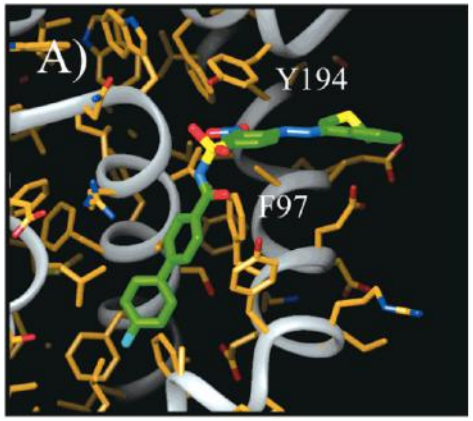
Murray, C.W.; Rees, D.C. *Nat. Chem.* **2009**, *1*, 187.

Drug Discovery in 20th Century

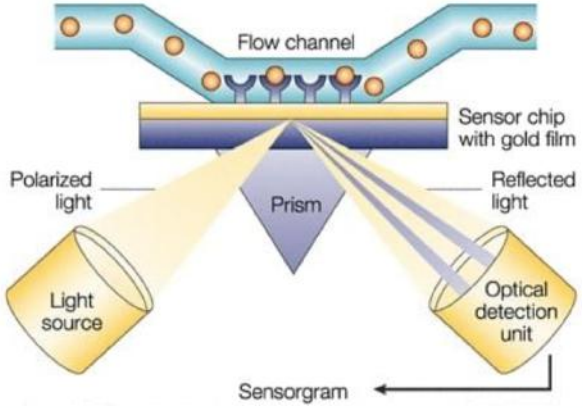
Advanced analytical techniques for HTS



NMR



X-ray



Surface plasmon resonance (SPR)

Systems biology

Genomics → Transcriptomics → Proteomics → Metabolomics

Targets & Biomarkers

Personalized therapy

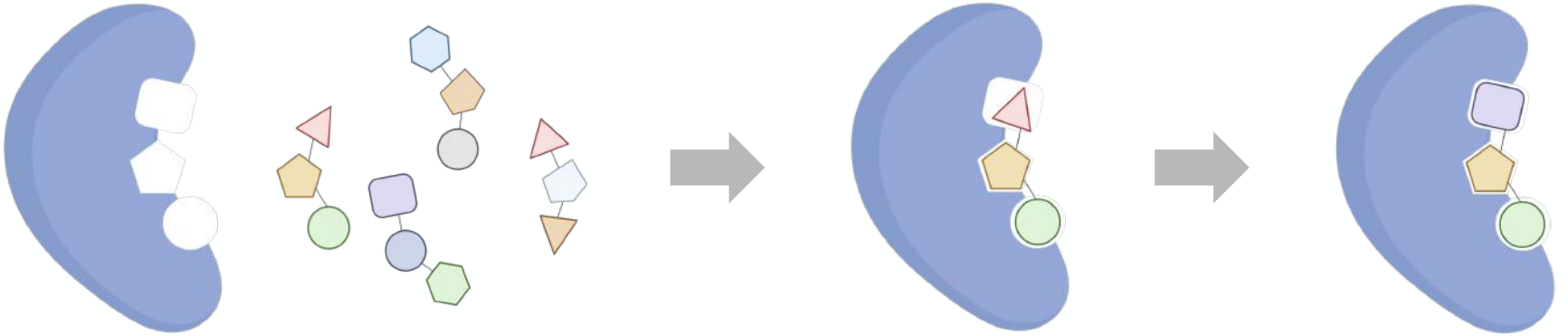
Mechanism & Interactions

Drug repurposing

Cooper, M.A. *Nat. Rev. Drug Discov.* **2002**, *1*, 515.
Murray, C.W.; Rees, D.C. *Nat. Chem.* **2009**, *1*, 187.

Fragment-Based Drug Discovery

Traditional HTS

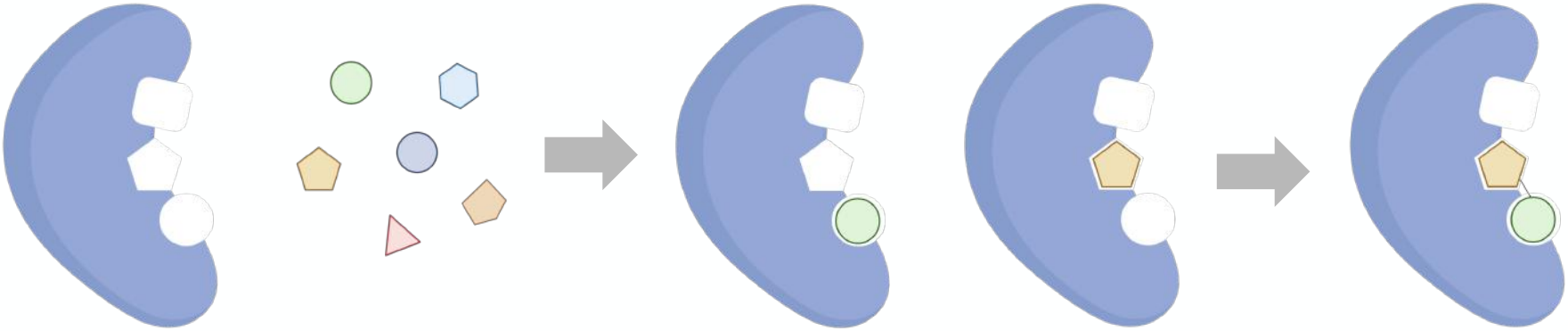


Ligand design and screening

Lead compound identification

Ligand optimization

Fragment-Based Drug Discovery



Ligand screening

Lead fragment identification

Kd ~ 100 μM

$\Delta G \sim 9–10$ kcal/mol

Fragment linking and growing

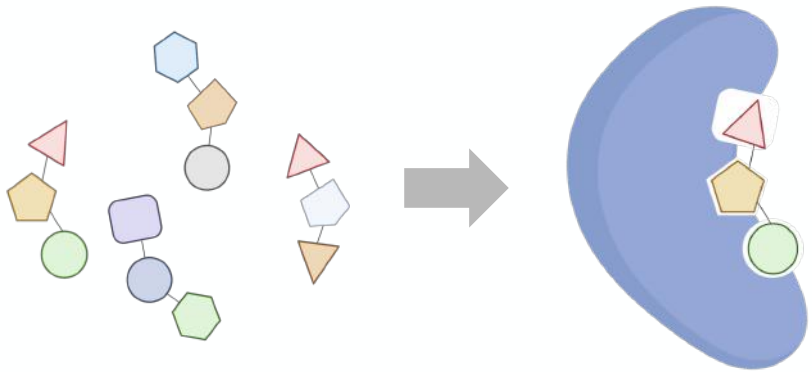
Kd ~ 3 nM

$\Delta G \sim \Delta G_1 + \Delta G_2 \sim 15–16$ kcal/mol

Murray, C.W.; Rees, D.C. *Nat. Chem.* **2009**, *1*, 187.

Fragment-Based Drug Discovery

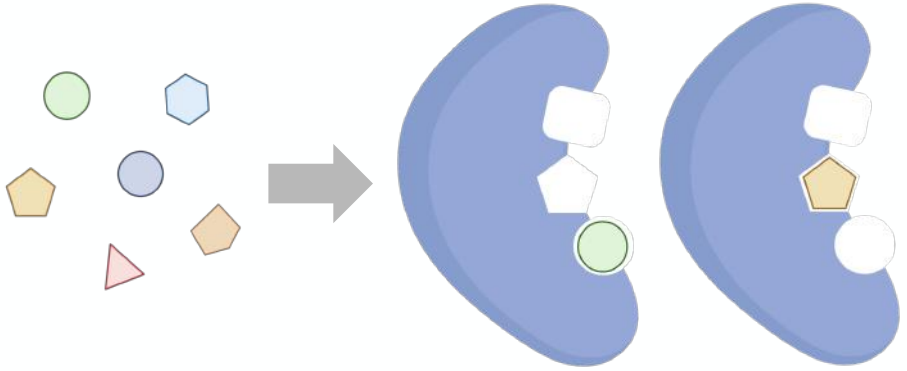
High-Throughput Screening (HTS)



Library size ***> 100000***

Molecular weight ***> 300 Da***

Fragment-Based Drug Discovery (FBDD)



Library size ***< 5000***

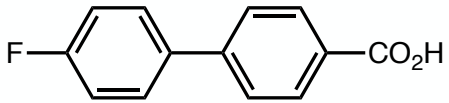
Molecular weight ***< 300 Da***

- *Reduced synthetic resources*
- *High throughput 2D-NMR method*
- *Successful drug development cases*

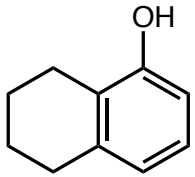
Abbott

Murray, C.W.; Rees, D.C. *Nat. Chem.* **2009**, *1*, 187.

Case Study: Navitoclax

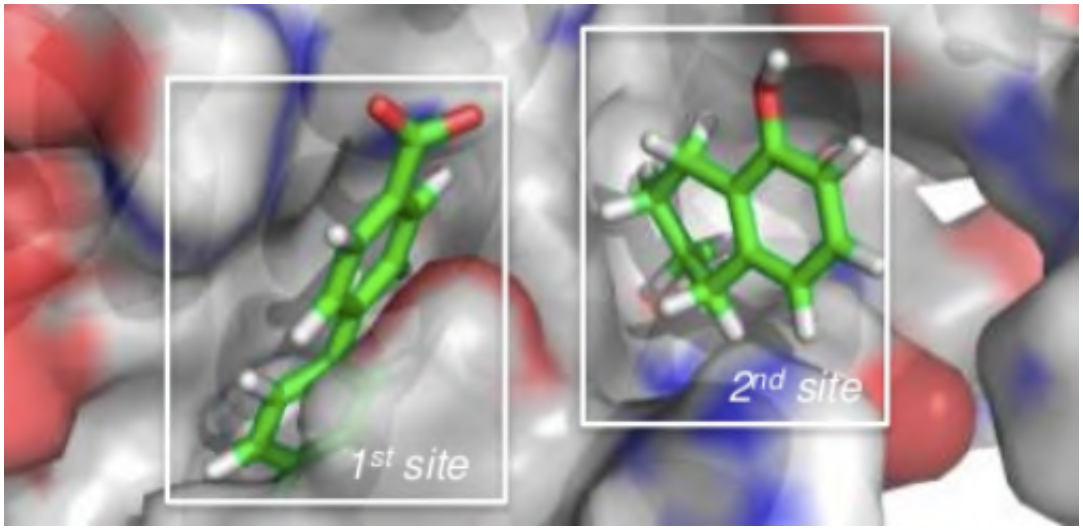


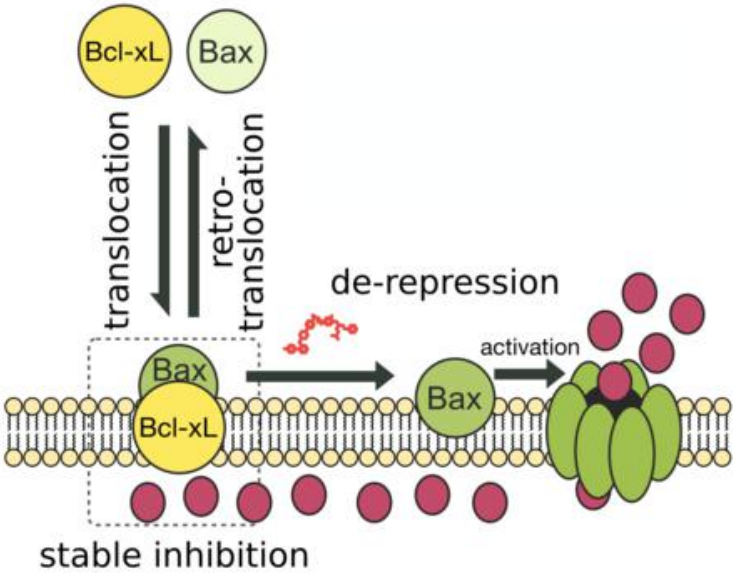
K_D = 0.3 mM



K_D = 4.3 mM

Bcl-X_L (antiapoptotic protein)





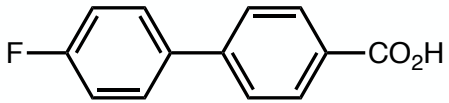
Bcl-X_L binds to Bax and inhibits apoptotic cell death.

Mutated Bcl-X_L turns off apoptotic pathway of cancer cells.

Targeting PPI between Bcl-X_L & Bax

Petros, A.M. *et al. J. Med. Chem.* **2006**, *49*, 656.
Bruncko, M. *et al. J. Med. Chem.* **2007**, *50*, 641.

