Chemical approaches to drugging “undruggable” proteins

Diego A. Granados

May 5th, 2023

Knowles Group Literature Meeting

Princeton University

Outline

- Introduction to undruggable proteins
  - What makes a protein “undruggable”?
  - Attempts to drug K-Ras mutations

- Activity-based approaches to finding “druggable” sites

- Success stories in covalent drugs
  - Ibrutinib and Bruton’s tyrosine kinase
  - Sotorasib and K-Ras G12C

- Conclusions
  - ”Yet to be drugged” instead of “undruggable”

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What makes something “undruggable”?

A protein is considered **undruggable** when traditional pharmacological strategies have failed

Journal of Hematology & Oncology **2022**, 15, 152

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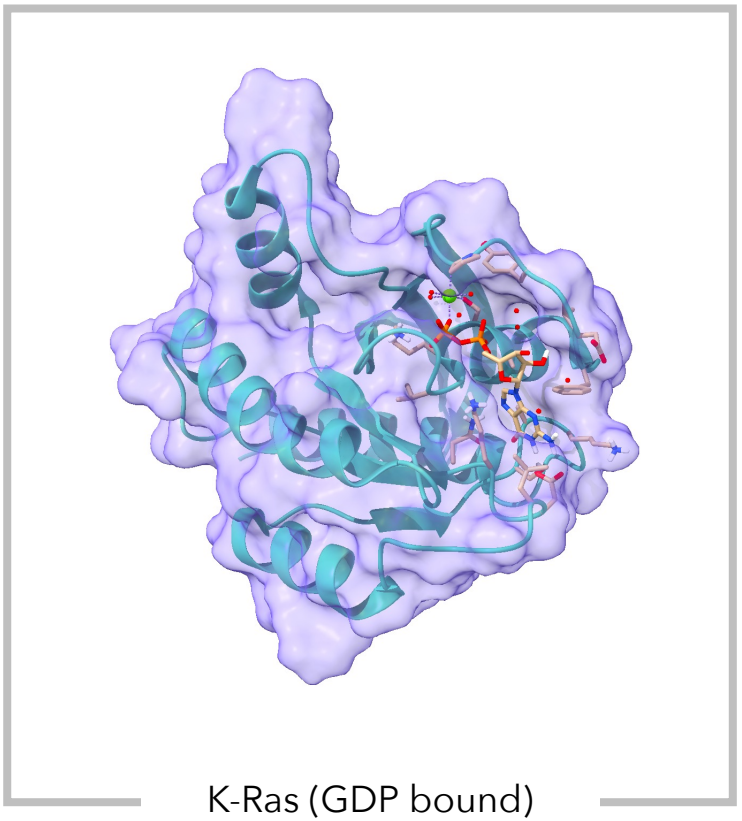
  - First discovered oncogene (1967)
  - GTP kinase (GTPase) involved in MAPK signal transduction (cell proliferation)



K-Ras (GDP bound)

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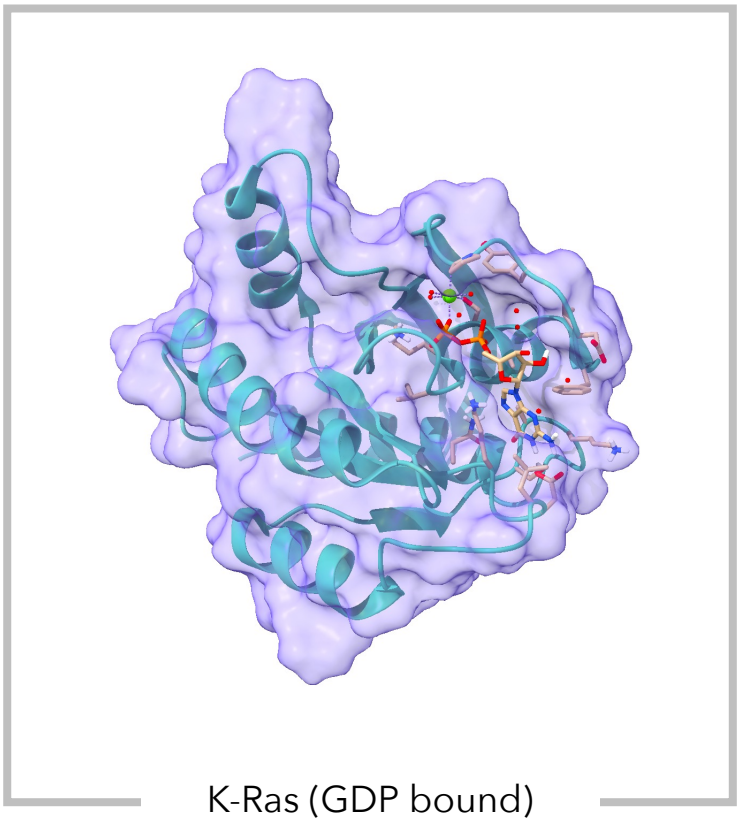

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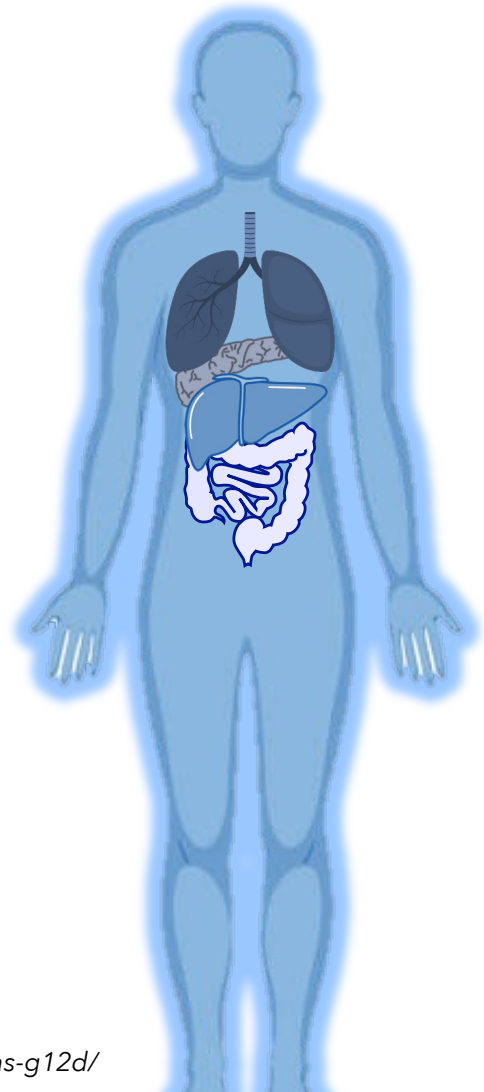
K-Ras mutations are implicated in a variety of cancers



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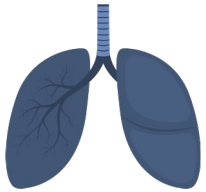
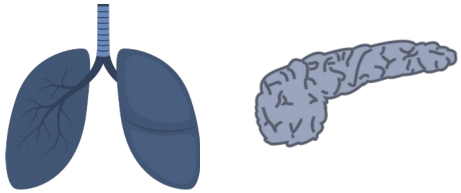
K-Ras G12C

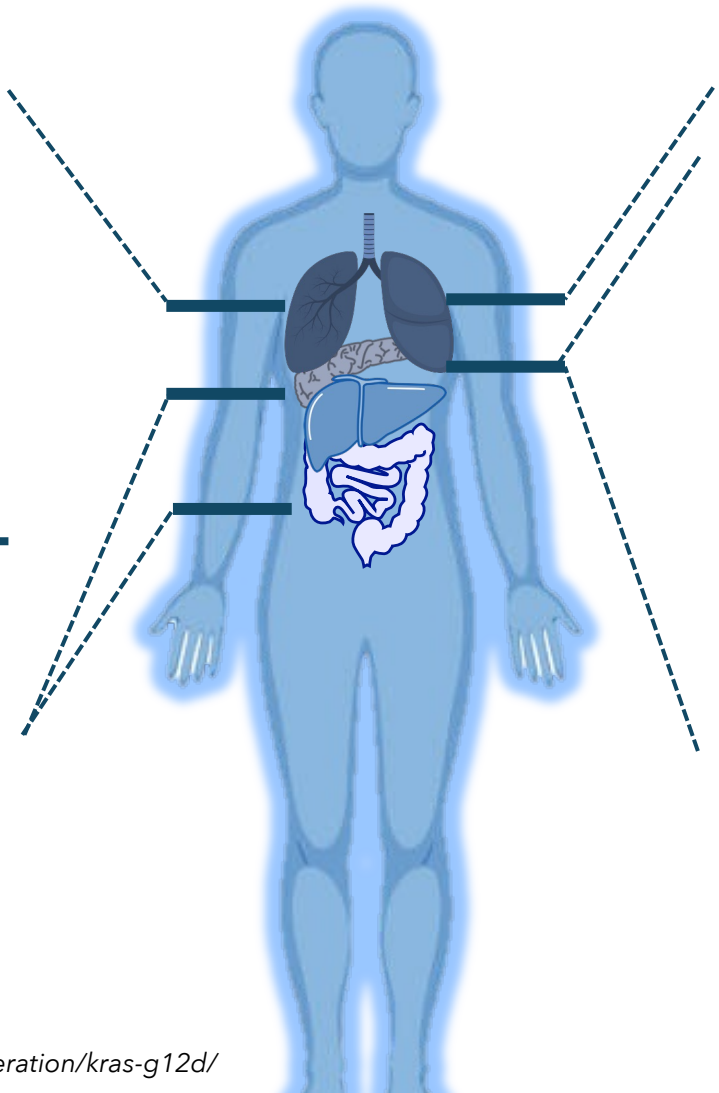


2.47% cases
Lung adenocarcinoma

K-Ras G12V



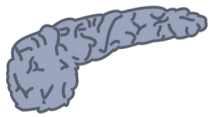
3.47% cases
Pancreatic, lung and colon/colorectal adenocarcinoma



K-Ras G12D

4.20% cases
Pancreatic, colon, and lung adenocarcinoma

K-Ras Q61R



0.17% cases
Pancreatic adenocarcinoma

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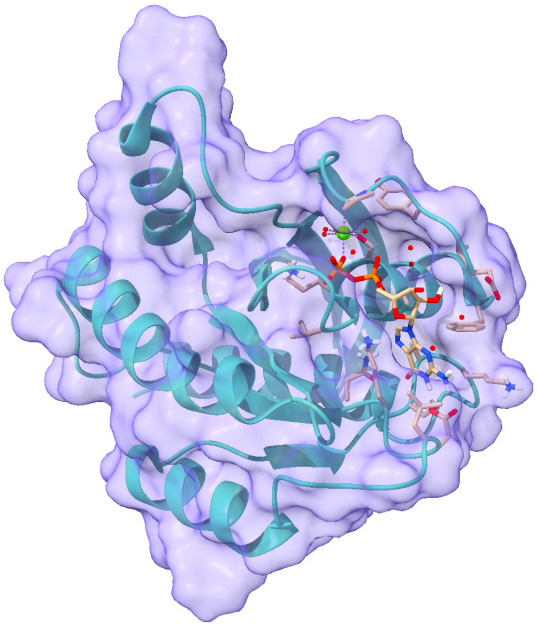

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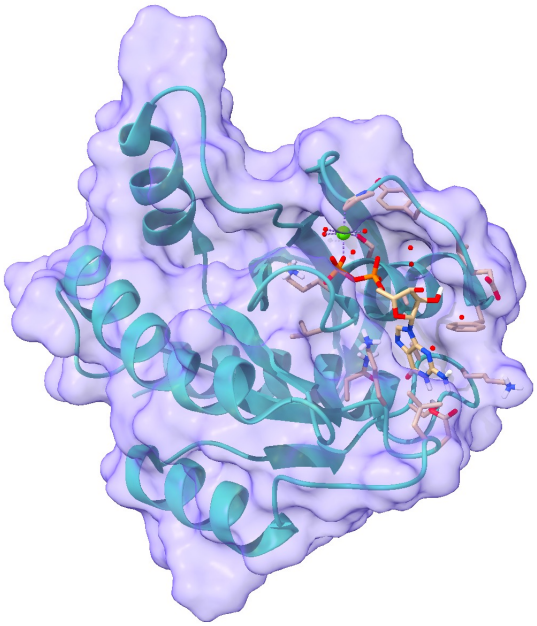

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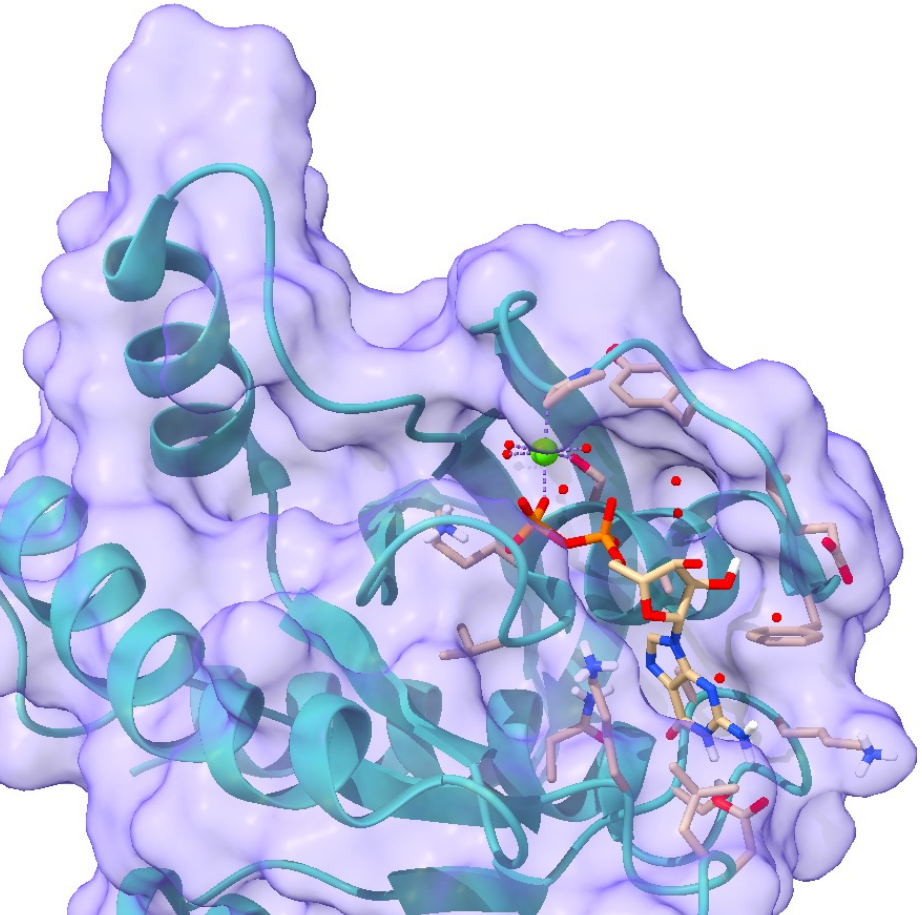
K-Ras mutations tend to affect phosphorylation state