Case Study: Navitoclax



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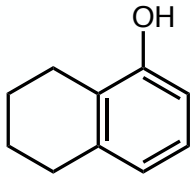
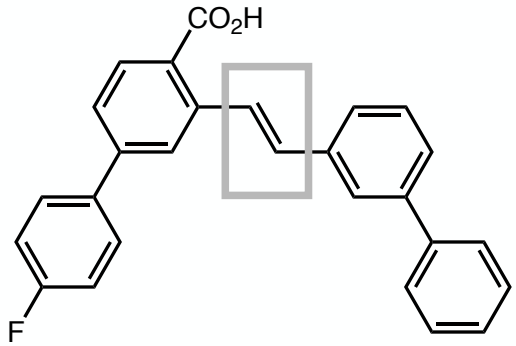
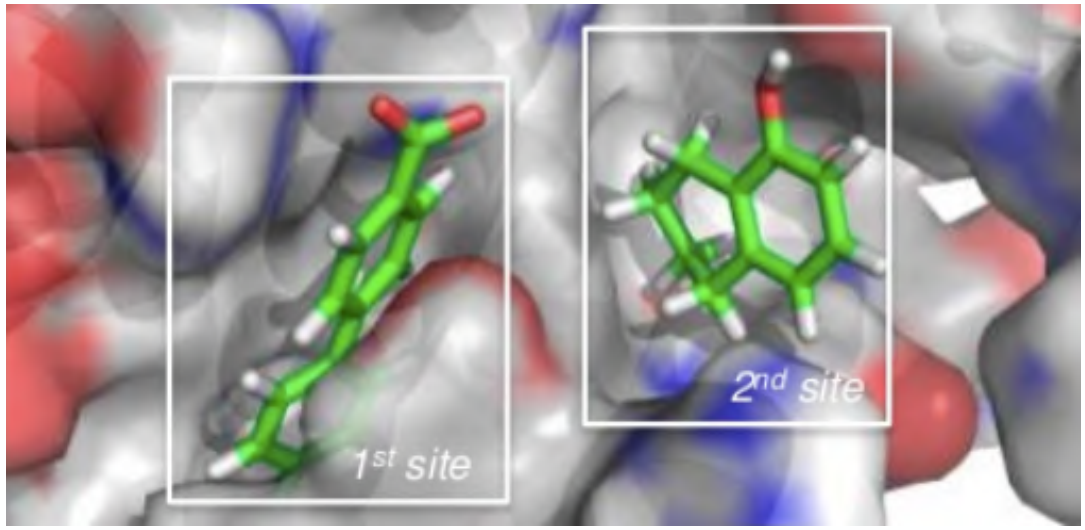


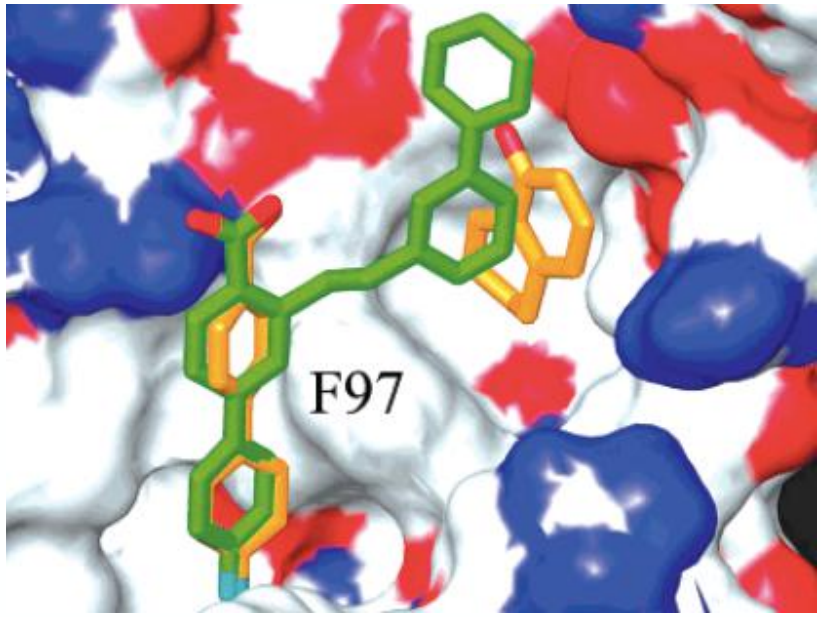
K_D = 4.3 mM



K_i = 1.4 μM

Bcl-X_L (antiapoptotic protein)





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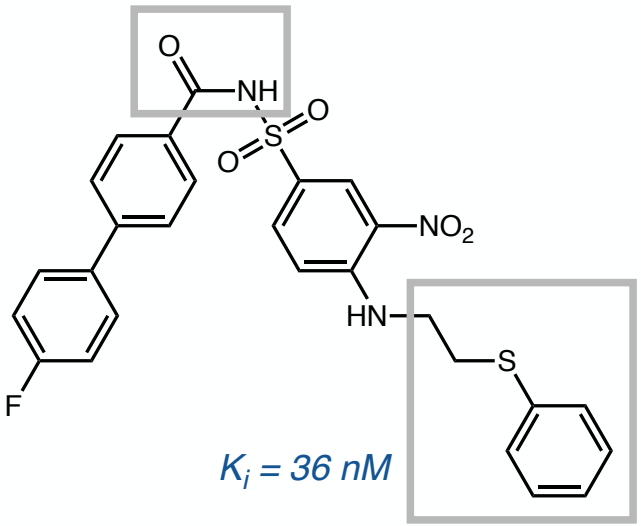
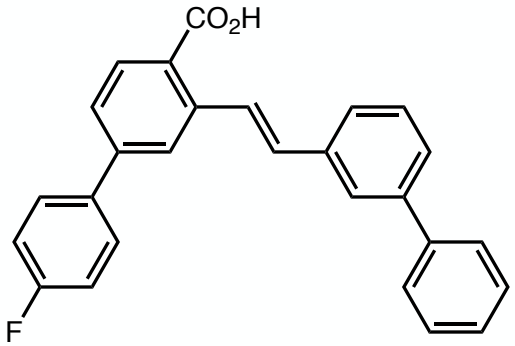
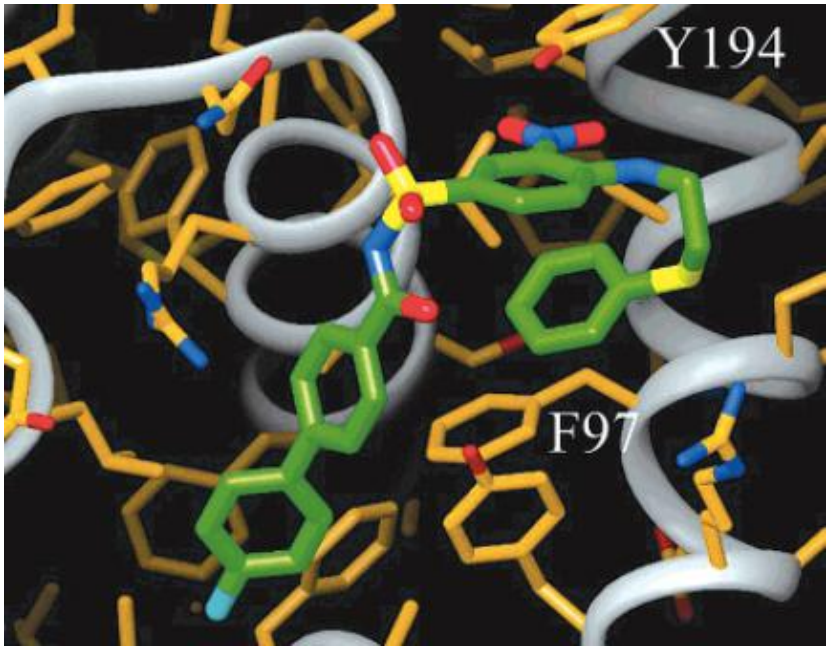
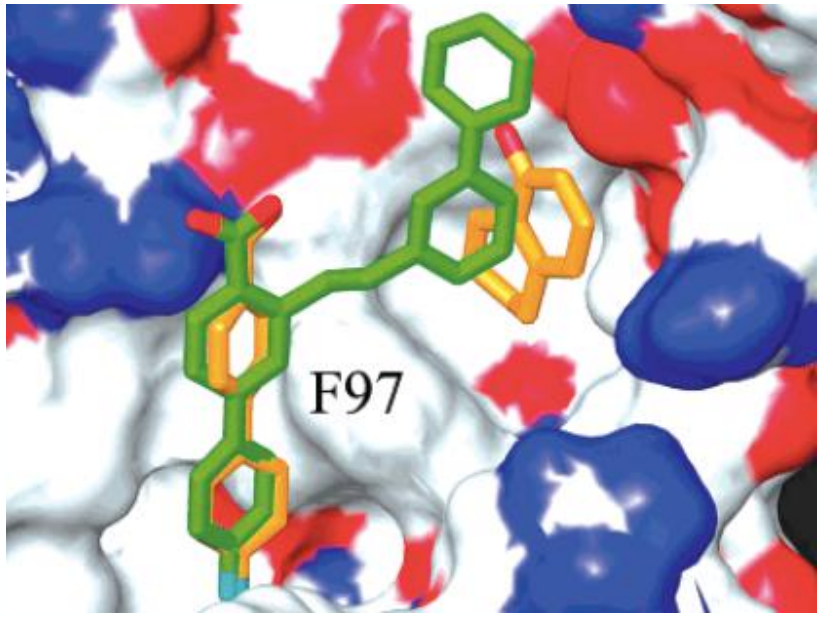


K_i = 36 nM



K_i = 1.4 μM





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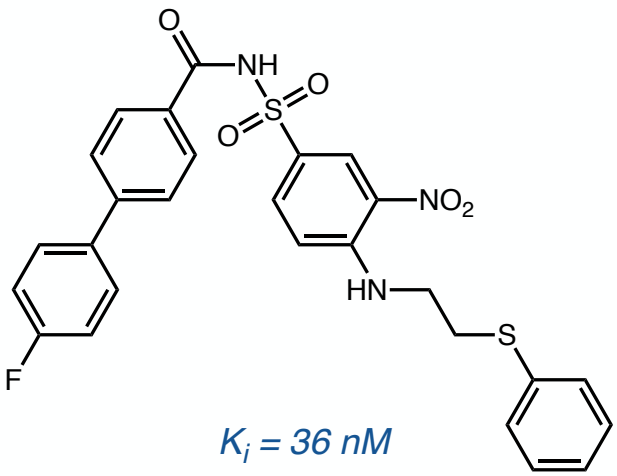

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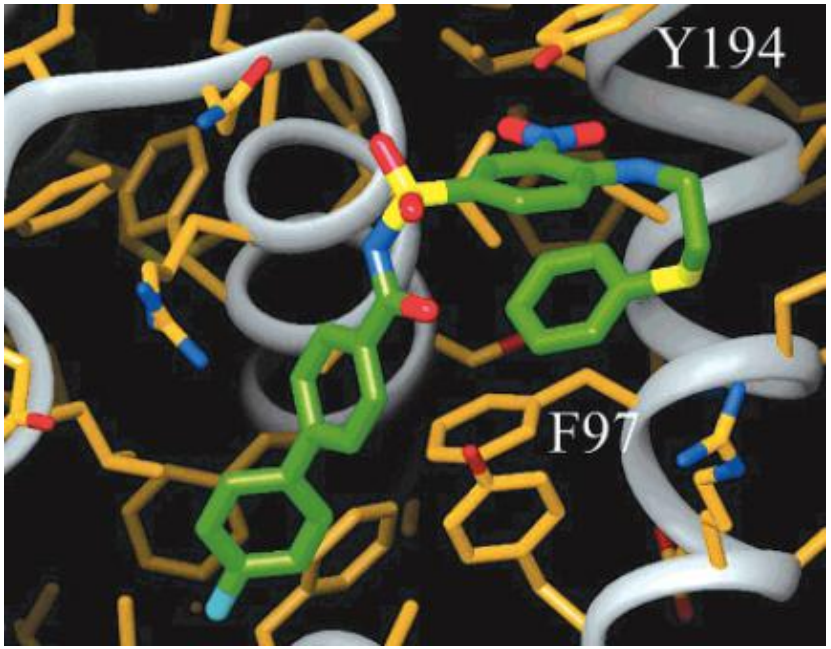
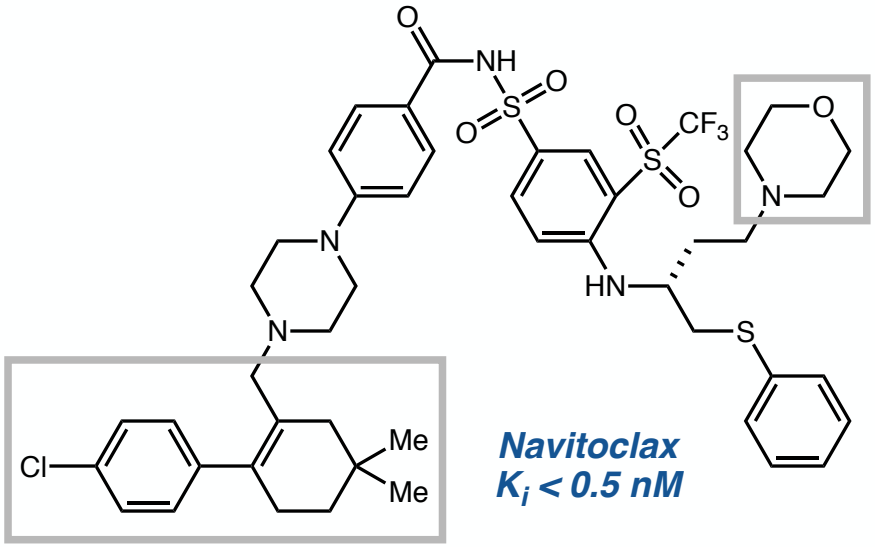
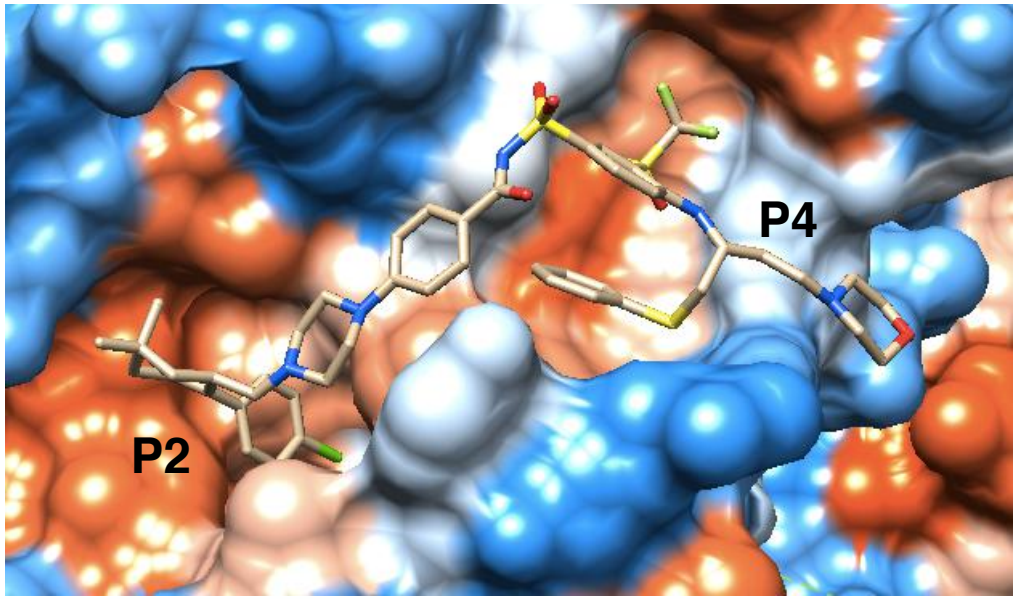




Navitoclax
K_i < 0.5 nM



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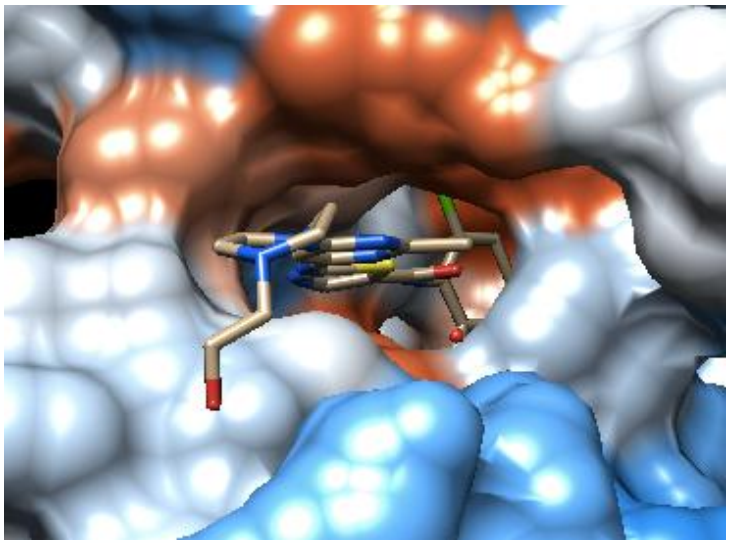
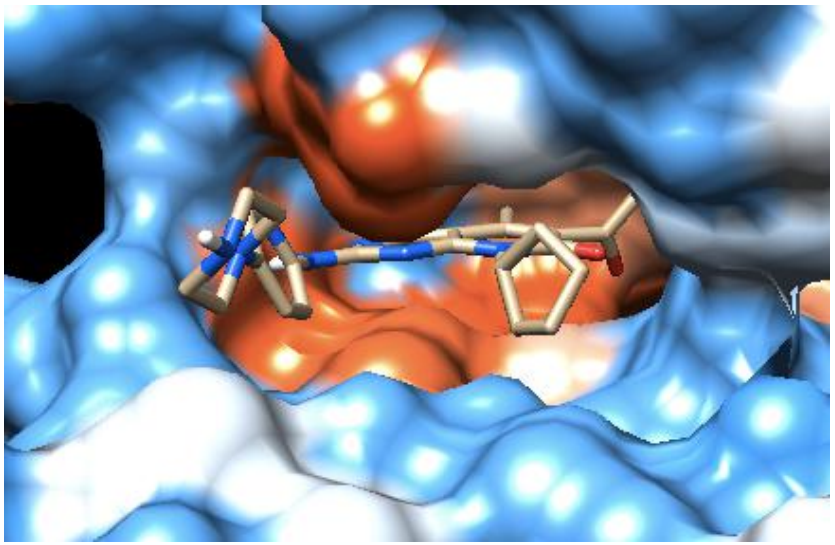
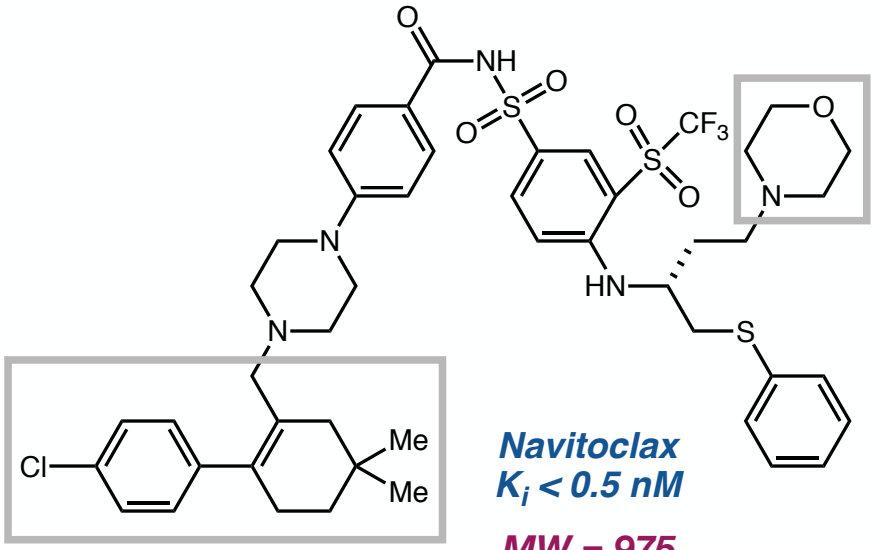
Case Study: Navitoclax



Dasatinib bound to ABL kinase (PDB: 2GQG)

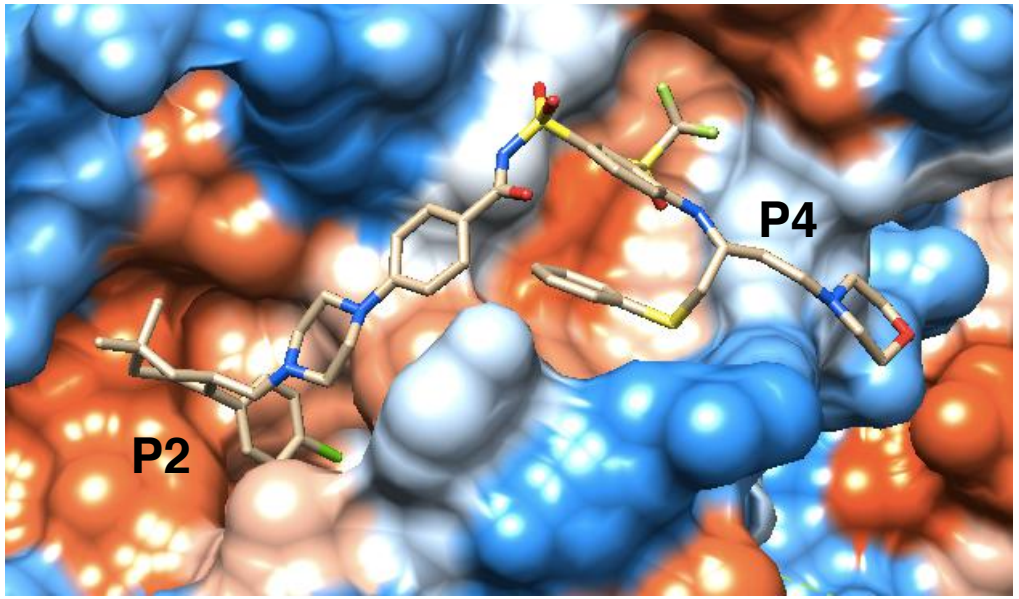


Palbociclib bound to CDK6 (PDB: 5L2I)



Navitoclax
K_i < 0.5 nM

MW = 975



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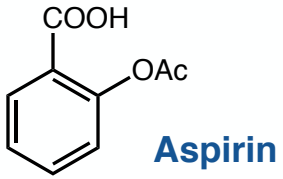
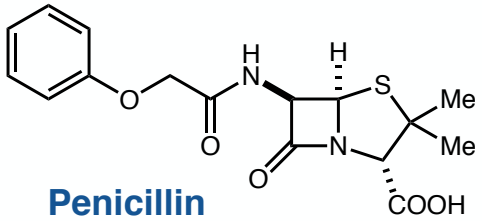
Resurgence of Covalent Drugs



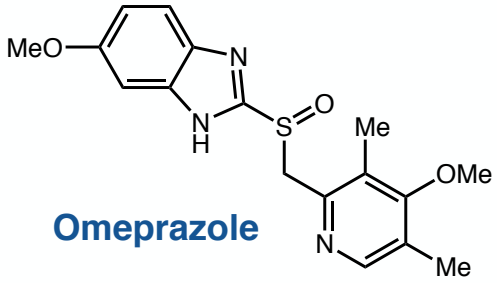
Aspirin

Bayer synthesized and distributes arpirin to patients



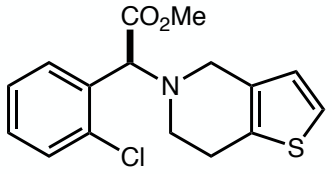
Penicillin

Discovery of penicillin



Omeprazole

Blockbuster proton pump inhibitor (omeprazole) approved



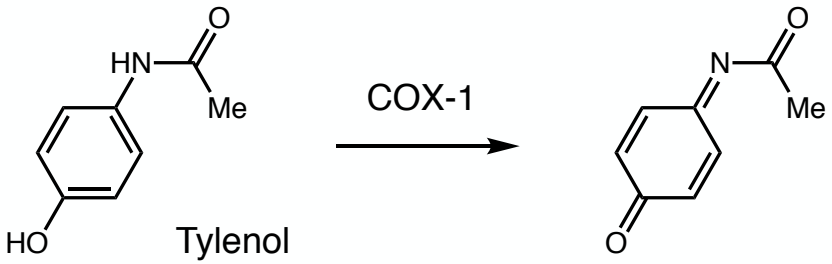
Clopidogrel

Blockbuster antiplatelet drug (clopidogrel) approved

1899 **1928** **1980s** **1990s**

Development of targeted covalent drugs

Acetaminophen-induced hepatotoxicity



Tylenol

Reactive quinone intermediate readily reacts with cysteine residues

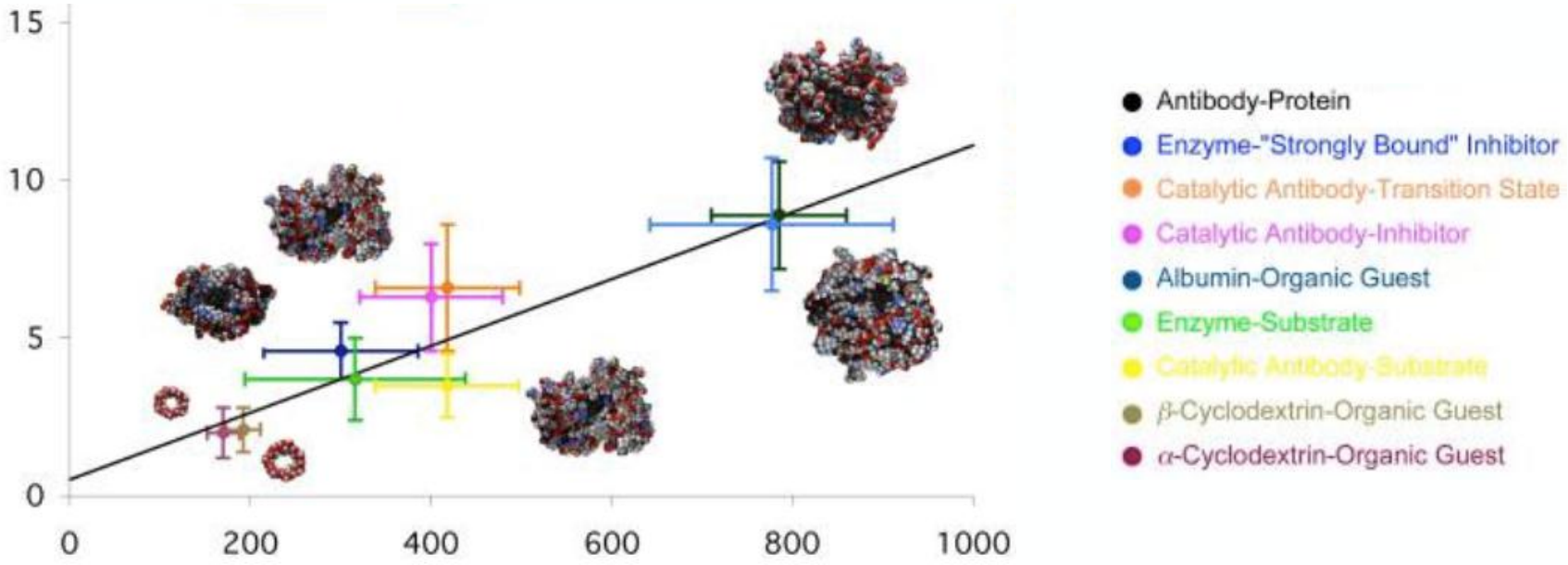
Nonselective covalent modification of proteins

Acute tissue injury

Immune system activation through haptenization

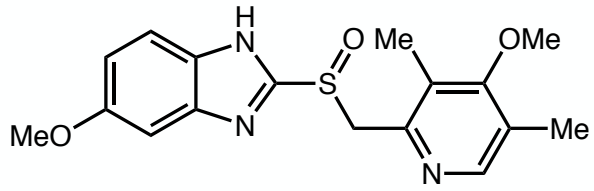
Singh, J.; Petter, R.C.; Baillie, T.A.; Whitty, A. *Nat. Rev. Drug Discov.* **2011**, *10*, 307.