Nature **2013**, 503, 548–551

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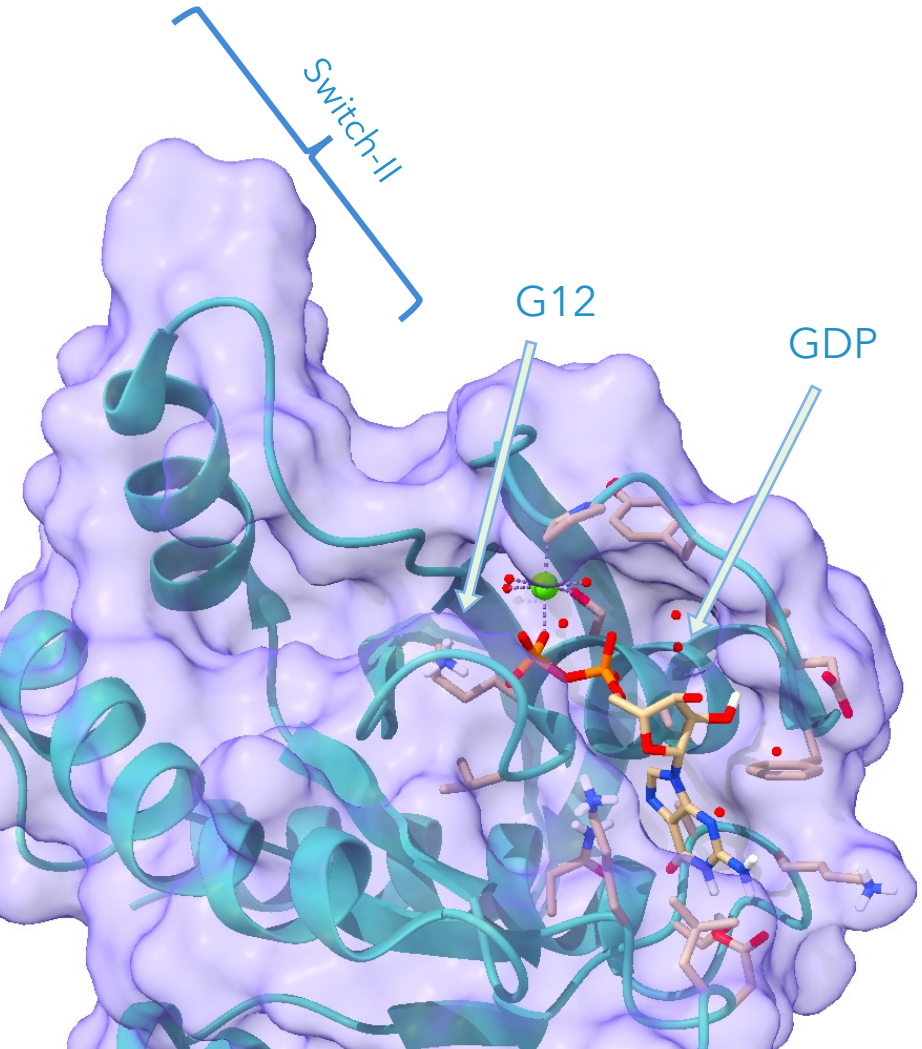

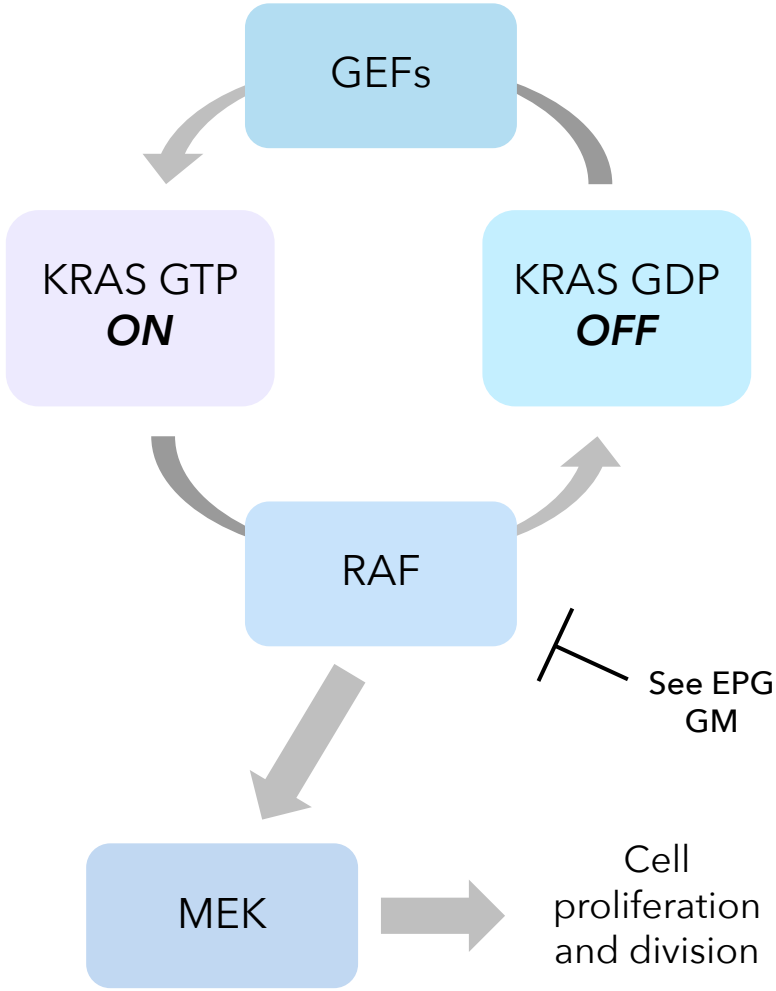

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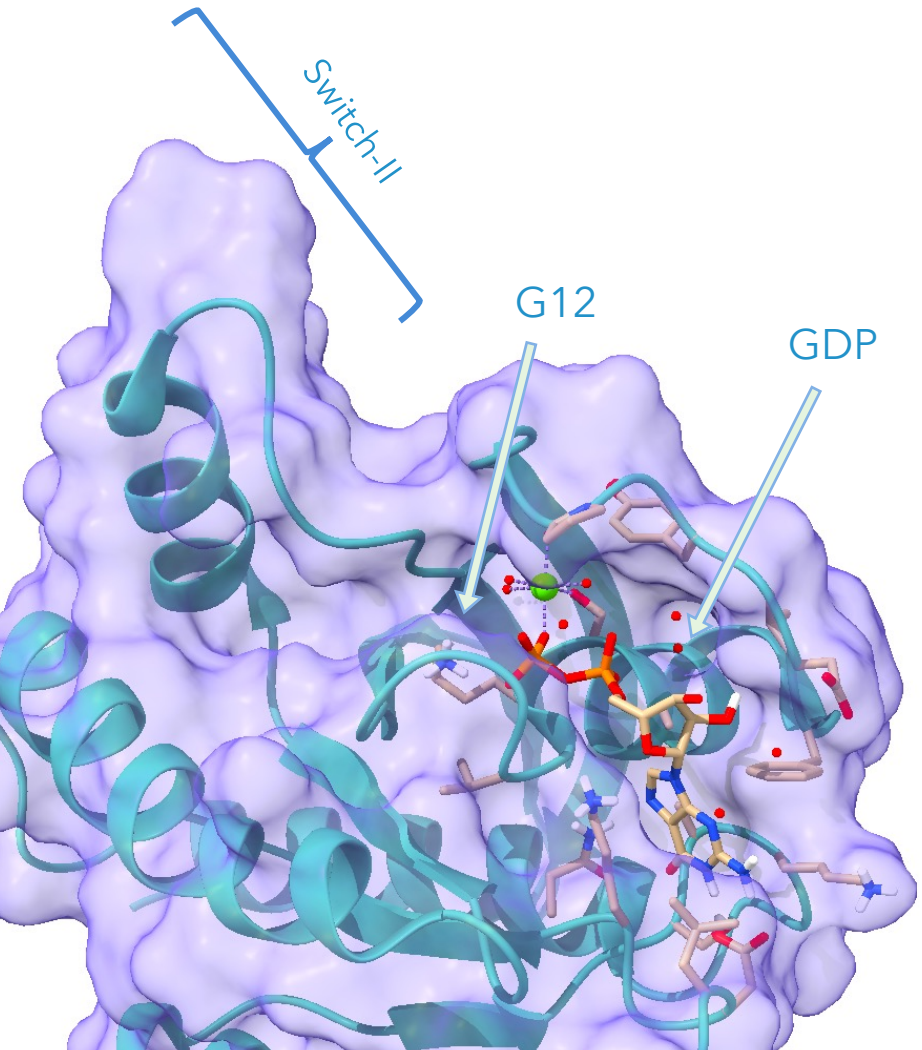

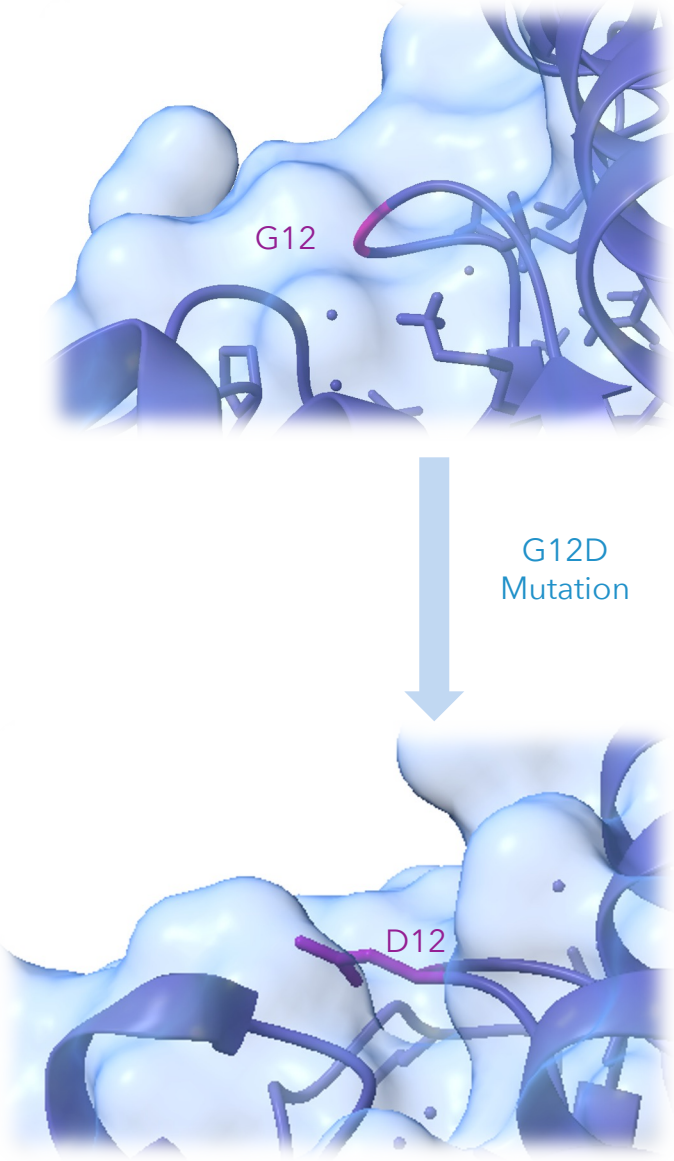

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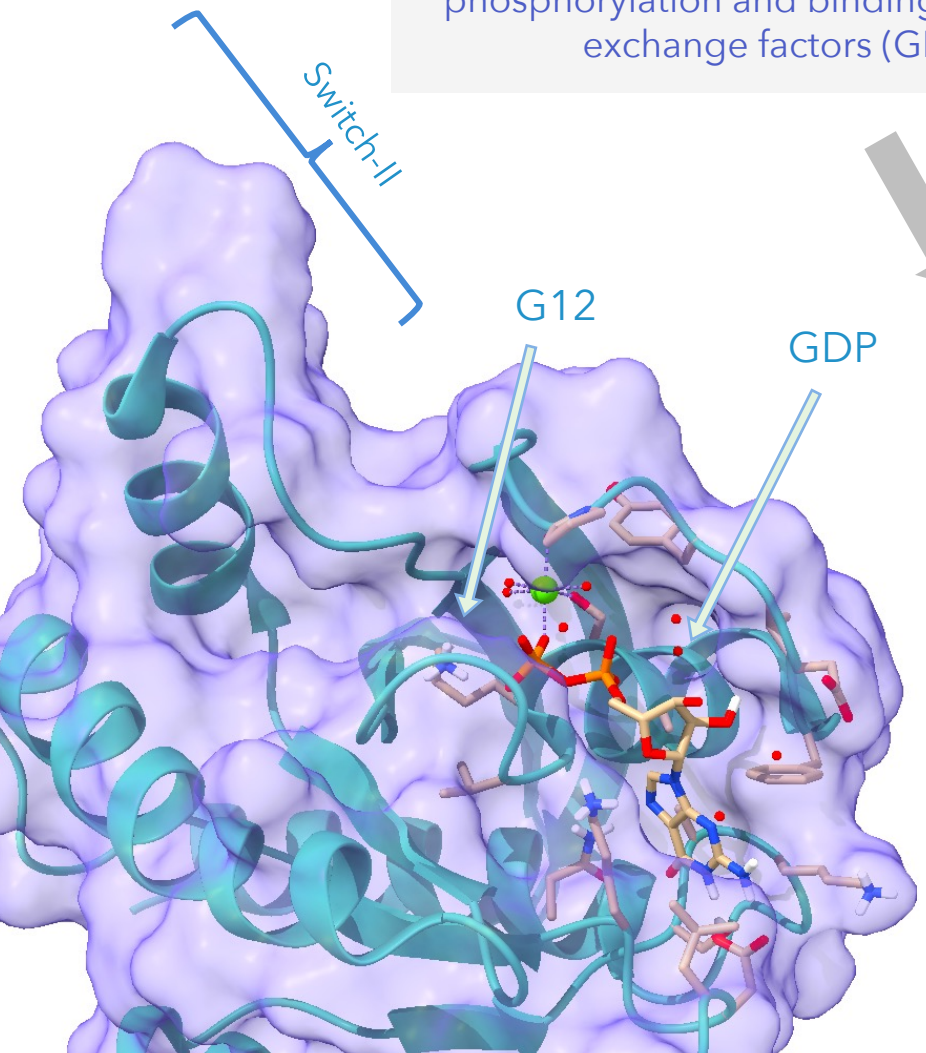
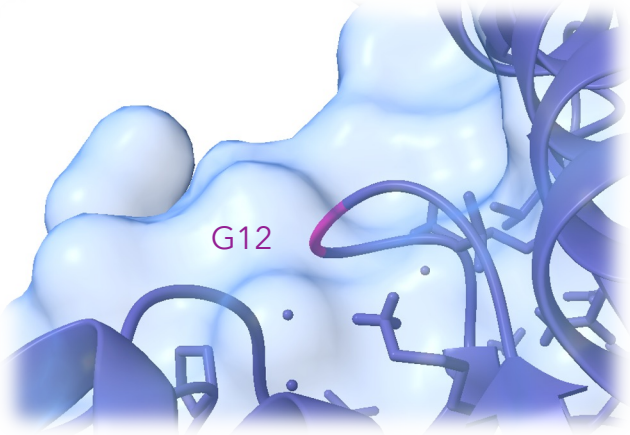
K-Ras mutations tend to affect phosphorylation state