Features of Covalent Drugs



Traditional ligand efficiency (~ 0.3 kcal/mol per heavy atom)

- *Covalent interactions exceed these ligand efficiency limits*



Omeprazole

- *Shorter exposure, longer effect*

Pharmacokinetic half life = 1–2 hr

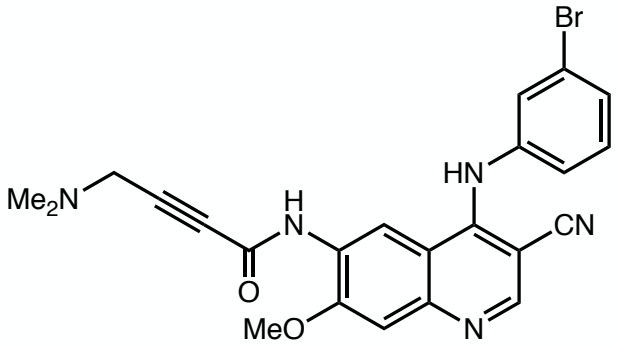
ATPase resynthesis half life = 54 hr

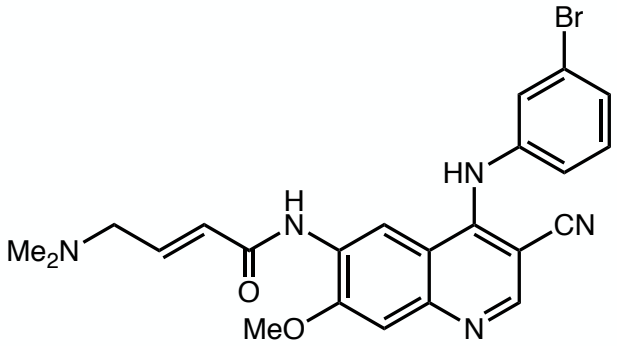
Duration of inhibition = 28 hr

Singh, J.; Petter, R.C.; Baillie, T.A.; Whitty, A. *Nat. Rev. Drug Discov.* **2011**, *10*, 307.

Features of Covalent Drugs

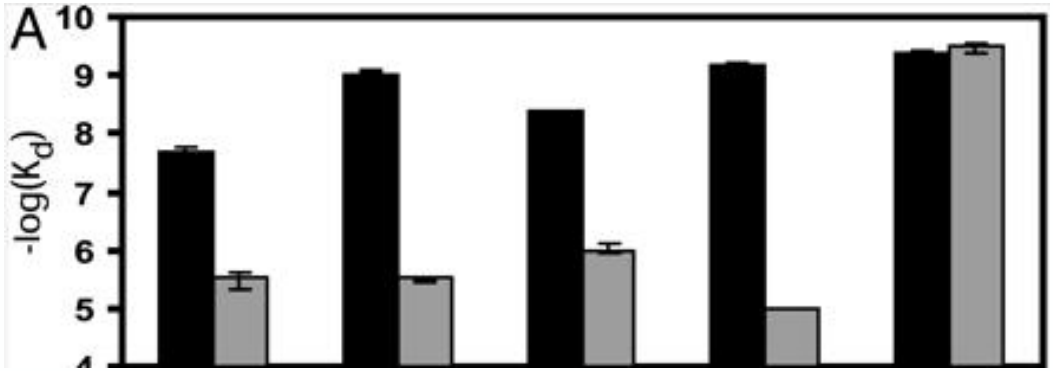
- *High degrees of discrimination between closely related proteins*





	IC_{50} (μM)	
EGFR	0.09	0.79
HER-2	**0.18**	**5.66**
A431	0.11	0.14
SKBR3	**0.12**	**0.03**
SW620	0.30	1.17

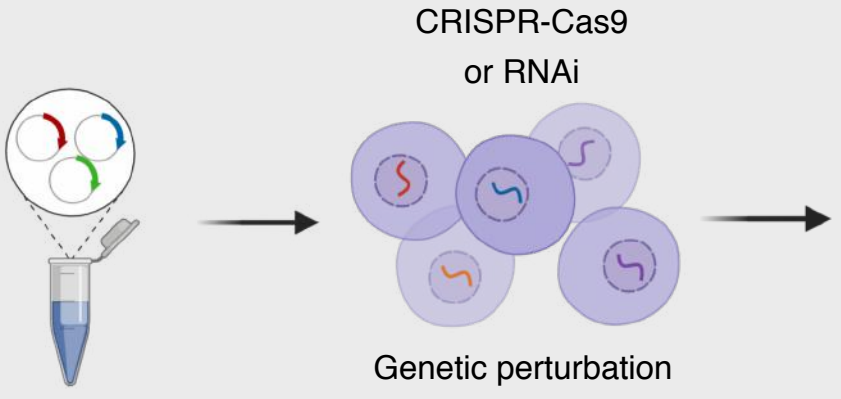
- *Less susceptible to resistance mutants*



Wisnner, A. *et al. J. Med. Chem.* **2003**, *46*, 49.
Singh, J.; Petter, R.C.; Baillie, T.A.; Whitty, A. *Nat. Rev. Drug Discov.* **2011**, *10*, 307.

Activity-Based Protein Profiling

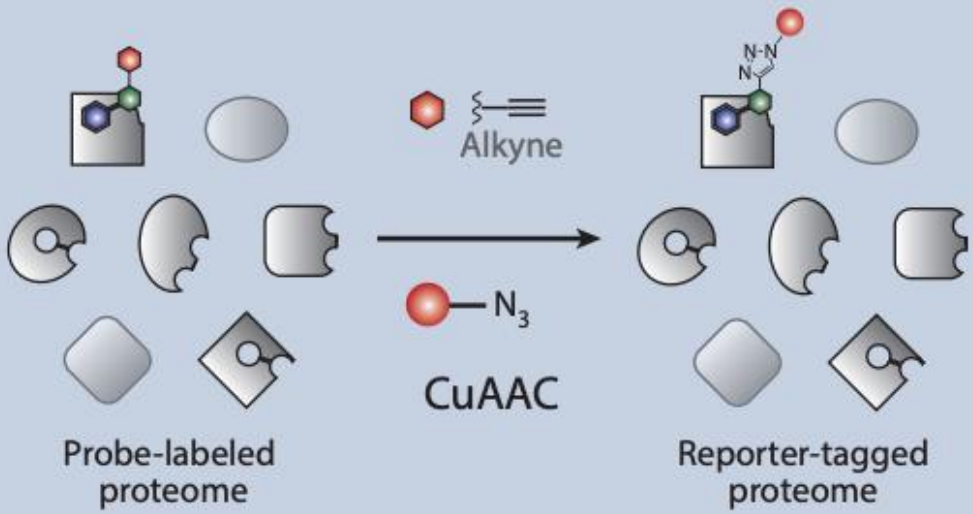
Chemical genetics-based drug discovery



Target ID & lead optimization

Cannot address post-translational modifications

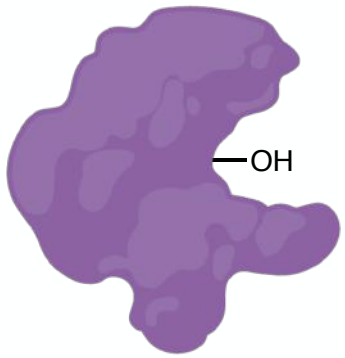
Chemical proteomics-based drug discovery

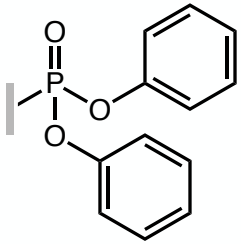


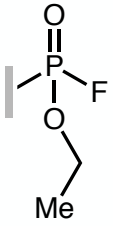
Target ID & lead optimization

Not applicable for HTS

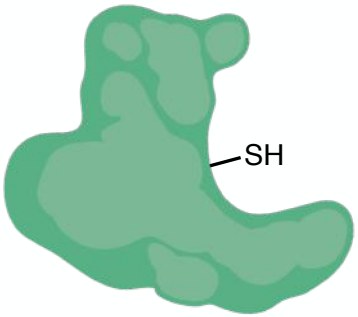
Activity-Based Protein Profiling

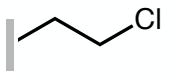


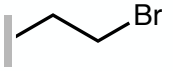


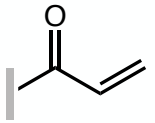


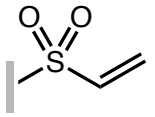
Serine hydrolase





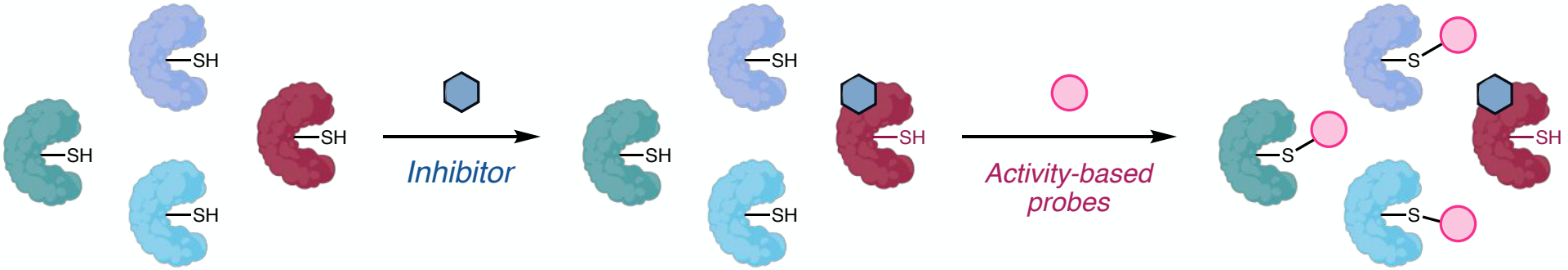






Cysteine protease

Inhibitor discovery by competitive ABPP



Cysteine proteome

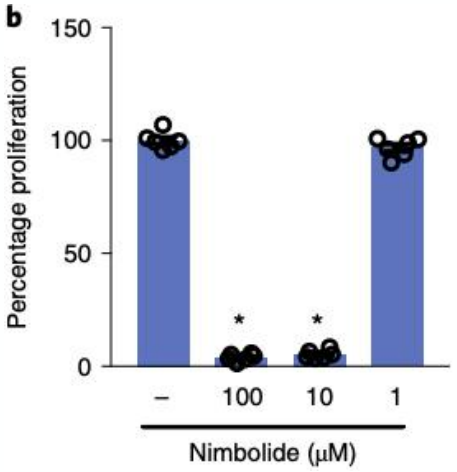
Inhibited proteins
loose activity

No modification of protein & small molecules

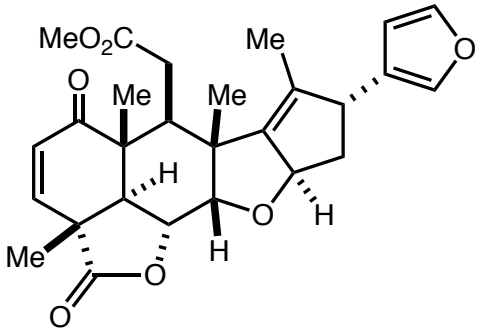
Qualitative measurement of activity inhibition

Carvatt, B.F.; Wright, A.T.; Kazarich, J.W. *Annu. Rev. Biochem.* **2008**, *77*, 383.

Activity-Based Protein Profiling

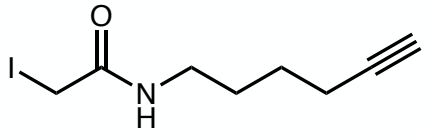


active against triple negative breast cancer cell 231MFP

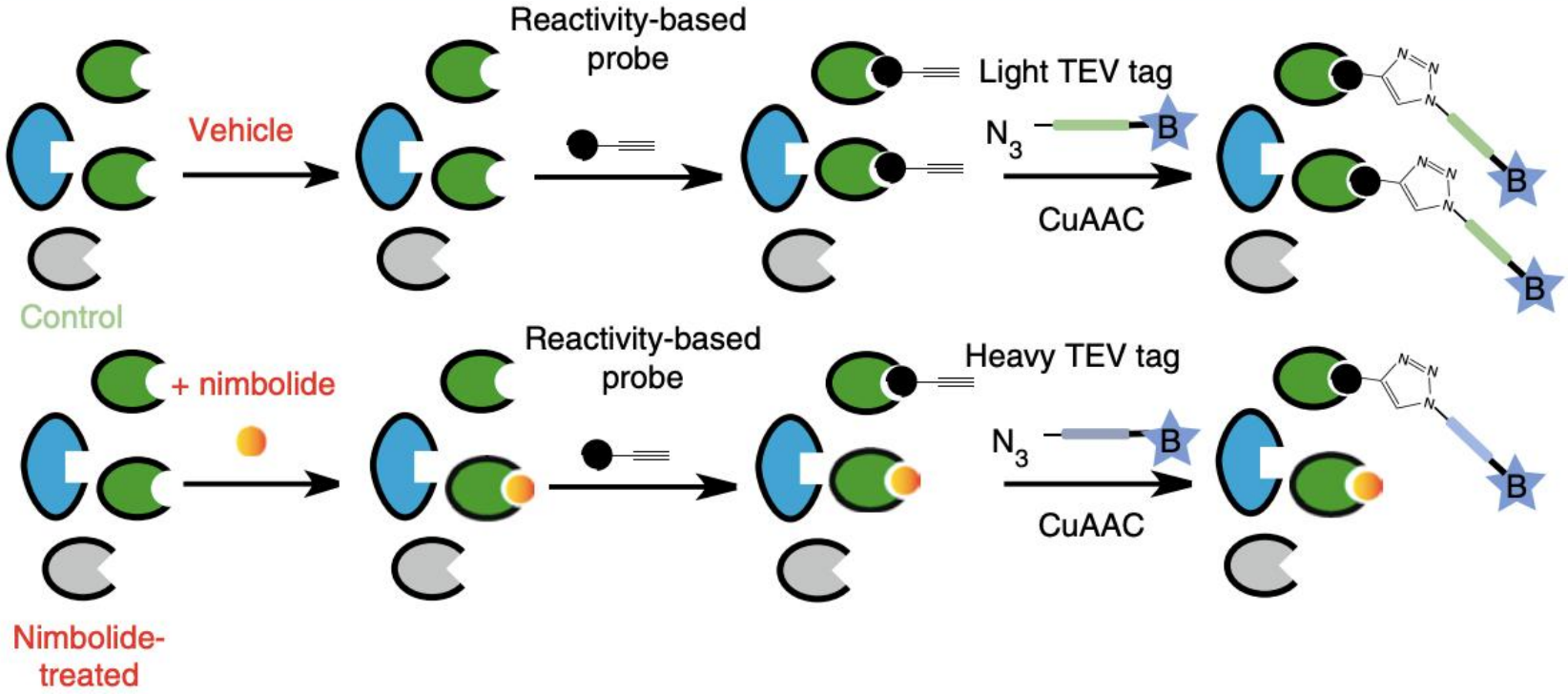


Nimbolide

vs.

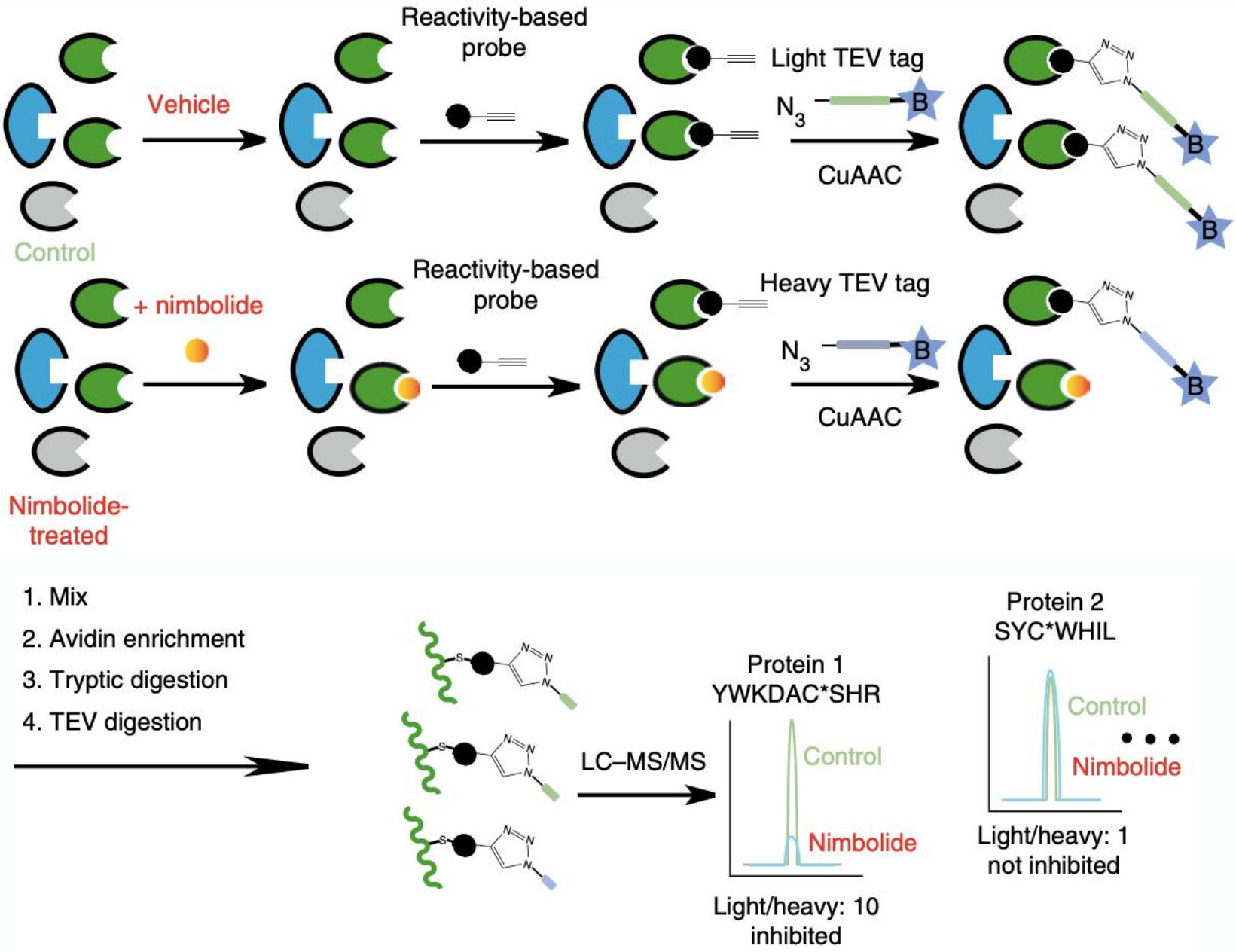


Reactivity-based probe



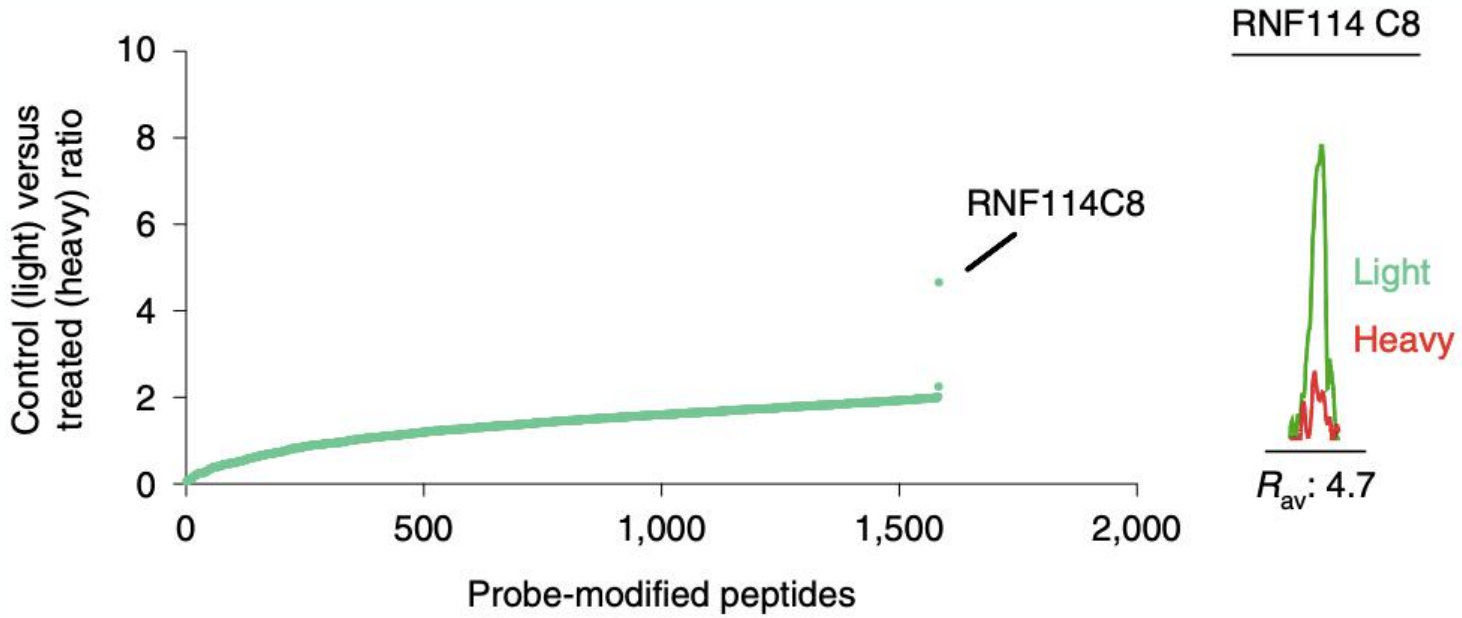
Spradlin, J.N. *et al. Nat. Chem. Biol.* **2019**, *15*, 747.
Carvatt, B.F.; Wright, A.T.; Kazarich, J.W. *Annu. Rev. Biochem.* **2008**, *77*, 383.

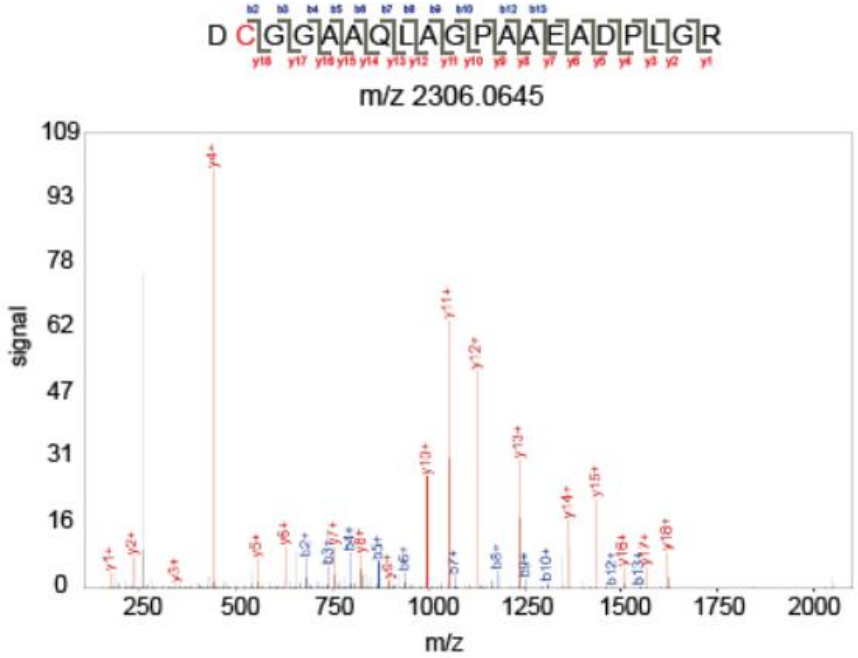
Activity-Based Protein Profiling

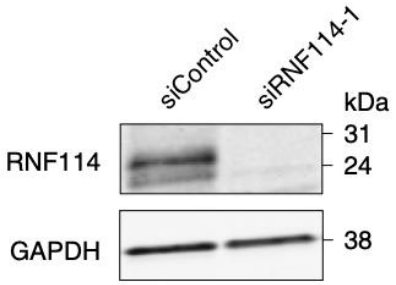


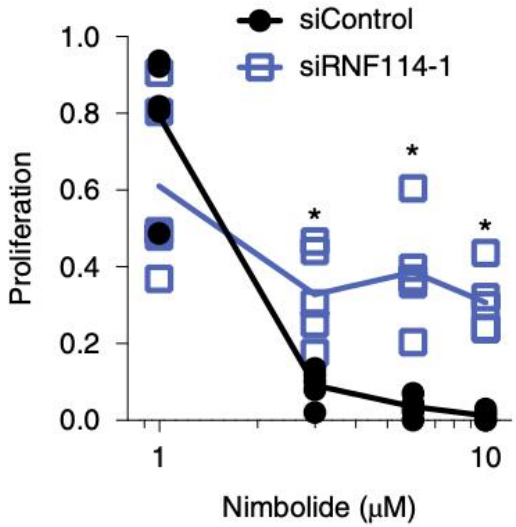
Spradlin, J.N. *et al. Nat. Chem. Biol.* **2019**, *15*, 747.
Carvatt, B.F.; Wright, A.T.; Kazarich, J.W. *Annu. Rev. Biochem.* **2008**, *77*, 383.

Activity-Based Protein Profiling







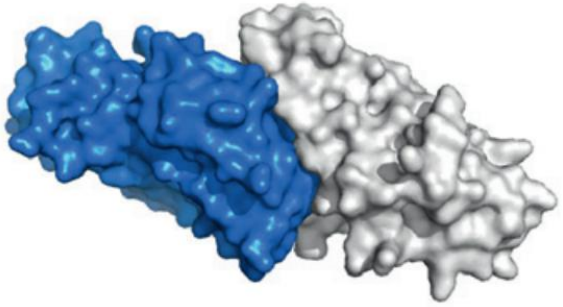


no dose dependent response to nimbolide

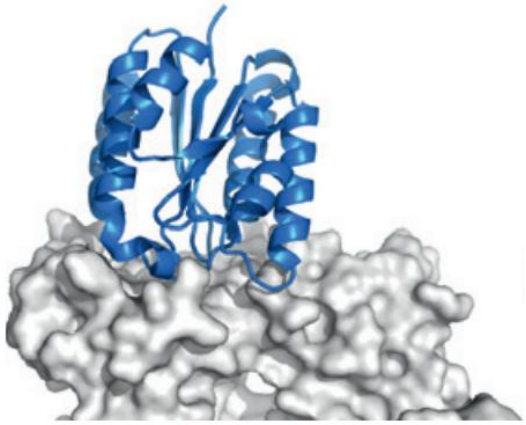
Spradlin, J.N. *et al. Nat. Chem. Biol.* **2019**, *15*, 747.
Carvatt, B.F.; Wright, A.T.; Kazarich, J.W. *Annu. Rev. Biochem.* **2008**, *77*, 383.