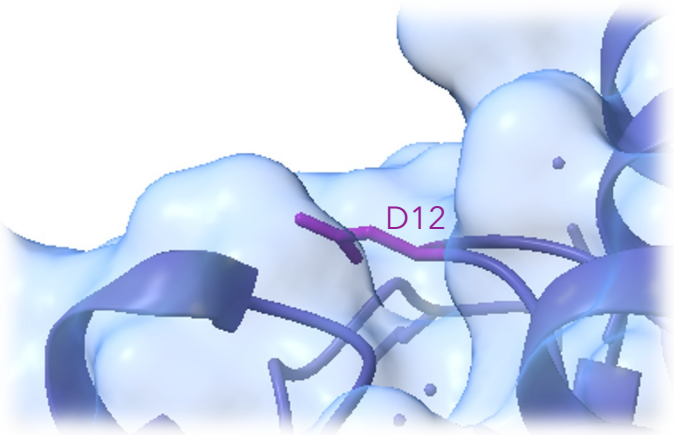
Mutations at G12 prevent efficient phosphorylation and binding to guanine exchange factors (GEFs)



Dysregulated cell division



G12D Mutation



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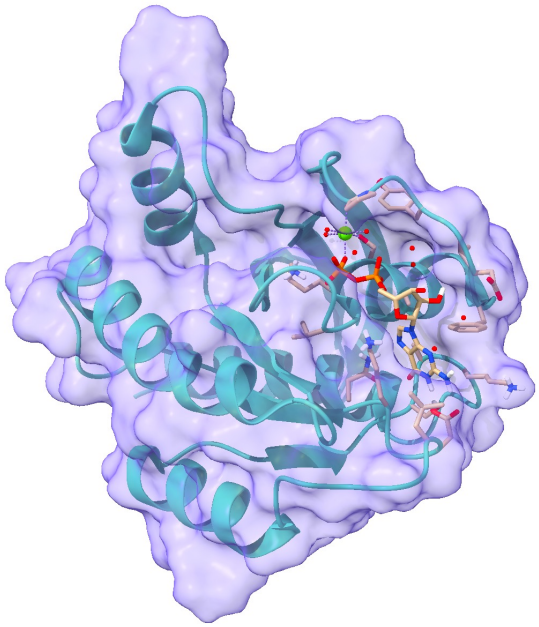
Challenges in drugging K-Ras



pM binding affinity to GTP/GDP

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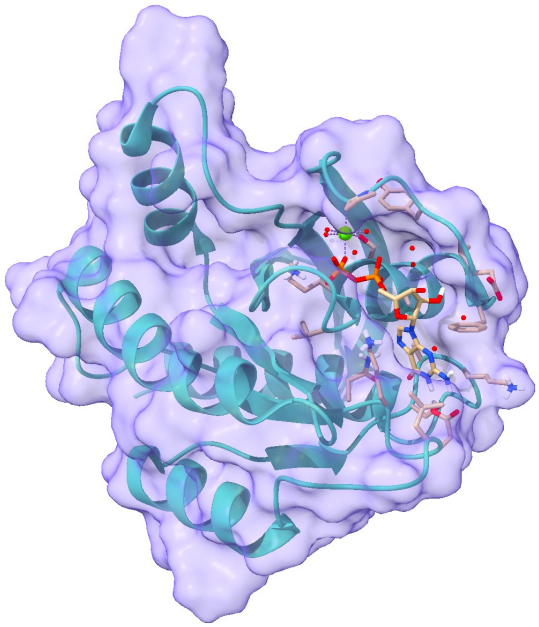
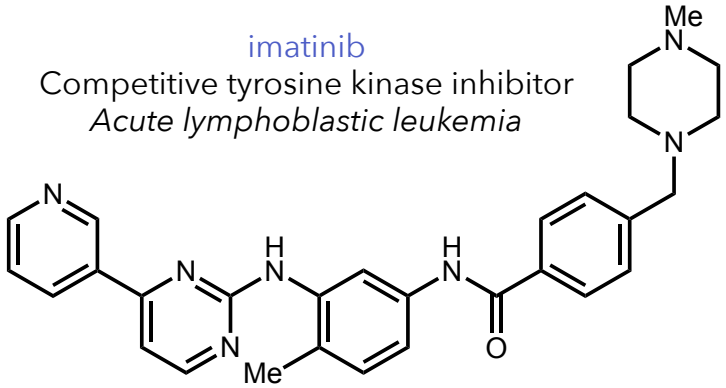


pM binding affinity to GTP/GDP

- Previous strategies to drug ATPases involving ATP-mimics

imatinib
Competitive tyrosine kinase inhibitor
Acute lymphoblastic leukemia



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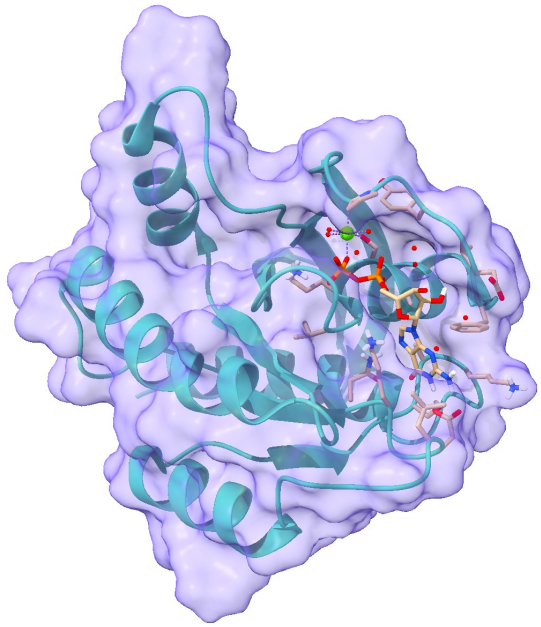

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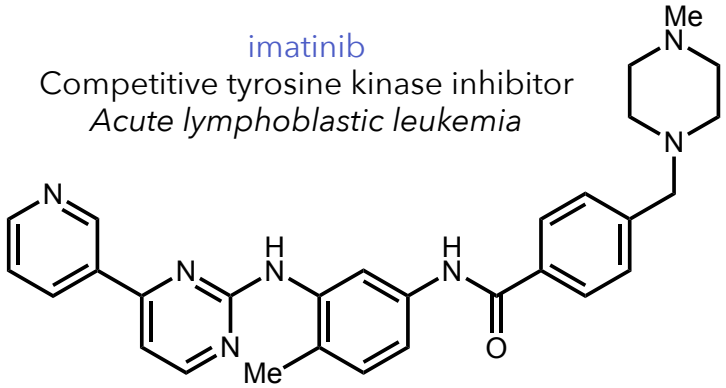
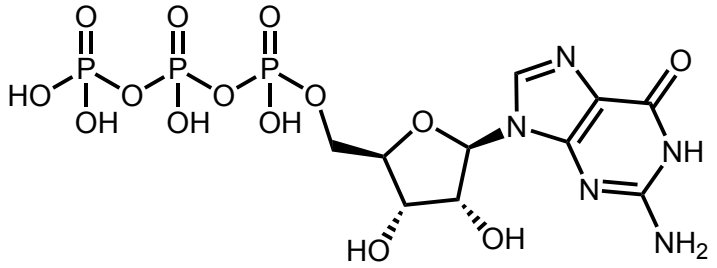


- Kinase competitive inhibitors are unsuccessful for K-Ras
- Unable to outcompete GTP binding



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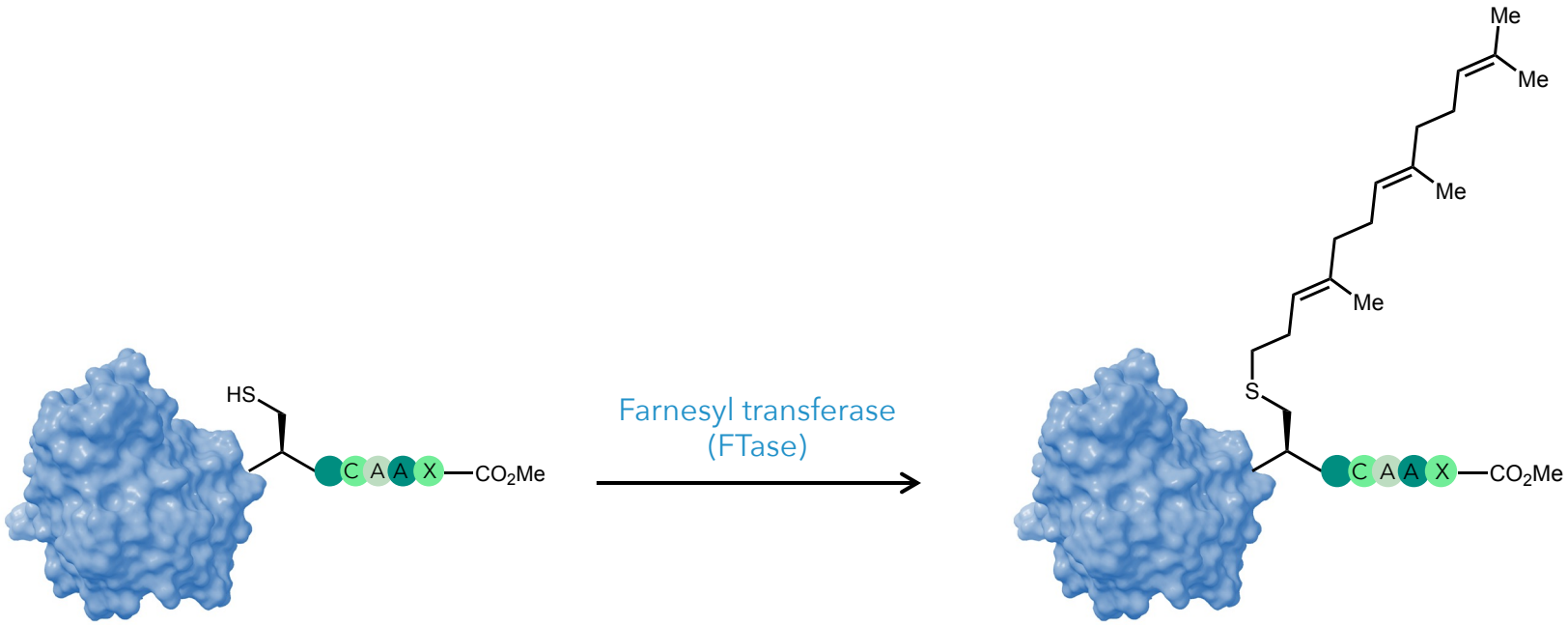
Could inhibiting membrane localization drug K-Ras?

- Key finding: K-Ras is unable to function unless properly membrane localized



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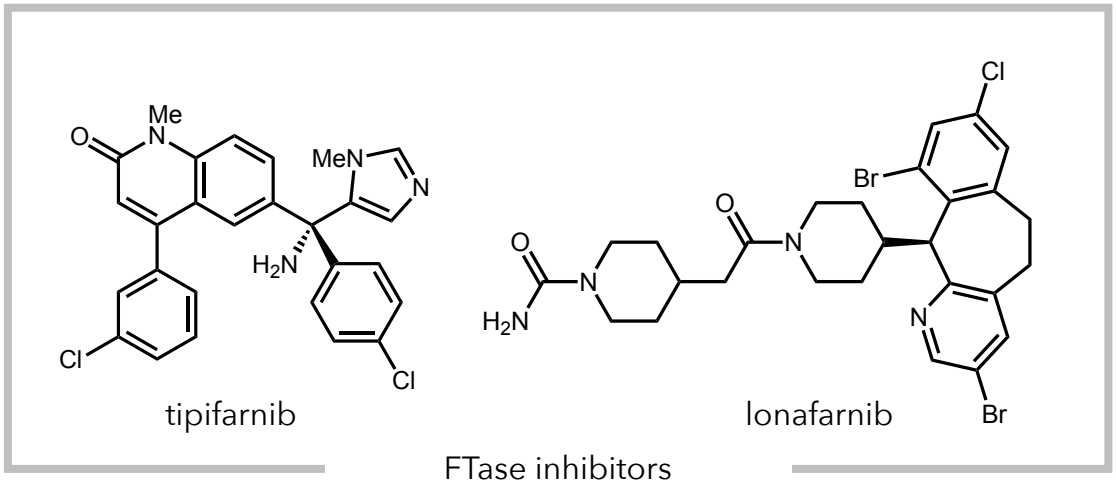

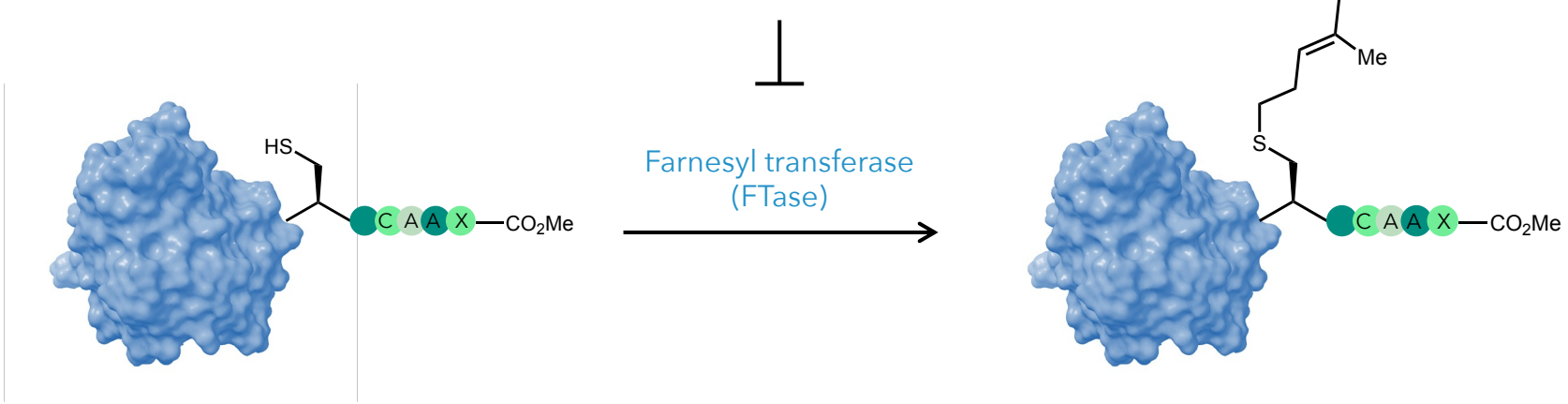

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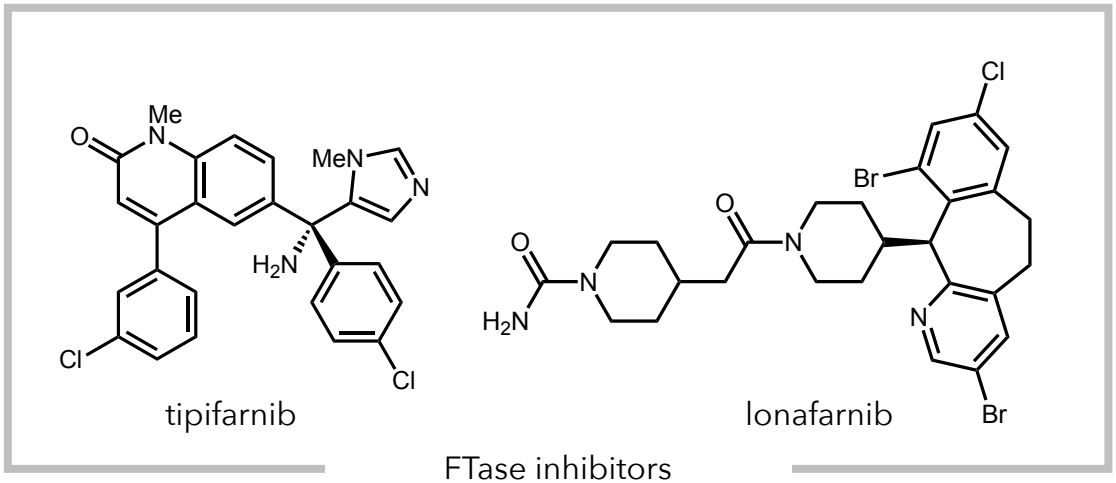