Targeting "Undruggable" Proteins

Protein–Protein interaction



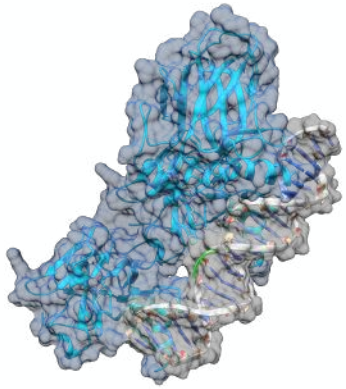
No dominant ***Hot Segment***



Surface area $< 2500\ Å^2$

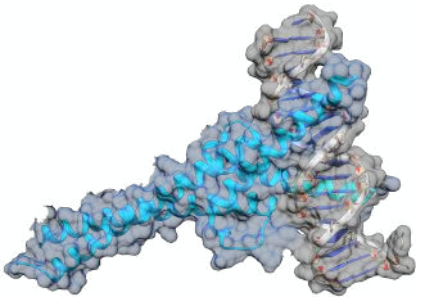
$K_d < 200\ nM$

Main drivers of cancer



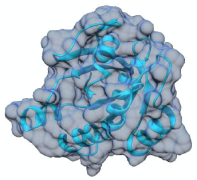
p53

Guardian of genome



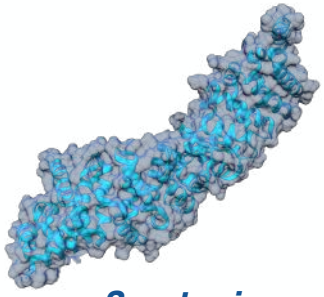
Myc

Master regulator



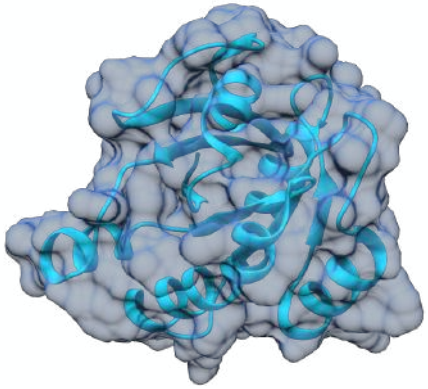
Ras

Beating heart of cancer



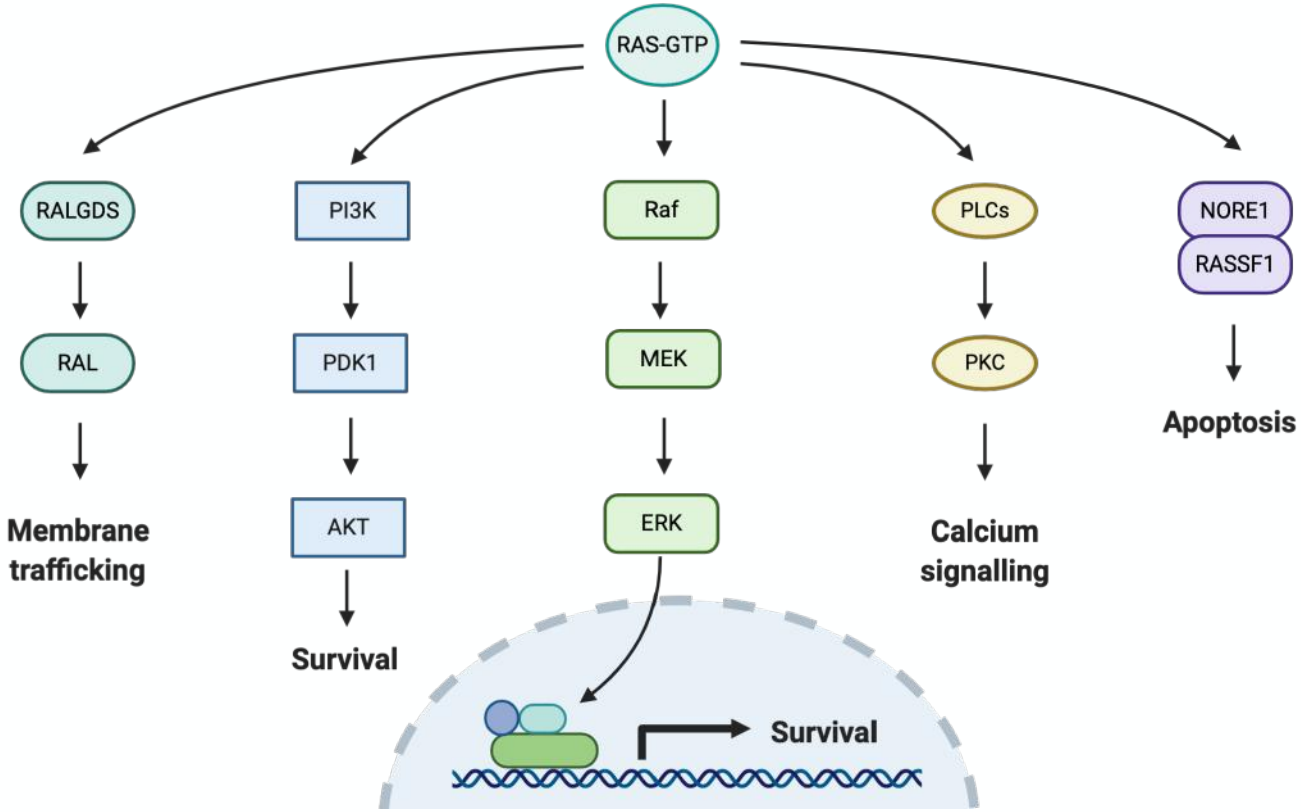
ß-catenin

Targeting KRAS Directly



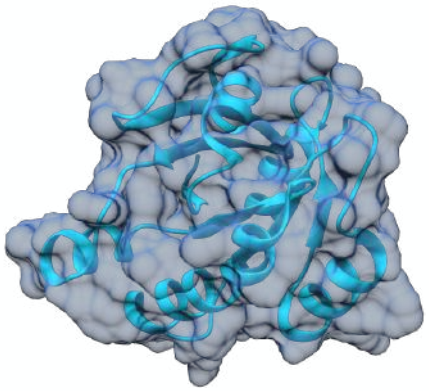
RAS

- Three isoforms: **KRAS**, NRAS, HRAS
- Mutated in approximately 25 percent of all human cancers
  - 90 percent of pancreatic cancers
  - 35–45 percent of colorectal cancers
  - 25 percent of lung cancers



Stolze, B.; Reinhart, S.; Bulllinger, L.; Frohling, S.; Scholl, C. *Scientific Reports* **2015**, *5*, 8535.

Targeting KRAS Directly



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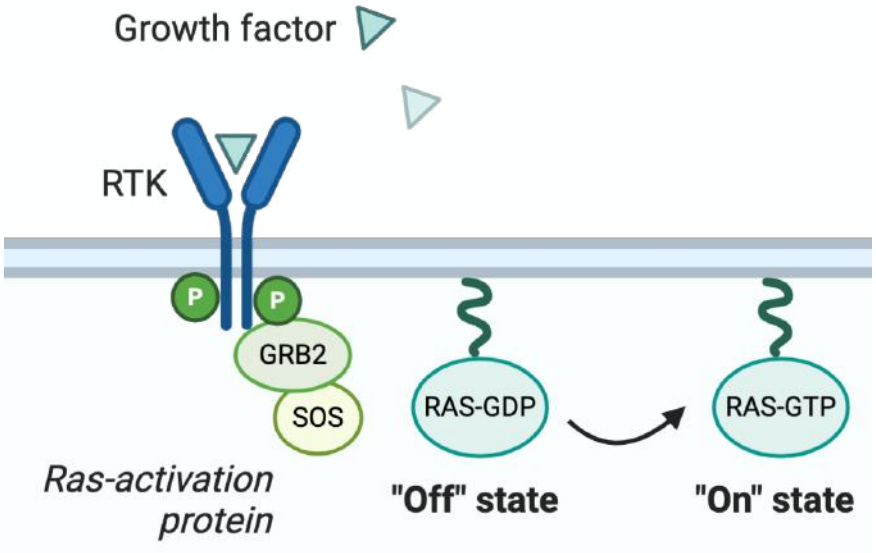
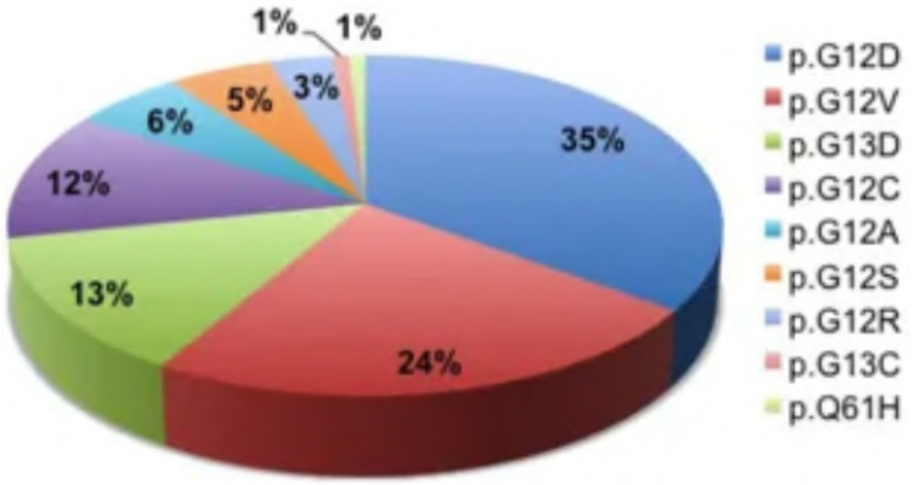
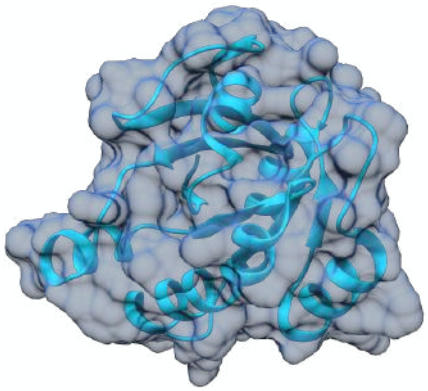


KRAS mutation frequency



McCormick, F. *Annu. Rev. Cancer Biol.* **2018**, *2*, 81.
Stolze, B.; Reinhart, S.; Bulllinger, L.; Frohling, S.; Scholl, C. *Scientific Reports* **2015**, *5*, 8535.

Targeting KRAS Directly



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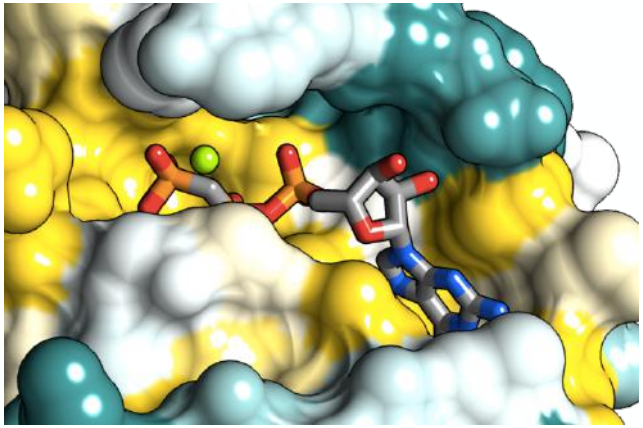
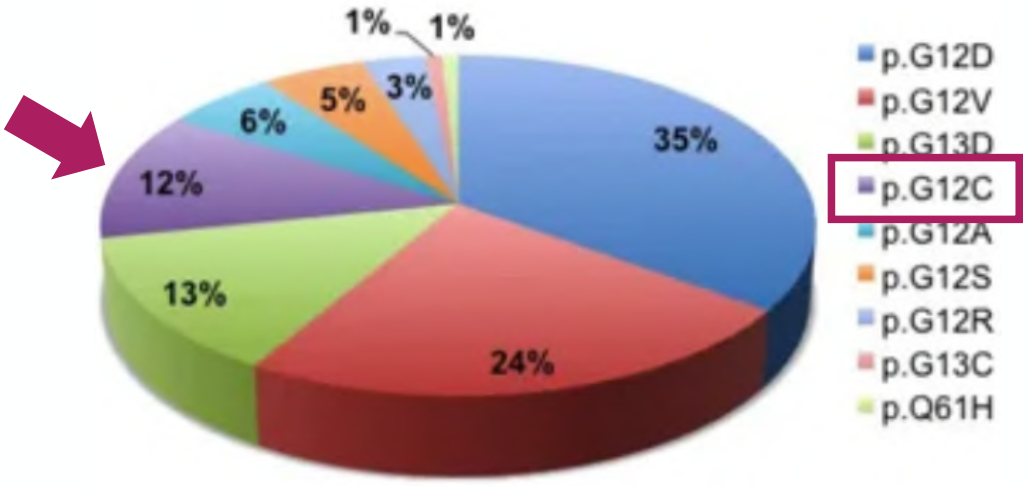


pM binding affinity w/ GTP

multiple downstream signals

KRAS mutation frequency

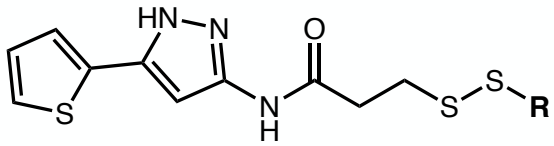


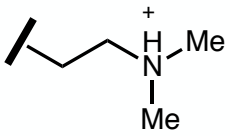
G12C – Smoking-associated lung cancer

McCormick, F. *Annu. Rev. Cancer Biol.* **2018**, *2*, 81.
Stolze, B.; Reinhart, S.; Bulllinger, L.; Frohling, S.; Scholl, C. *Scientific Reports* **2015**, *5*, 8535.