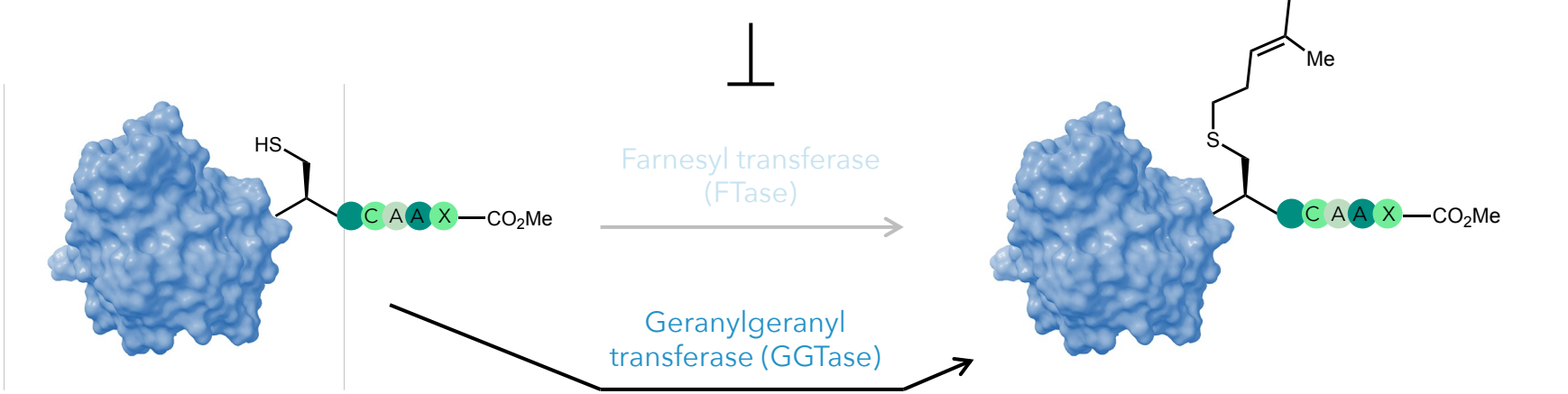

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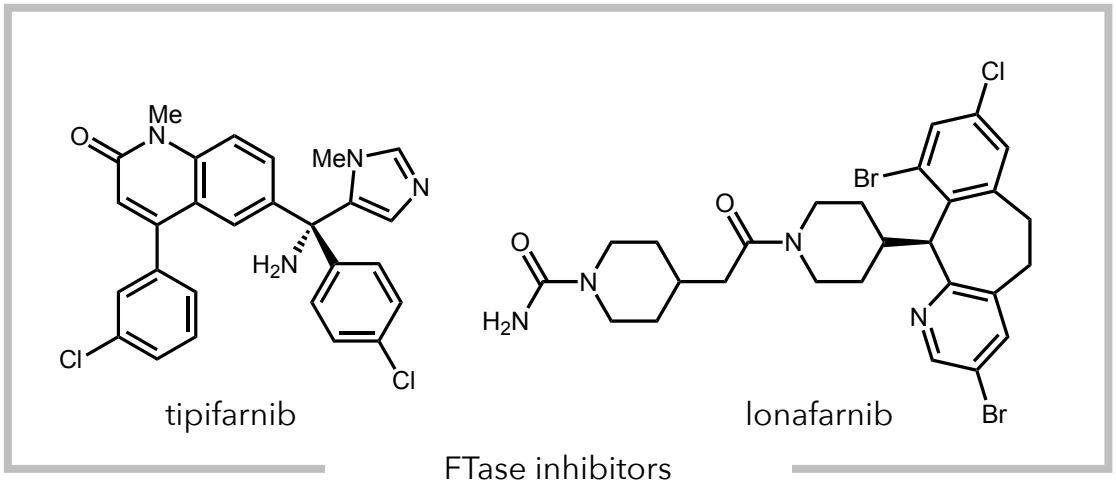

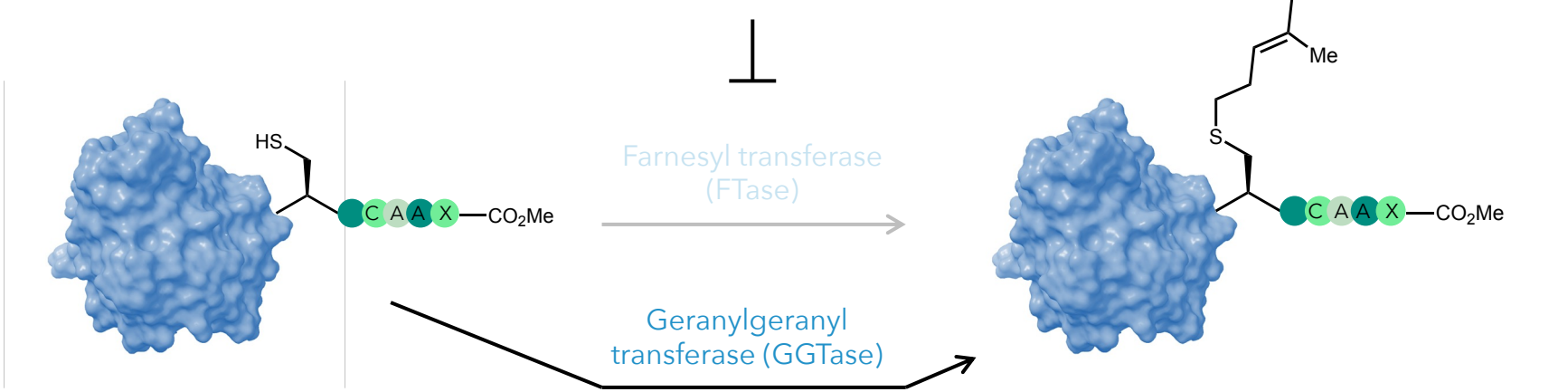

Combined inhibition of FTAse and GGTase proved too toxic to continue

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The complexity of the MAPK signaling

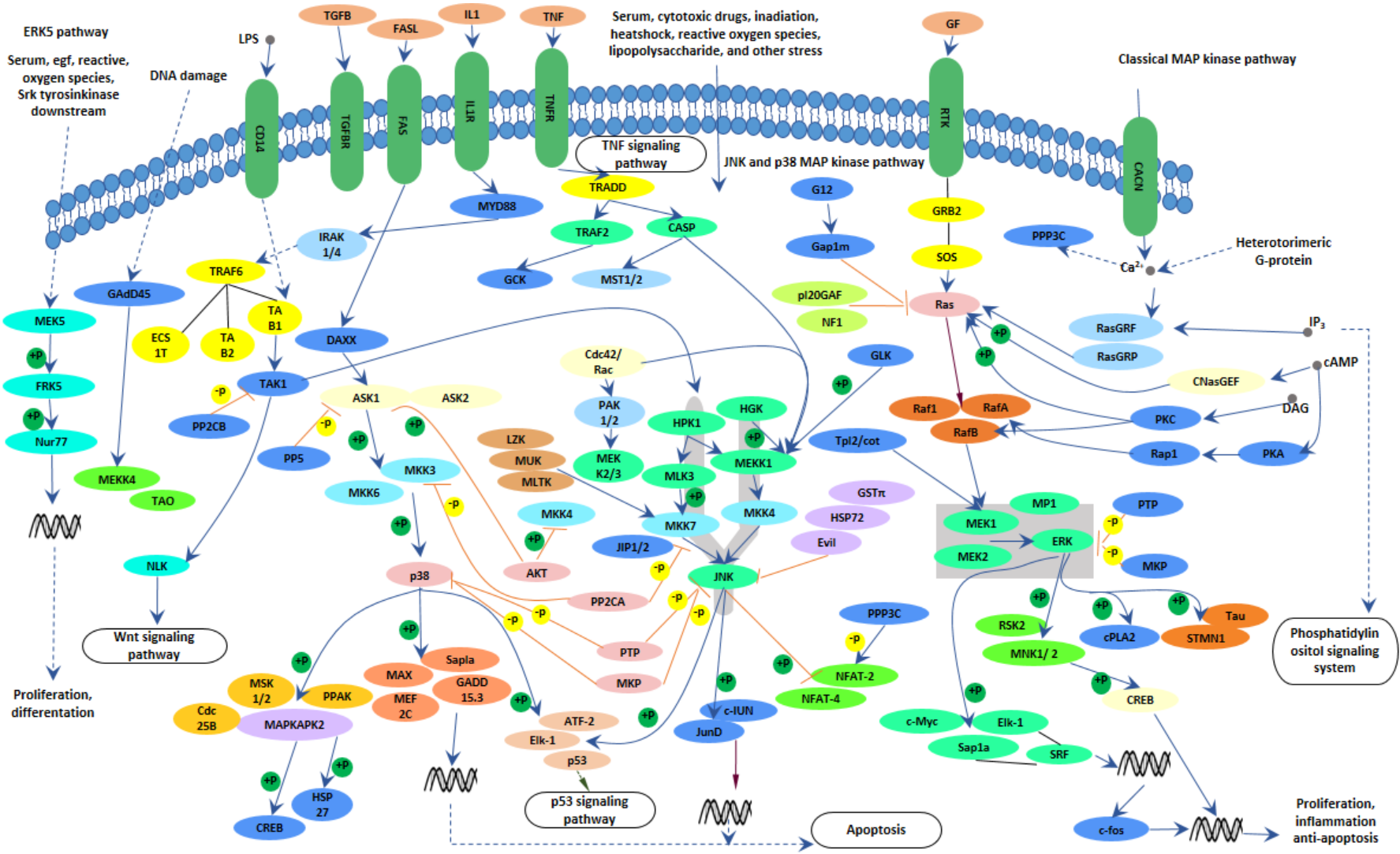

Image credit: https://www.cusabio.com/pathway/MAPK-signaling-pathway.html

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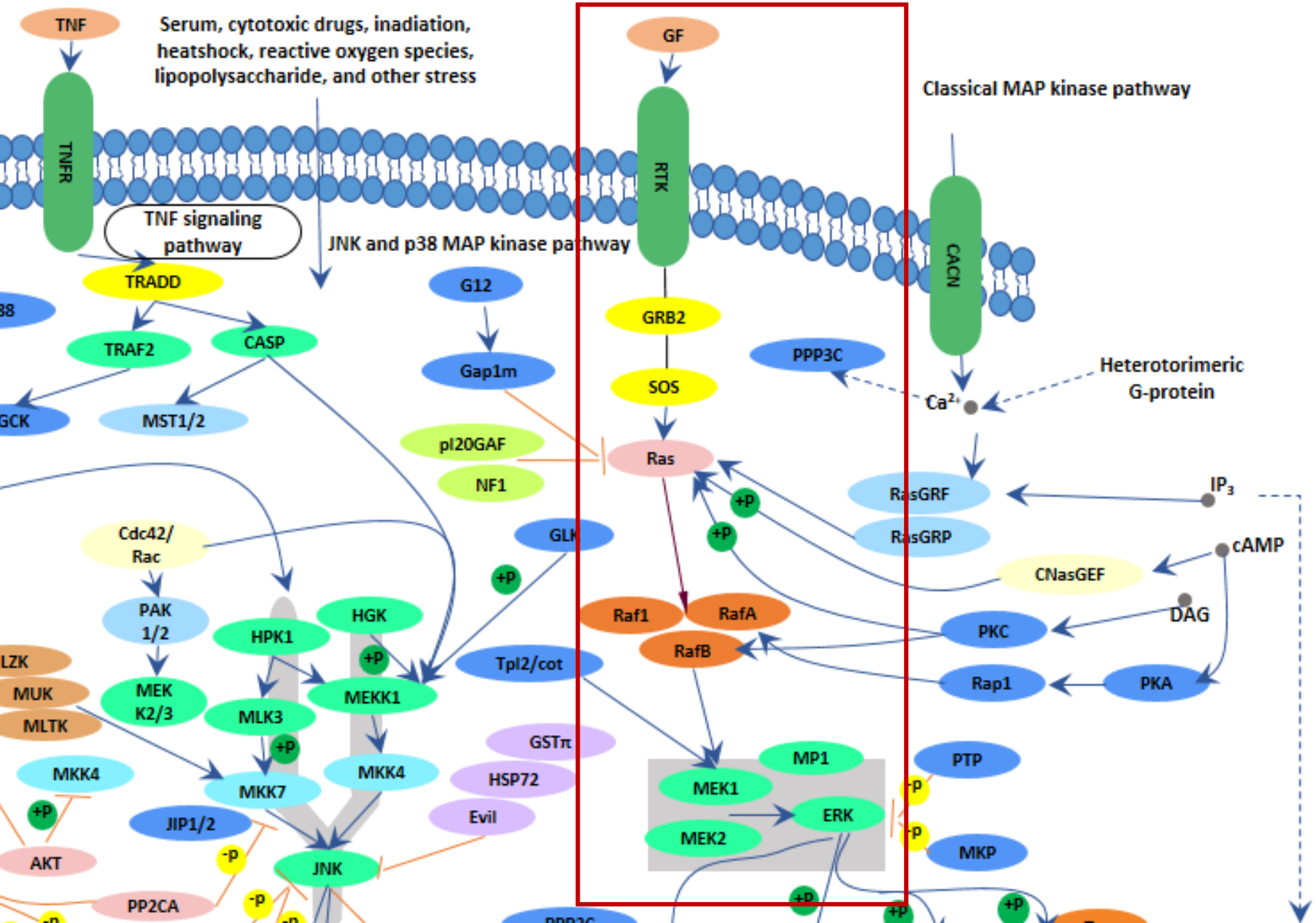

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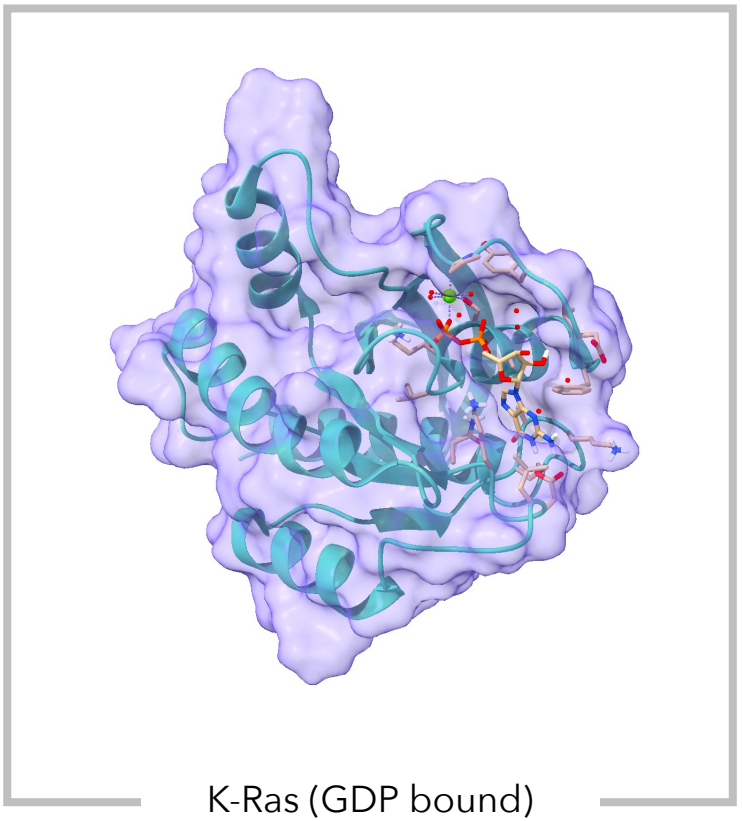

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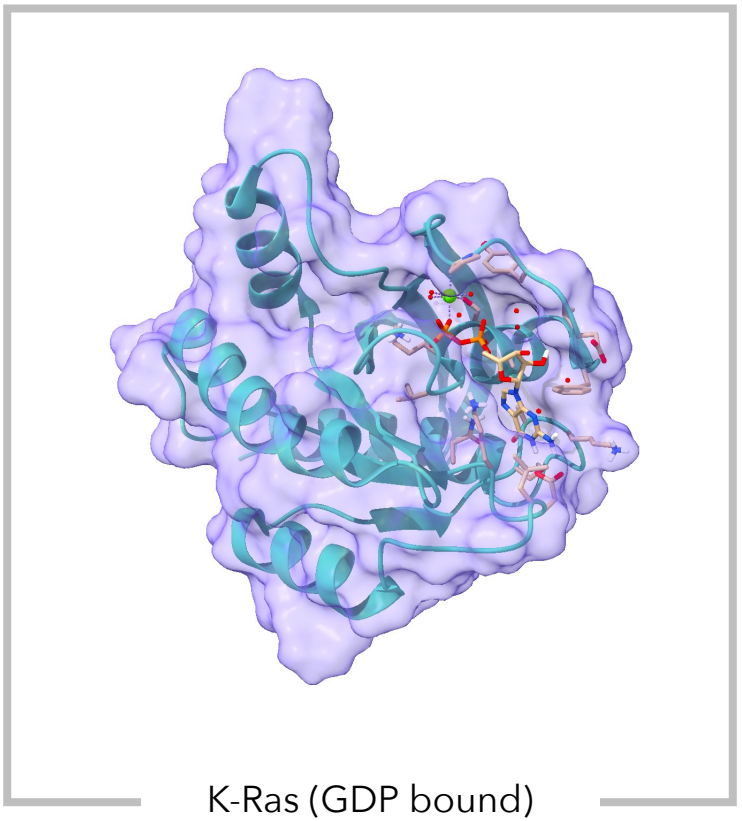

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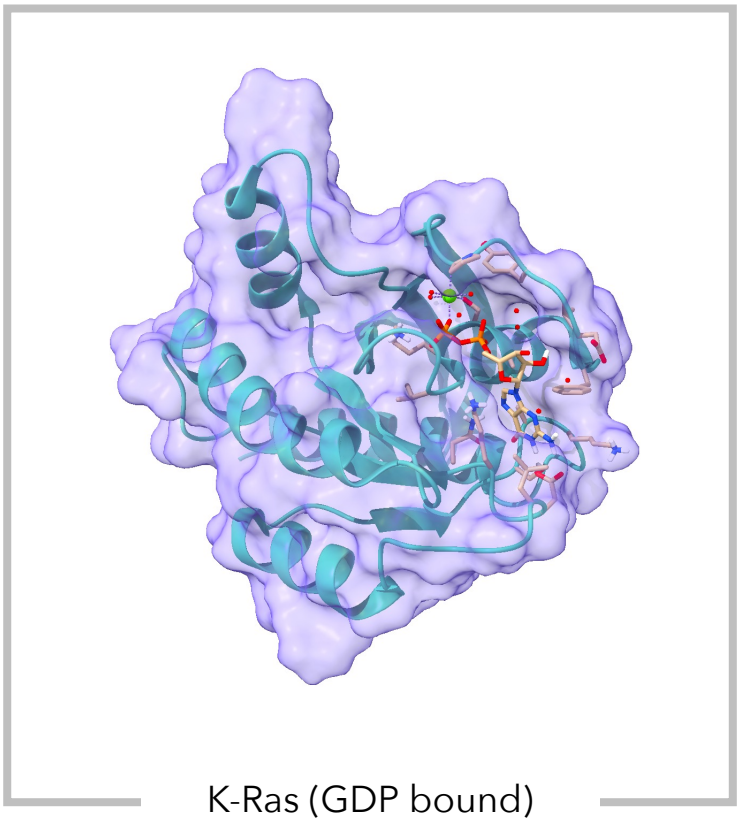
New methods to find "druggable" sites on proteins are necessary



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The advent of activity-based protein profiling and covalent drugs

Prof. Benjamin Cravatt III
Scripps Research Institute

- Known for **activity-based protein profiling (ABPP)**
- Allows for global analysis of **covalent** interactions with variety of proteins
- Proteomics (mass spectrometry) based approach focusing on *reactive* functionality rather than quantity

Cravatt, et al, *Science*, **1995**, 268, 1506-1509

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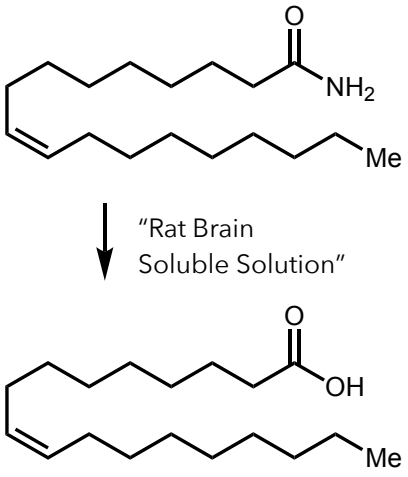
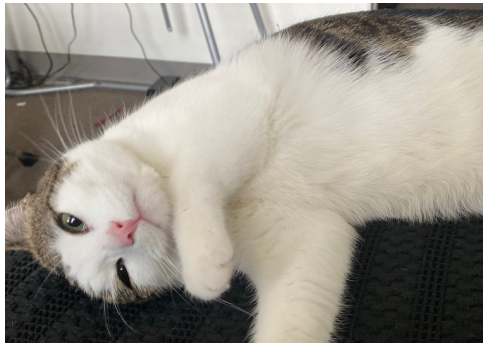
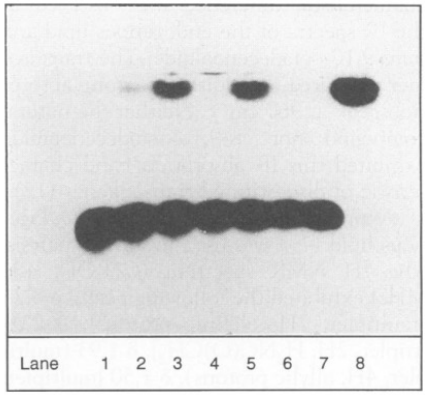




Isolated from cerebrospinal fluids of sleep deprived cats (like Ophelia)



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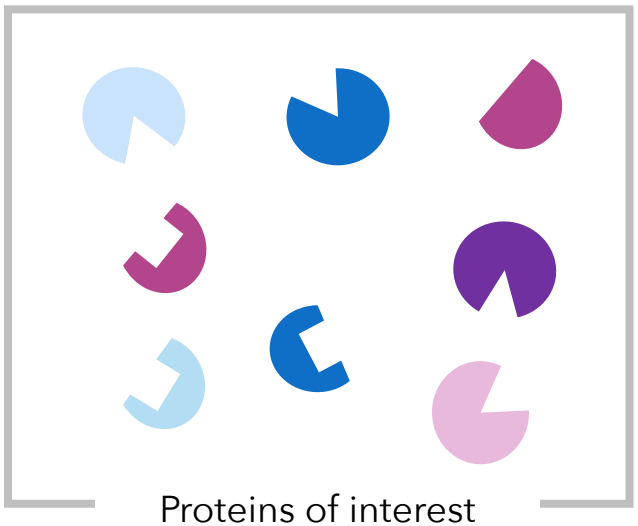
Principles of activity-based proteomics



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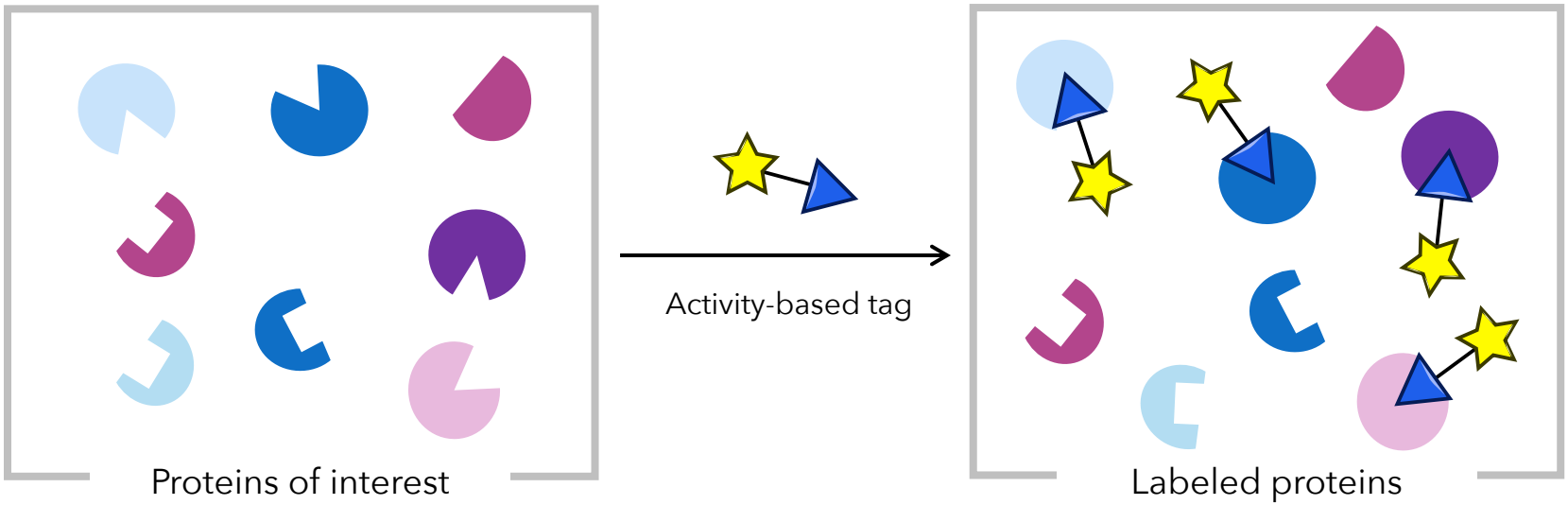

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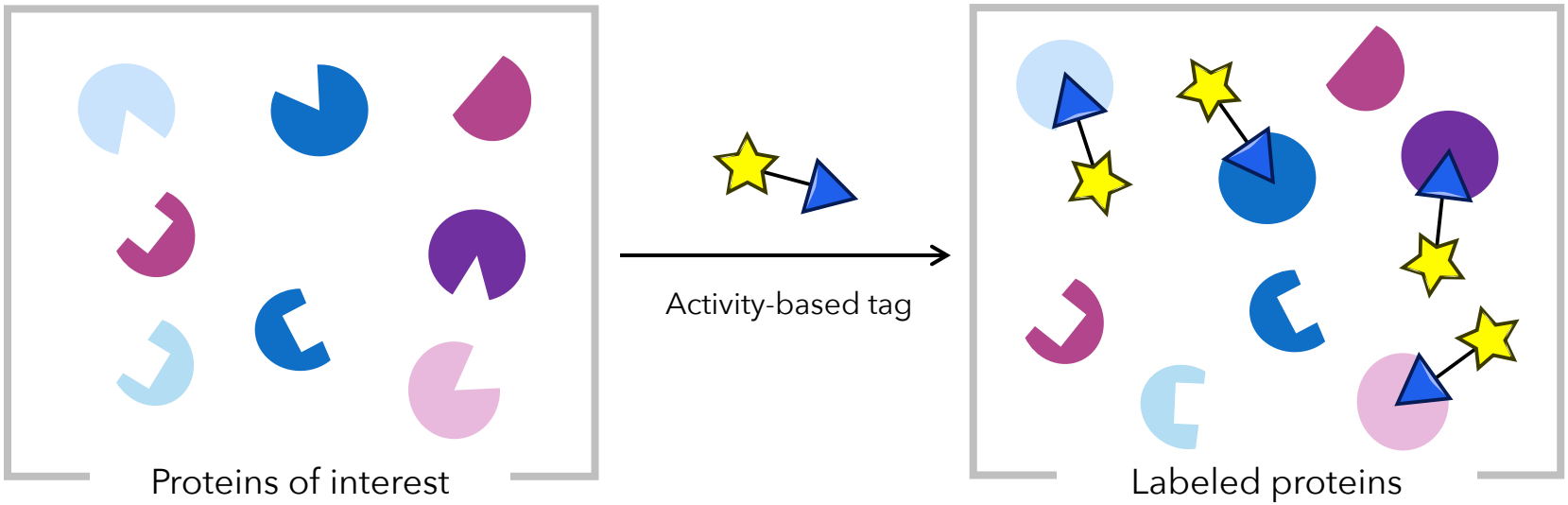