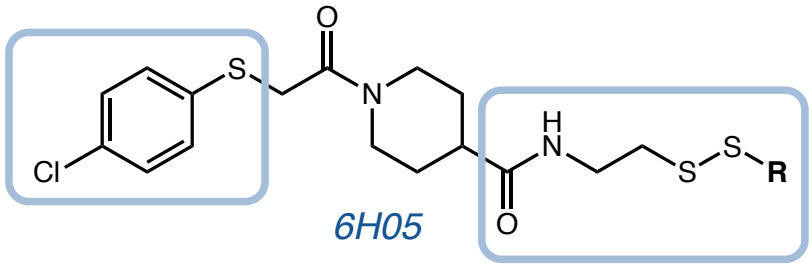
Seminal Report by Shokat and Coworkers

■ *Initial hits*



R = 

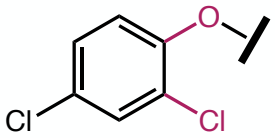
2E07

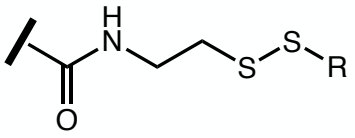


6H05

Relative potency

1





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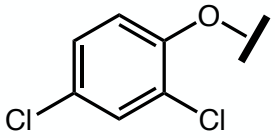

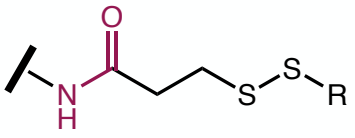

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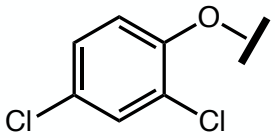

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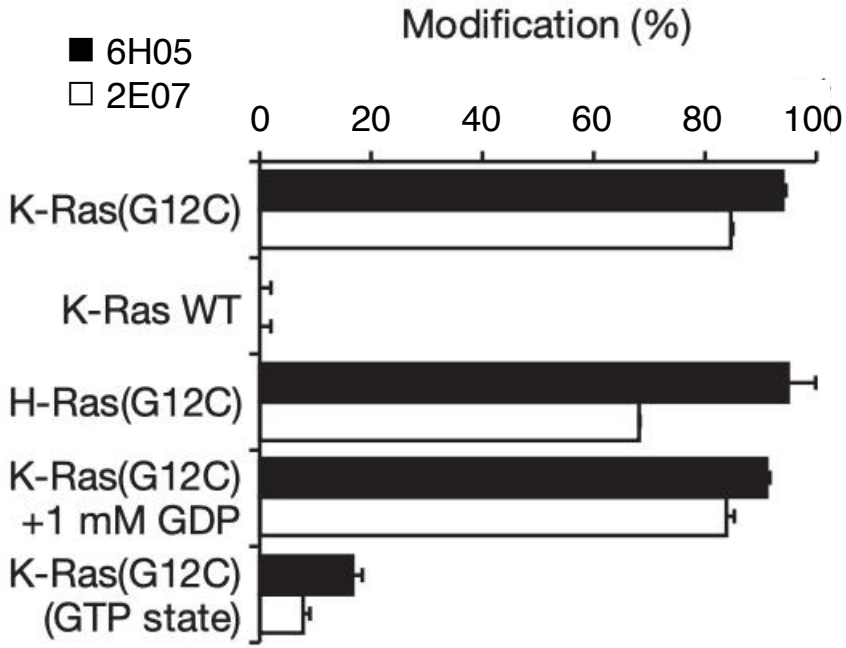
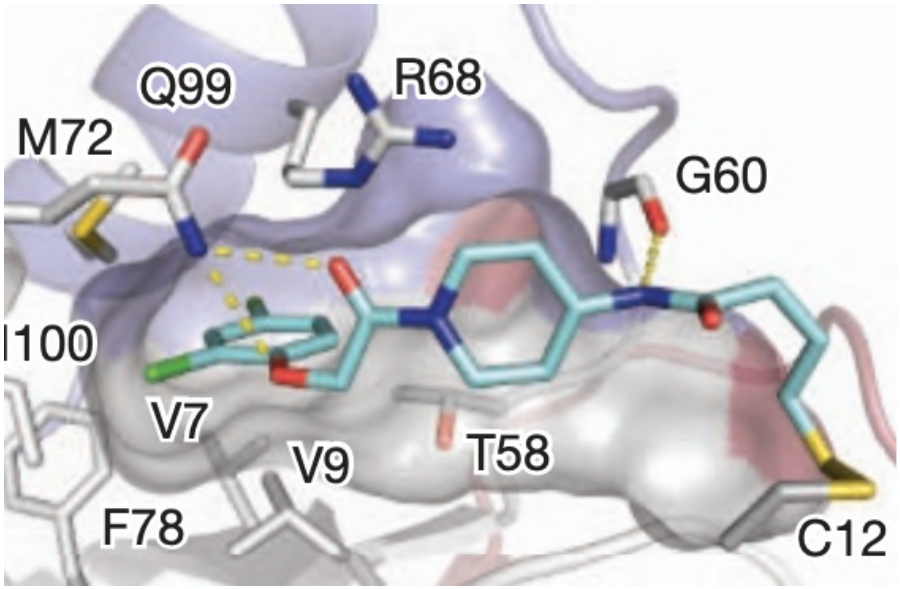
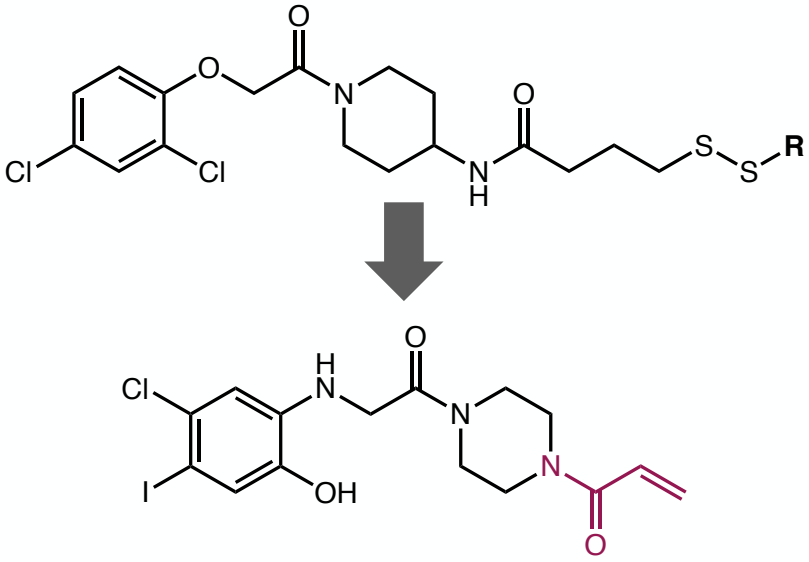


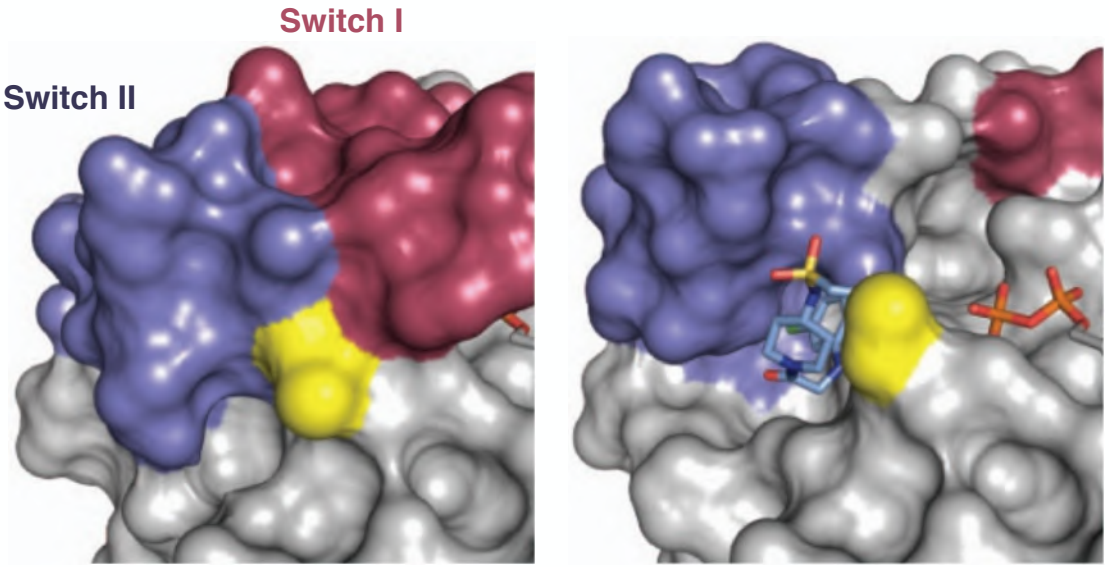


Ostrem, J.M.; Peters, U.; Sos, M.L.; Wells, J.A.; Shokat, K.M. *Nature* **2013**, *503*, 548.

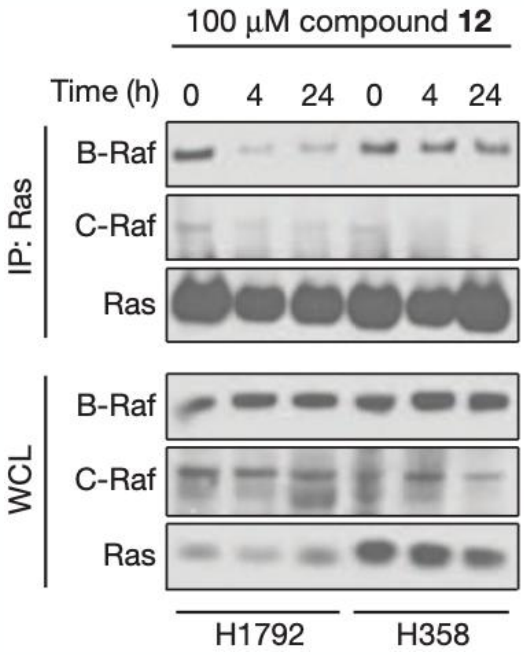
Seminal Report by Shokat and Coworkers

- *Irreversible covalent binder*

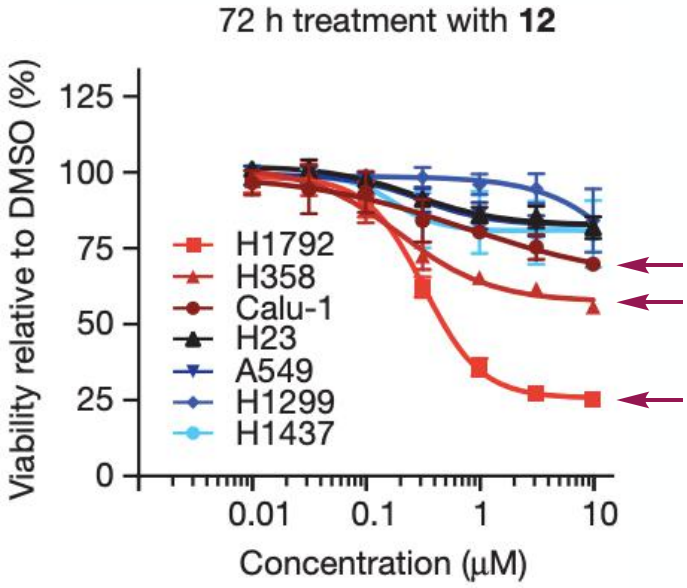




- *Shift of switch II and partial disordering of switch I*



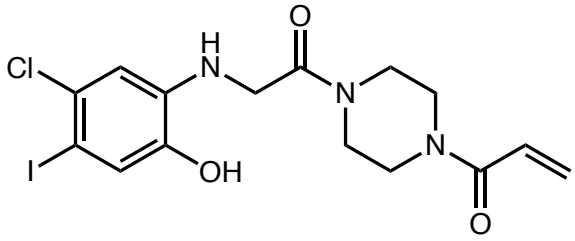
Raf protein did not co-immunoprecipitate after treatment



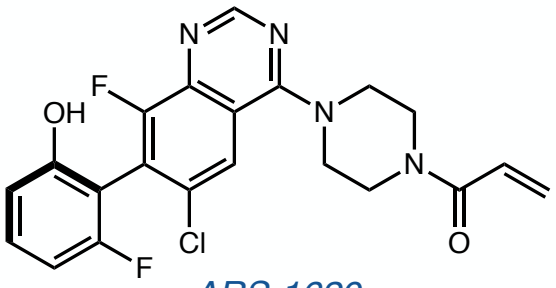
KRAS (G12C) mutant cell lines

Ostrem, J.M.; Peters, U.; Sos, M.L.; Wells, J.A.; Shokat, K.M. *Nature* **2013**, *503*, 548.

Lead Optimization

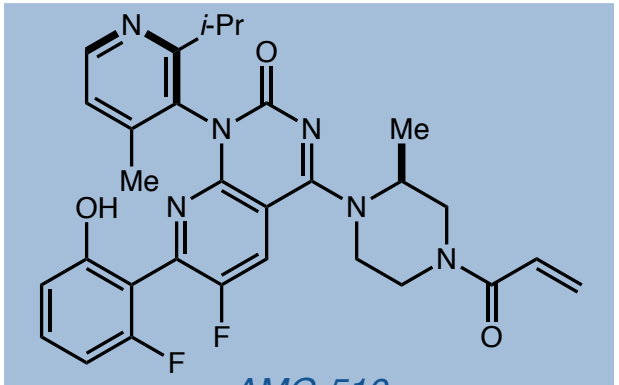


Shokat's covalent binder



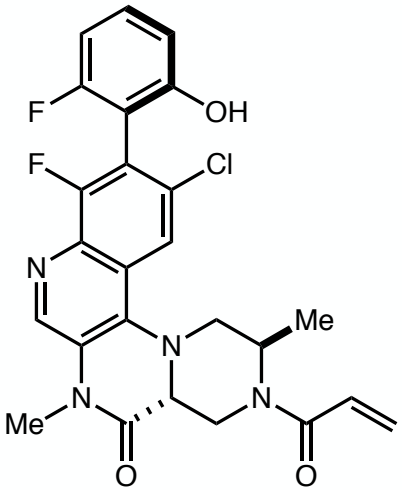
ARS-1620

Arazex Pharma/Wellspring Biosciences

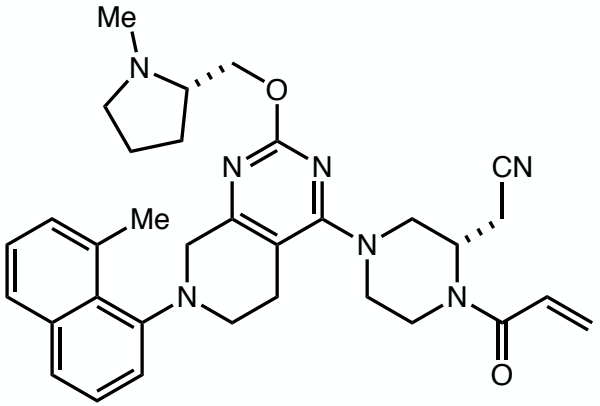


AMG-510

Amgen



AstraZeneca

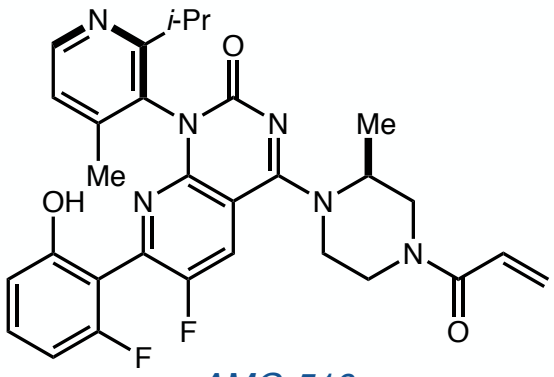


MRTX-1257

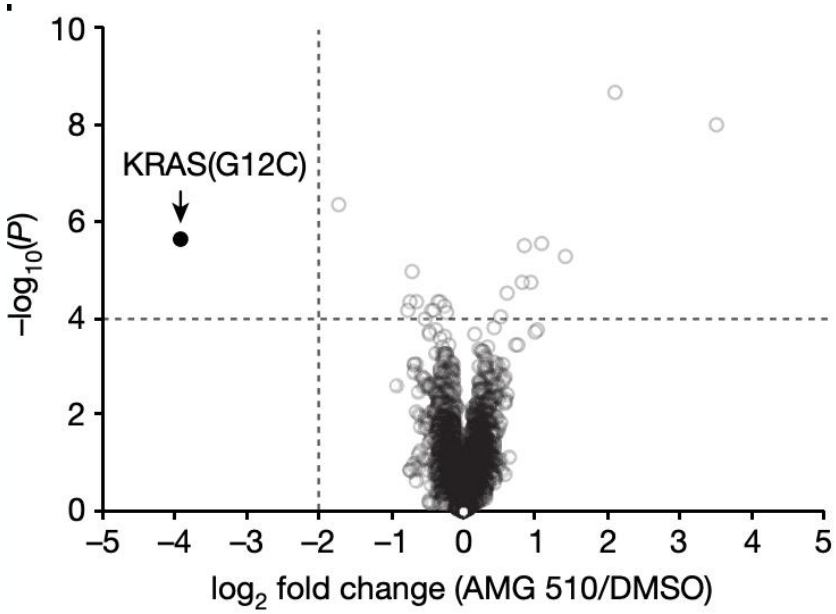
Mirati Therapeutics/Array Biopharma

Canon, J. *et al. Nature* **2019**, *575*, 217.