Visualization methods

- Fluorescence
- "Click"
- Streptavidin enrichment
- Mass spectrometry

Cravatt et al. *Proc. Natl. Acad. Sci.* **1999**, 96, 14694

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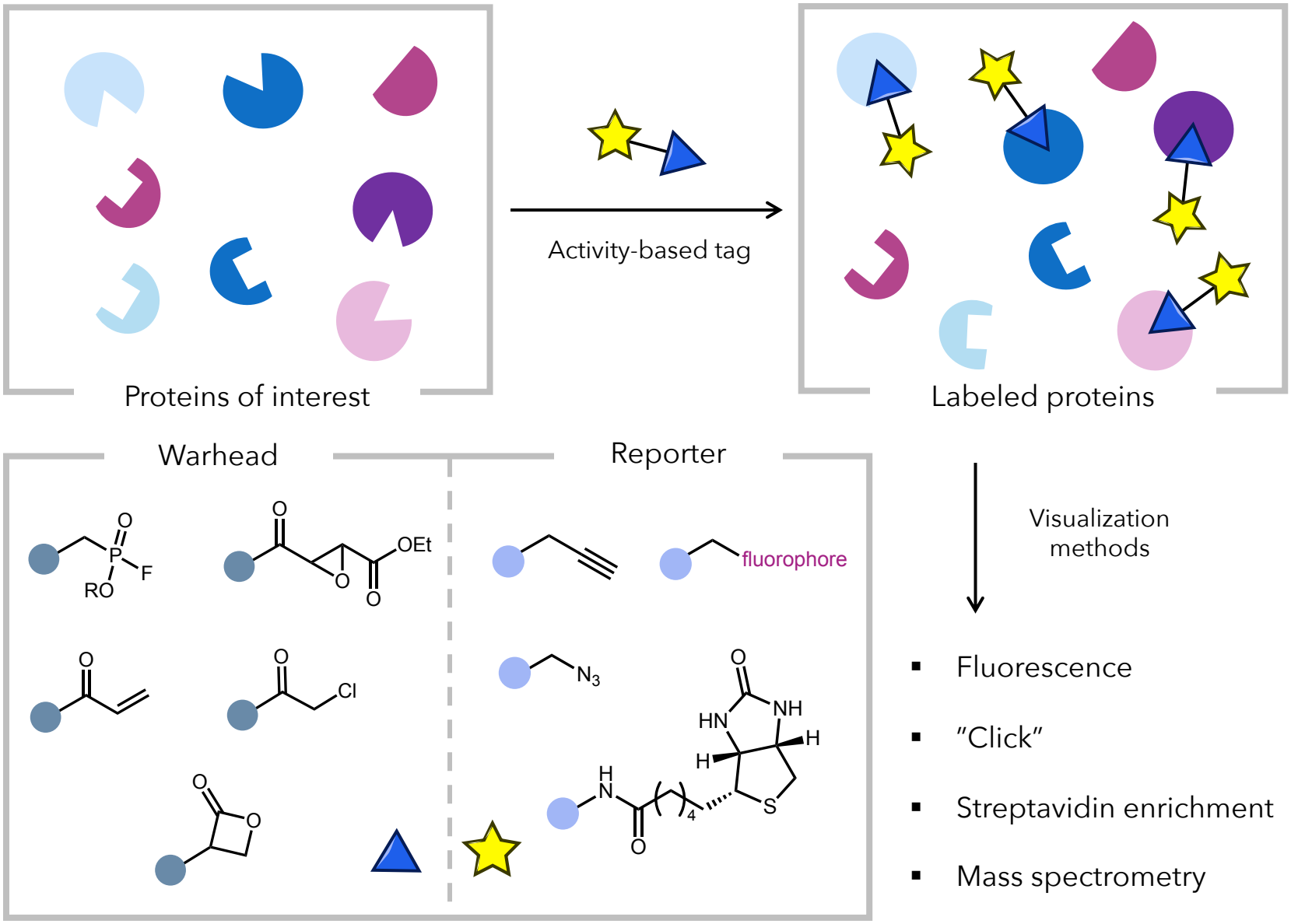

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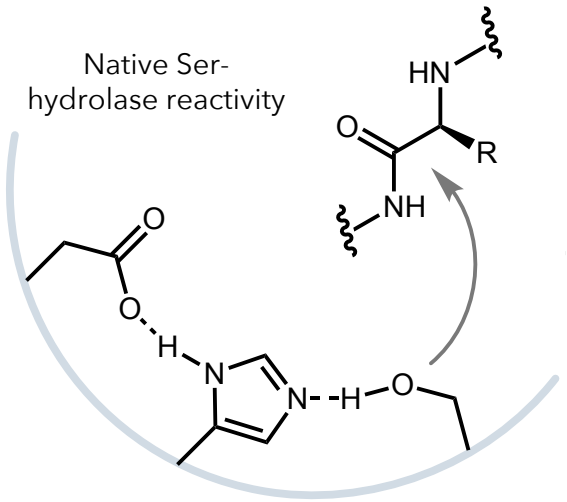
ABPP characterization of serine hydrolases



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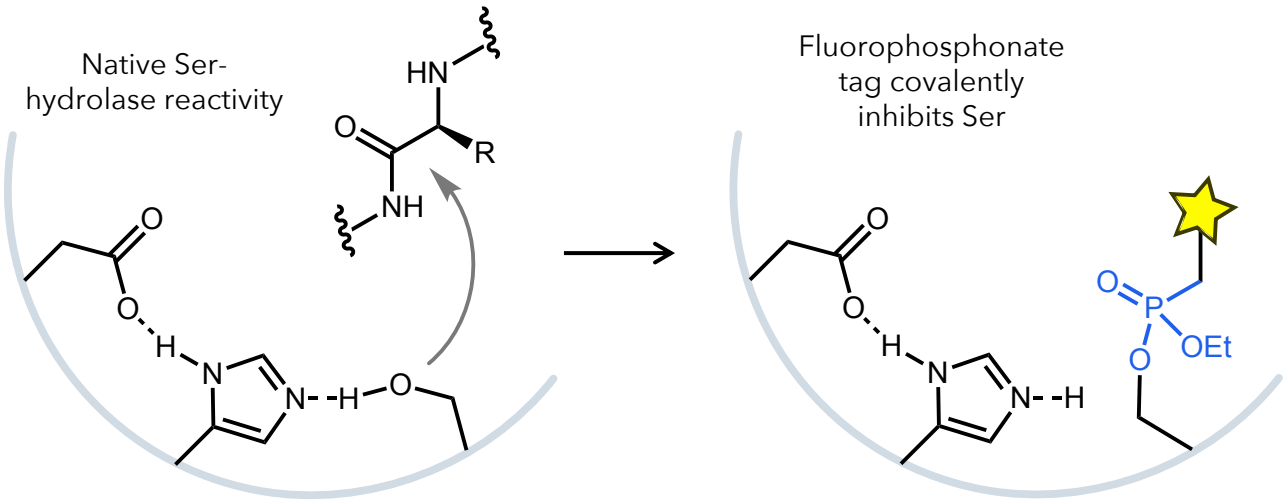

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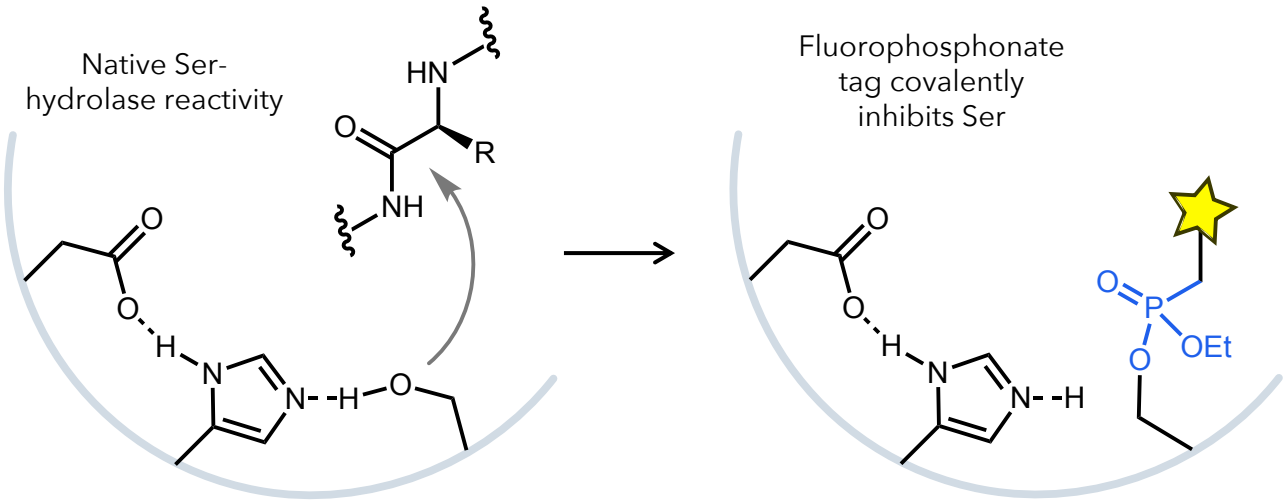
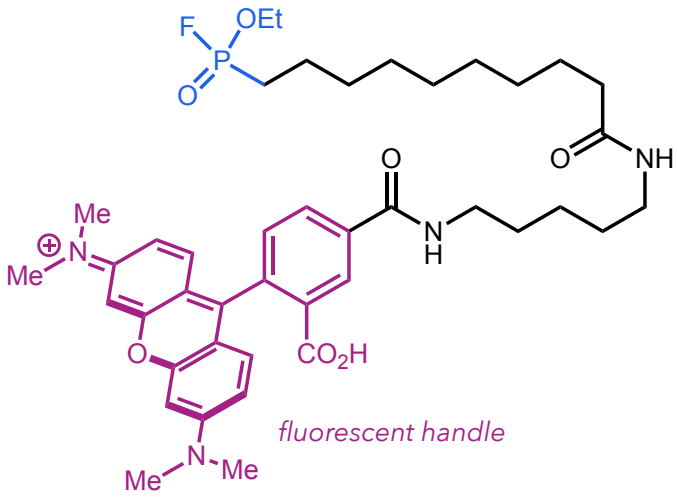


Serine hydrolase superfamily tag

electrophilic warhead



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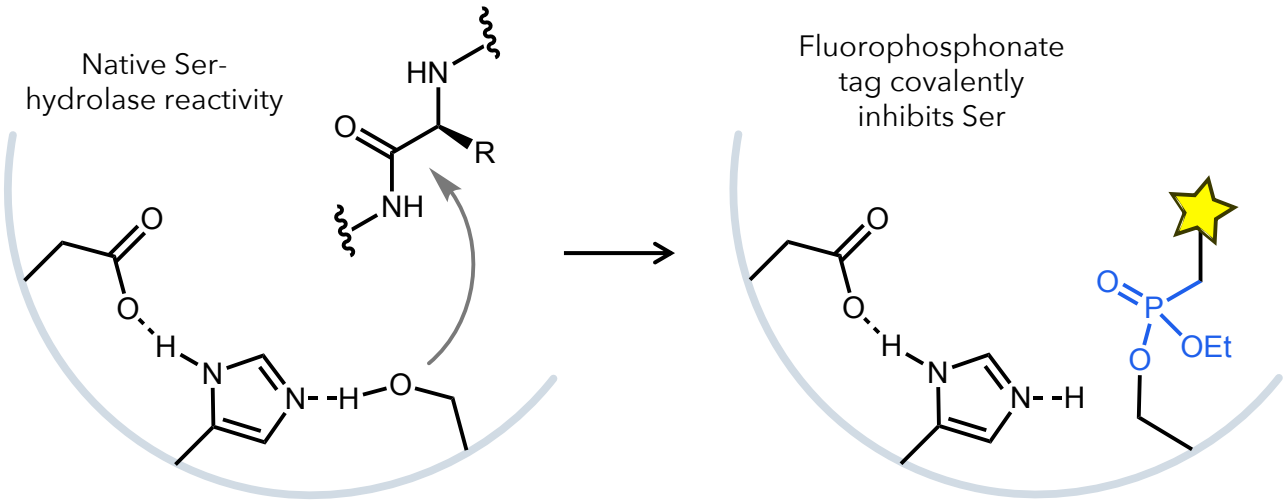

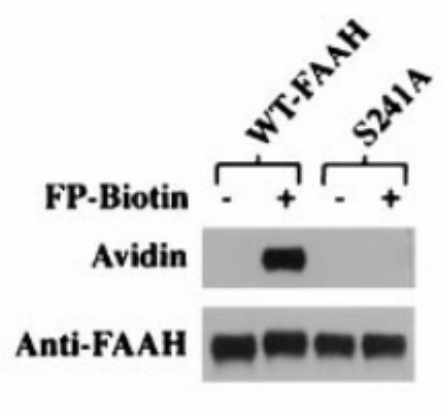

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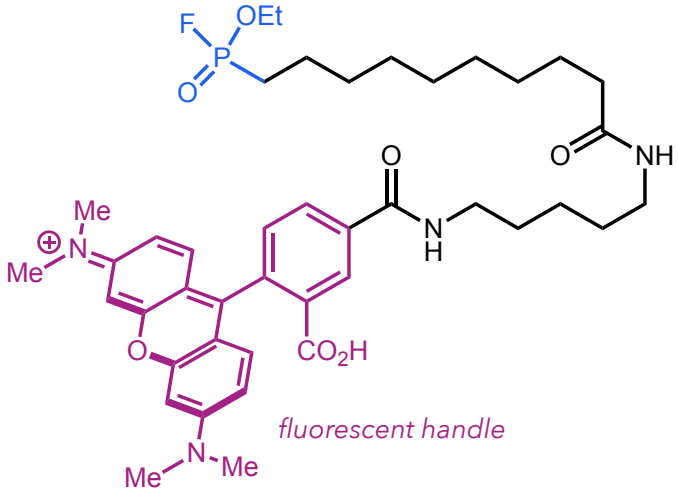

fluorescent handle

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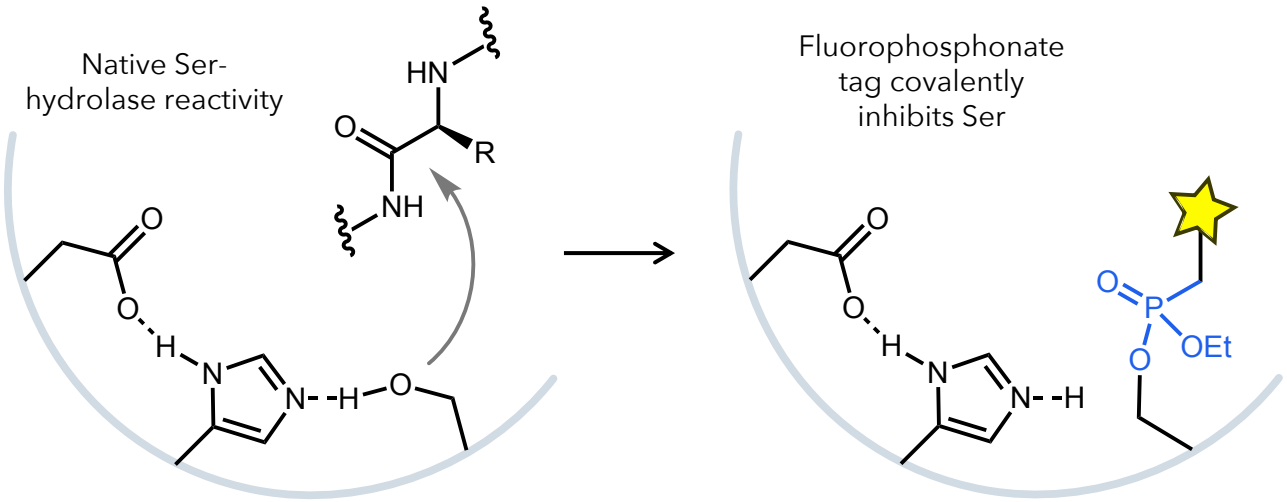

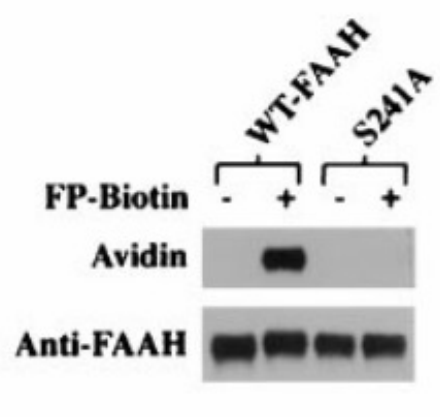

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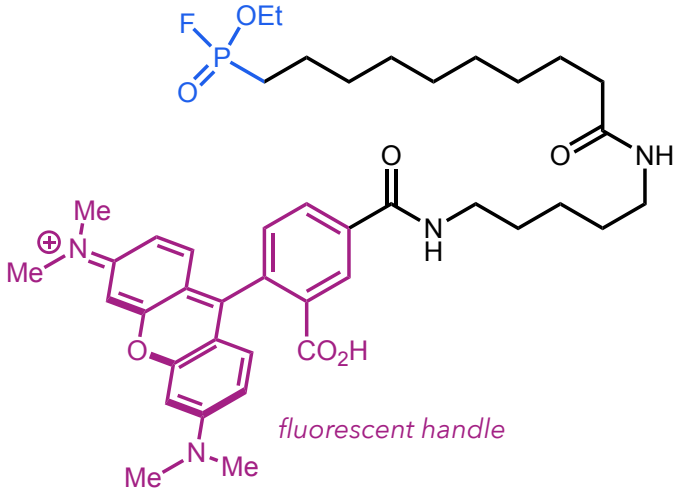

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- Serine hydrolases are involved in blood coagulation, inflammation, angiogenesis, amongst other processes

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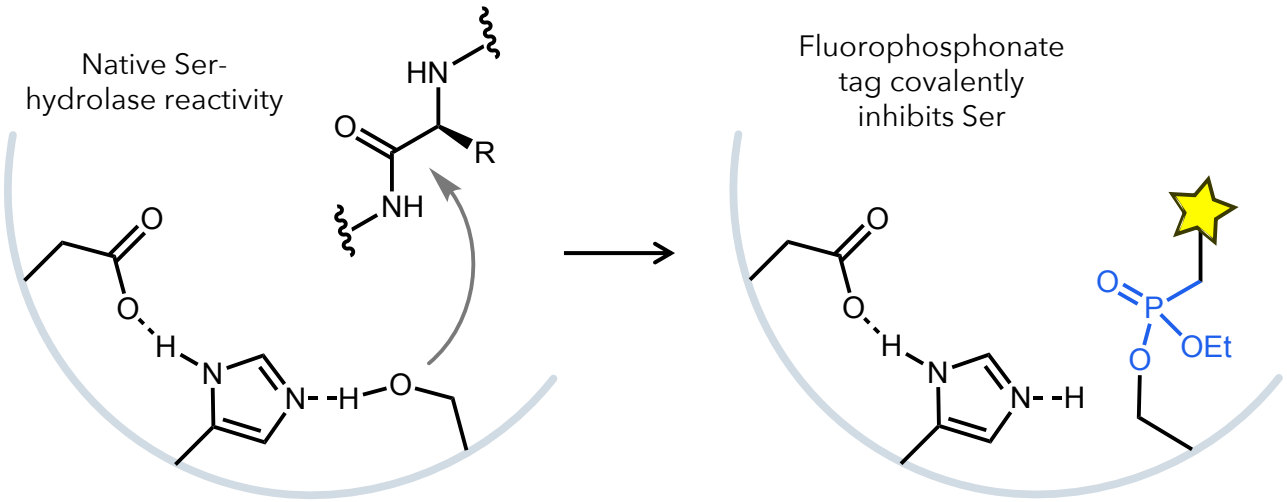

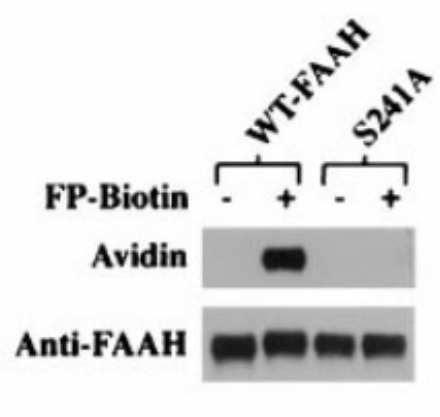

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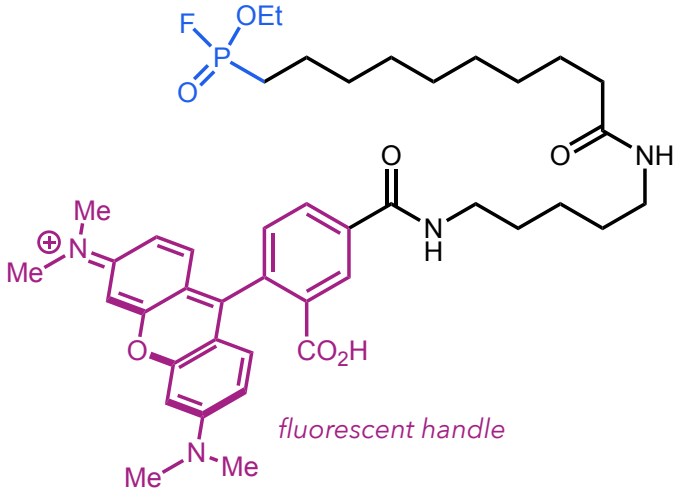

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- Frequently implicated in cancer, emphysema
- APBB allows for profiling of multiple potential hydrolases in **few experiments**

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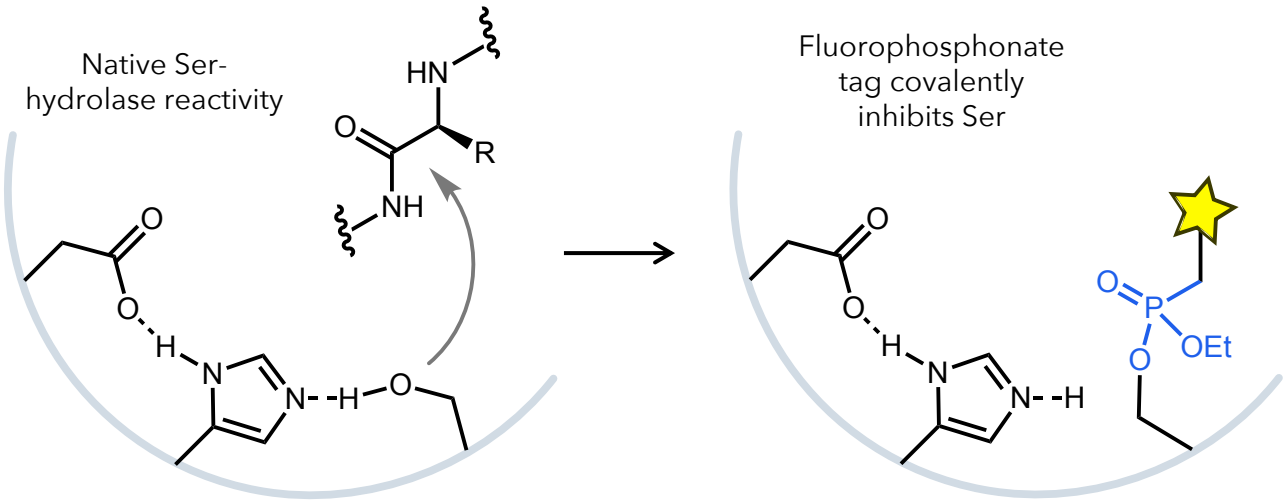

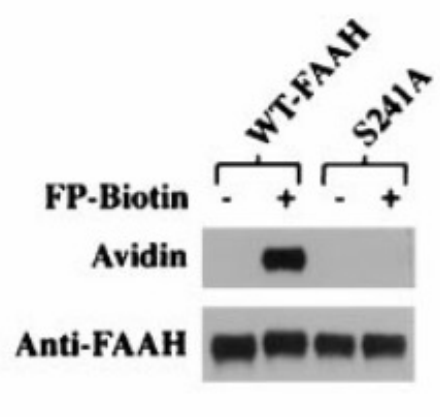

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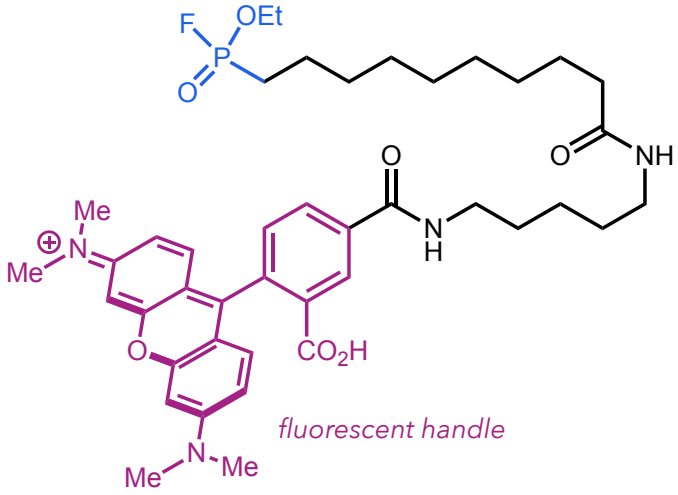

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- Frequently implicated in cancer, emphysema
- APBB allows for profiling of multiple potential hydrolases in **few experiments**
- This strategy is general and allows for characterization of specific protein classes dependent on activity

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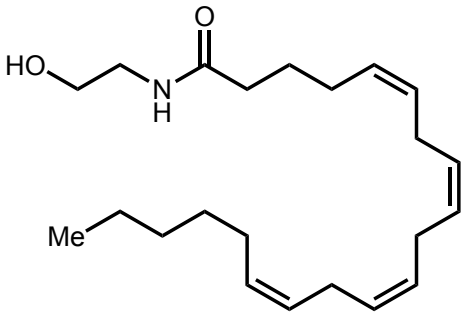
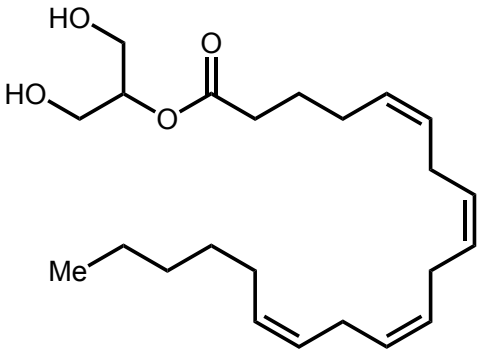
Fatty Acid Amide Hydrolase (FAAH)

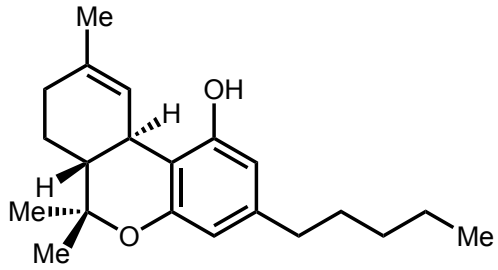
- Fatty acid amide hydrolase are serine hydrolases involved in lipid hydrolysis
- Endocannabinoid signaling pathway invoked in pain response, fear, and anxiety



anandamide



arachidonoyl glycerol



tetrahydrocannabinol

Cravatt et al. *Chemistry and Biology* ***2009***,16, 411-420; https://www.nytimes.com/2019/03/28/health/woman-pain-anxiety.html

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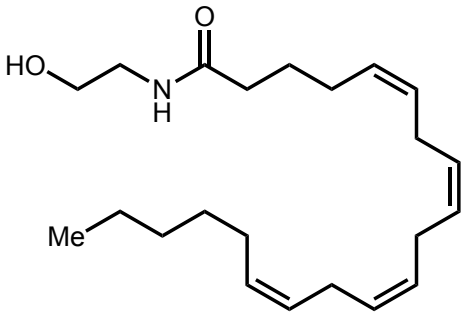

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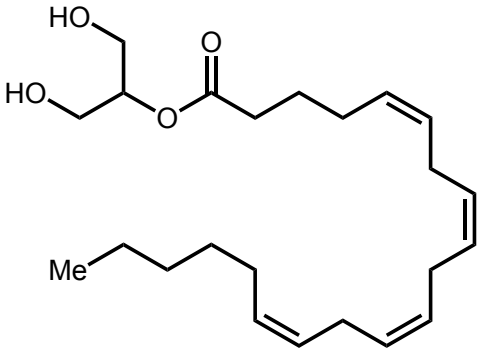

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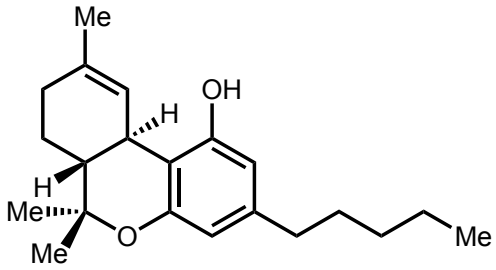

tetrahydrocannabinol

- Jo Cameron, 71, has never felt pain, fear, or anxiety
- Genetic studies reveal knockout of FAAH gene, with high levels of anandamide in brain
- Burns and cuts healed quicker than average

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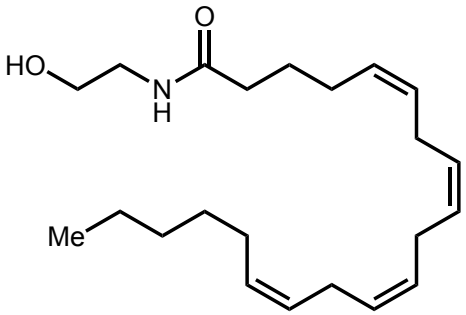

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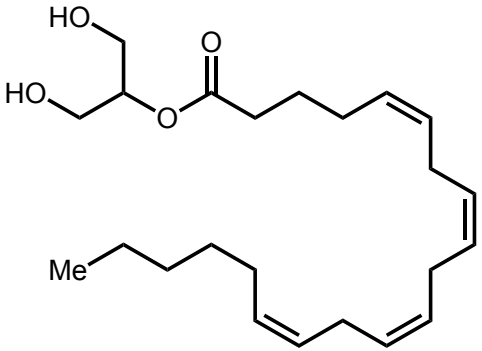

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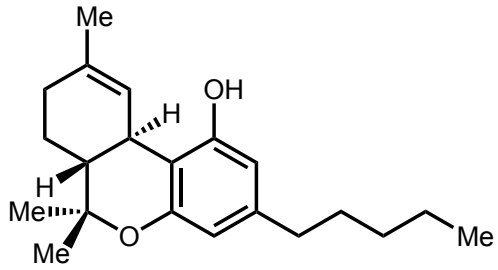

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Is FAAH inhibition a strategy to treat pain?