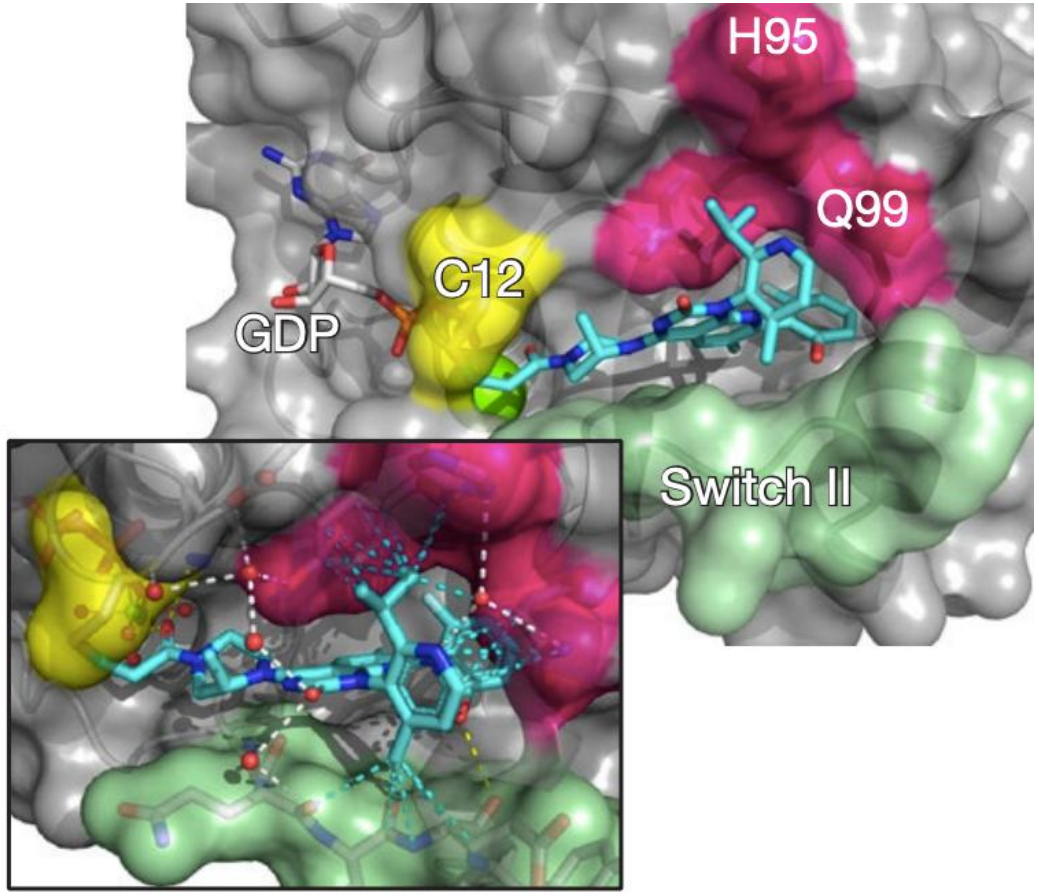
AMG-510



AMG-510
Amgen



Cystein proteomic profiling



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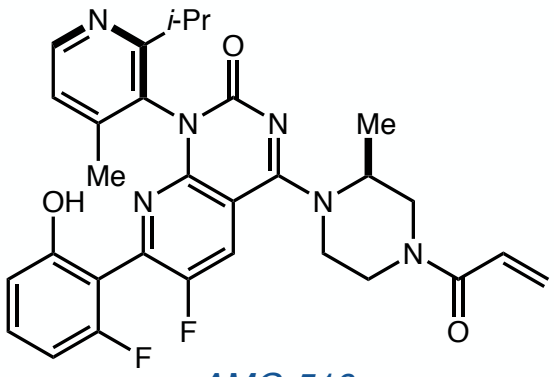
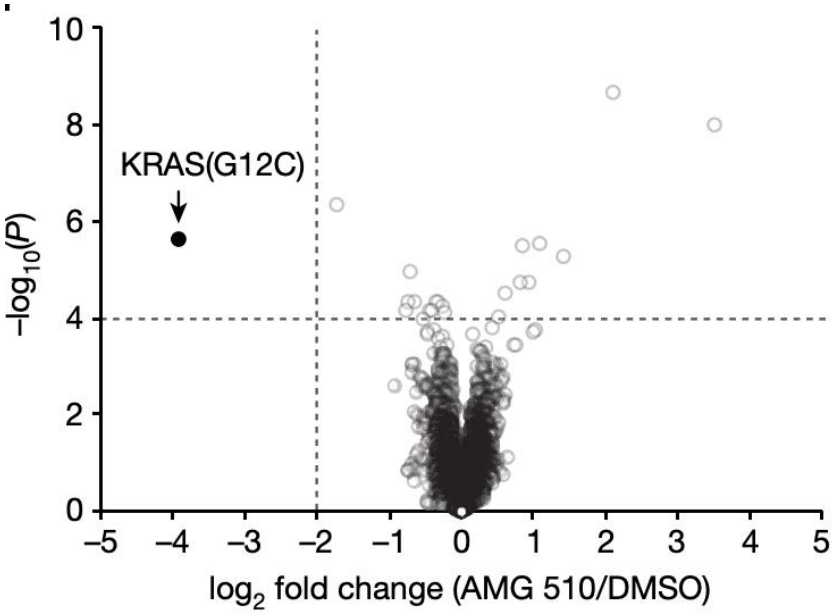


AMG-510
Amgen



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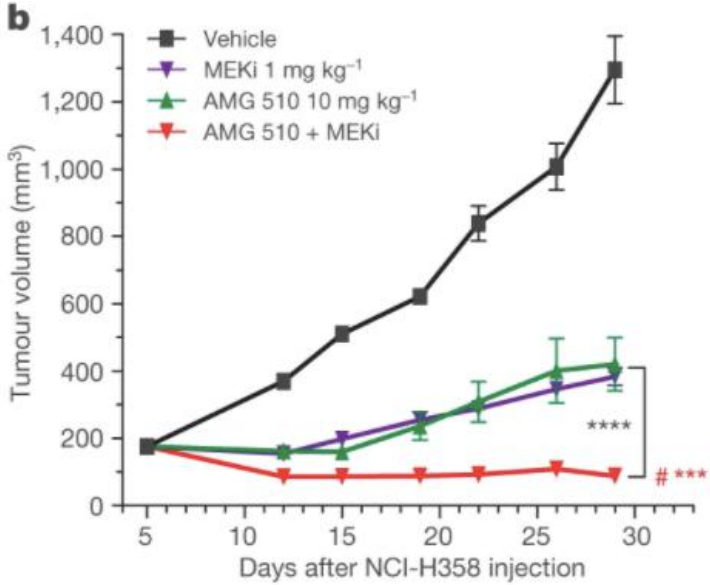

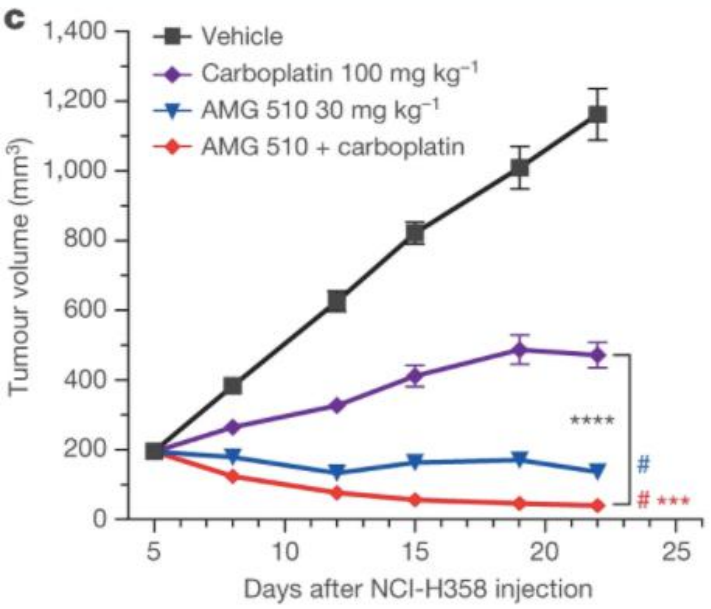

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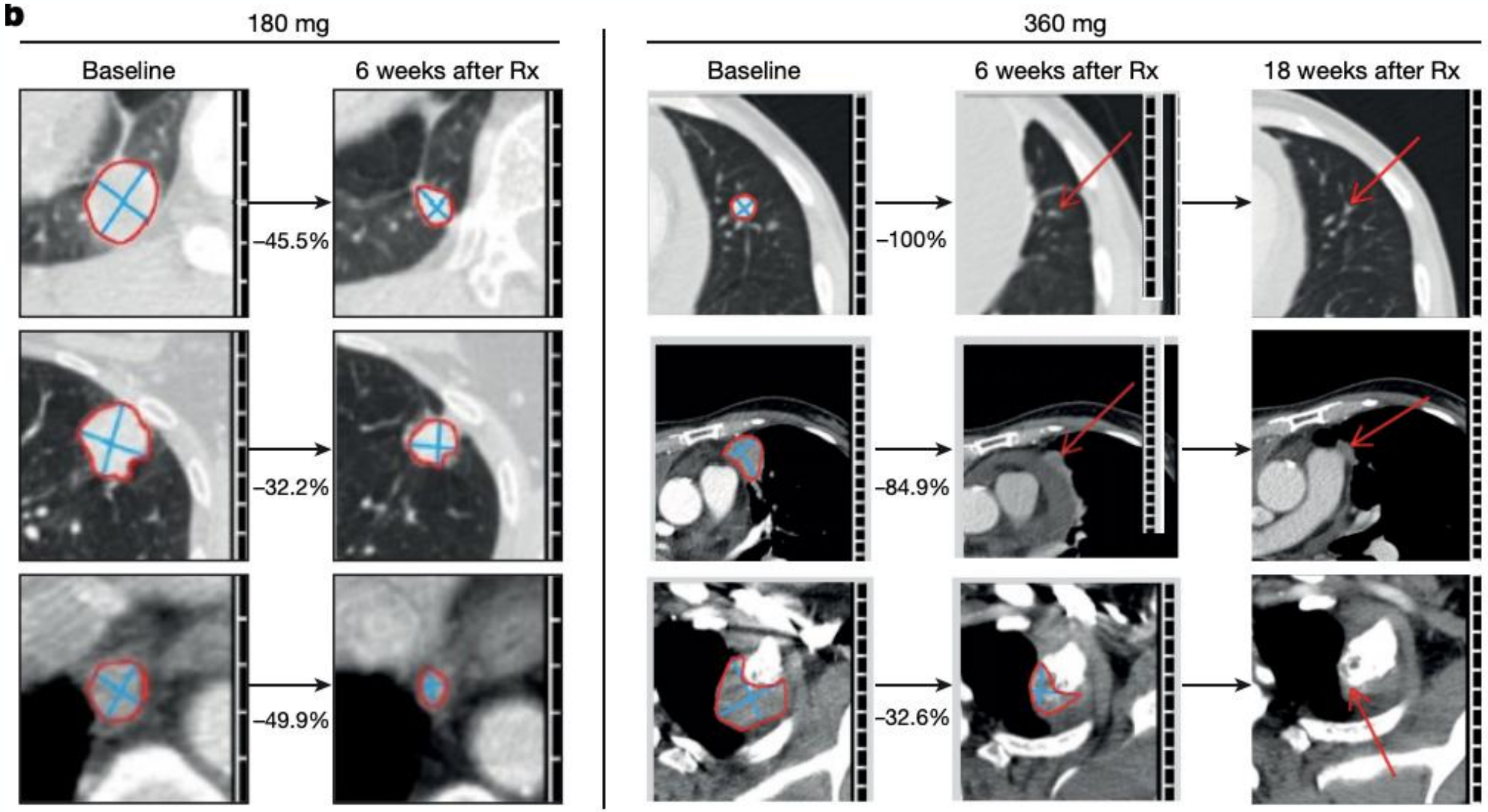
Clinical Activity of AMG-510 in Patients

$KRAS^{G12C}$ lung carcinoma patients



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