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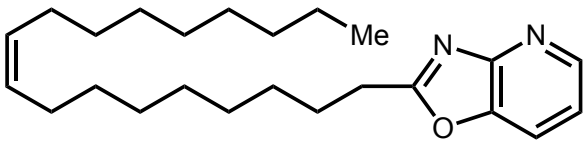
Fatty Acid Amide Hydrolase (FAAH) Inhibitors



reversible FAAH inhibitor

Binding affinity not strong enough, non-specific targeting

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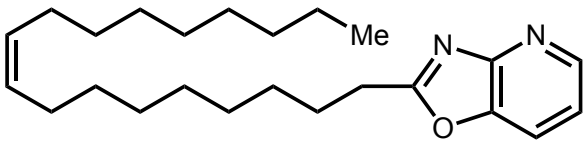

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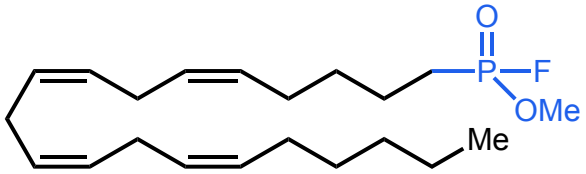

covalent FAAH inhibitor

Good binding affinity, too much off-target Ser-hydrolase inhibition

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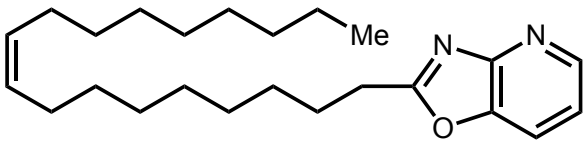

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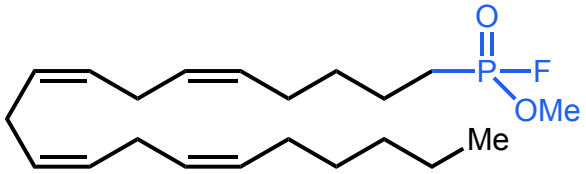

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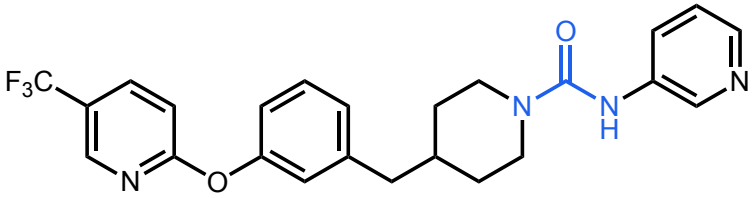

Pfizer PF-3845

ABPP shows remarkable selectivity and potent inhibition of FAAH

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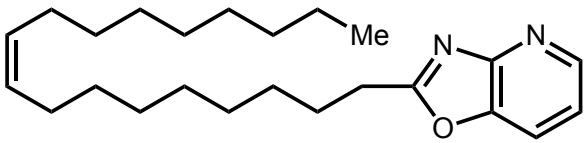

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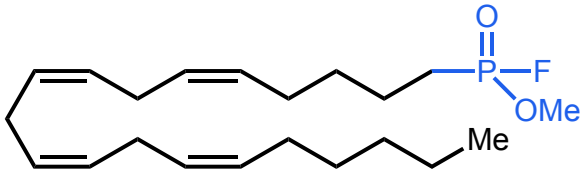

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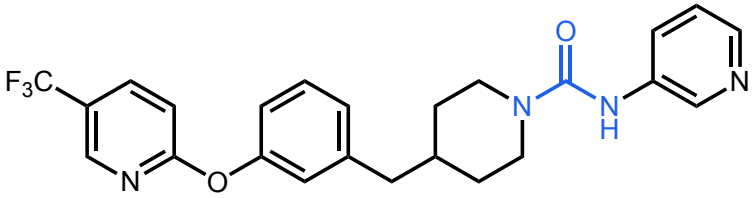

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Could covalent inhibitors serve as viable candidates for drug discovery?

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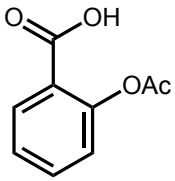
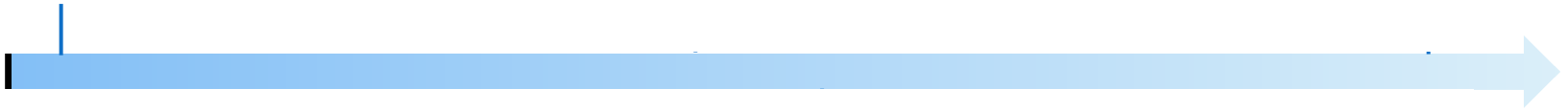
Are covalent inhibitors a viable strategy for drug discovery?



1890s - aspirin



J. Med. Chem. **2009**, 52, 5, 1231–1246

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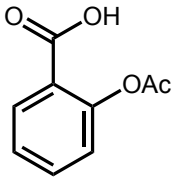

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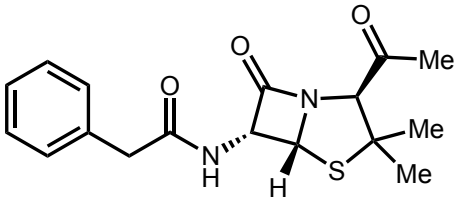

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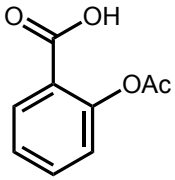

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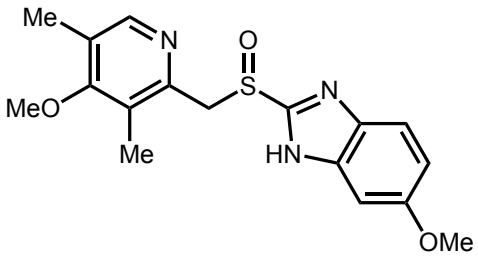

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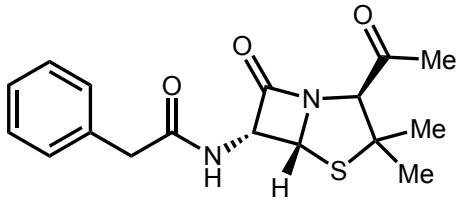

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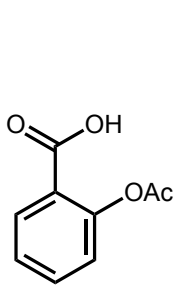

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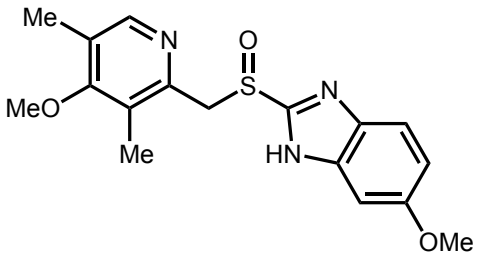

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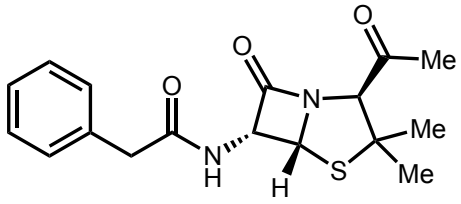
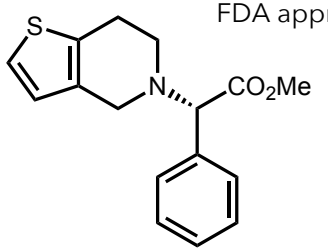


1997 – clopidogrel
FDA approval



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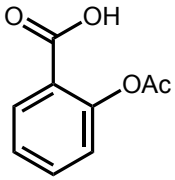

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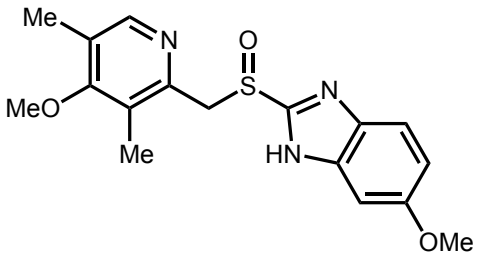

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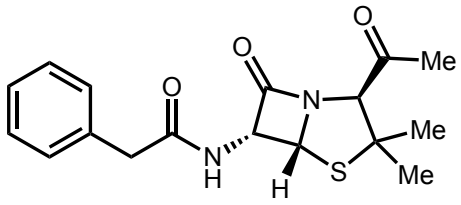
1930s - penicillin



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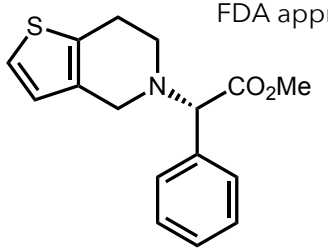

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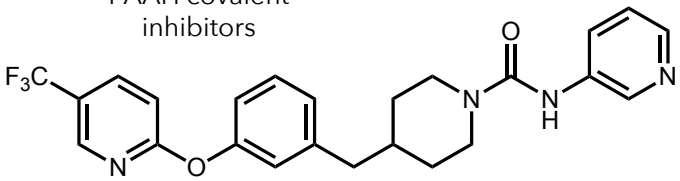

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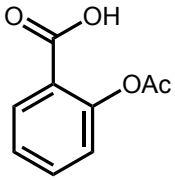

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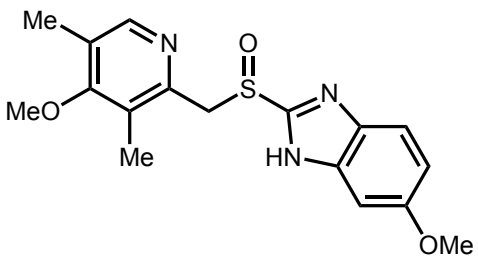

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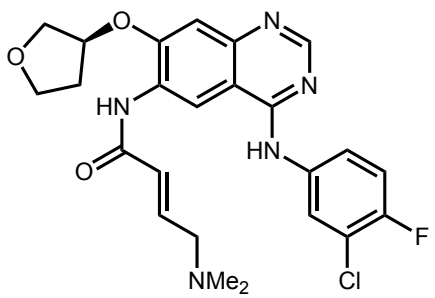
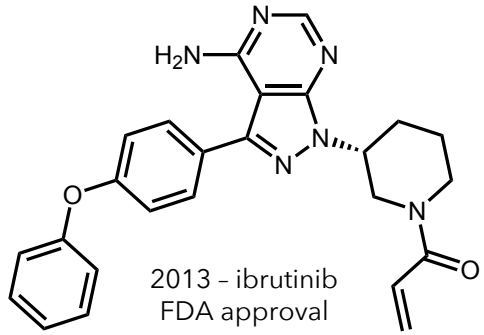


2013 – afatinib FDA approval



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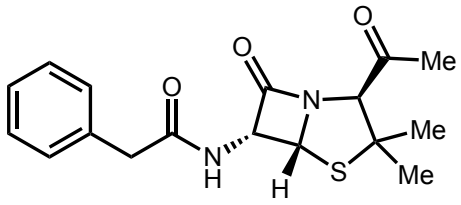

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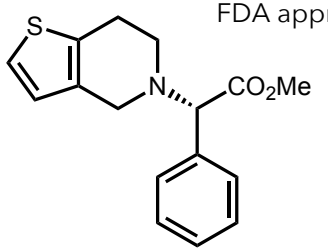

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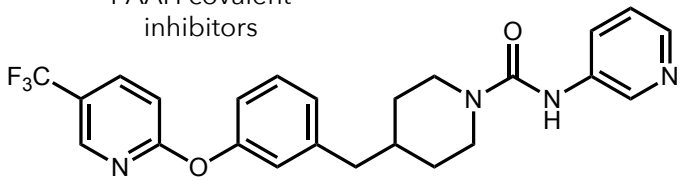

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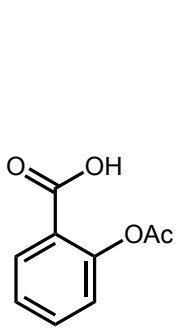

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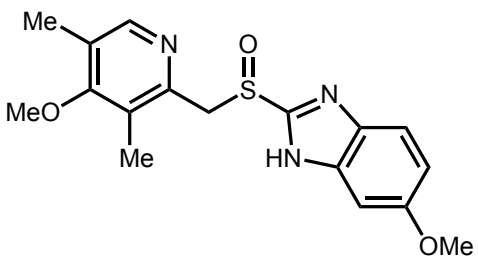

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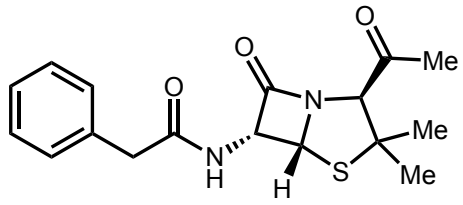

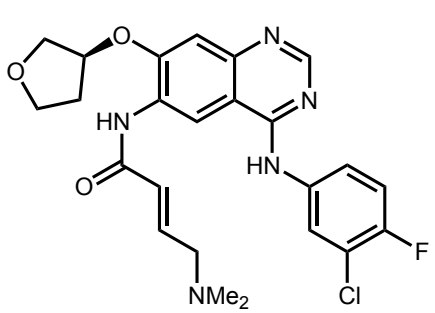

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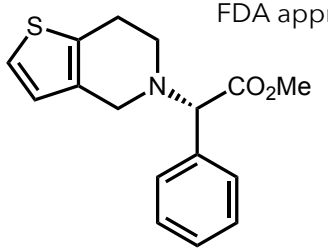

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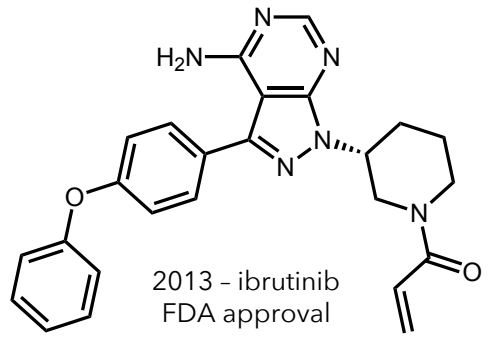

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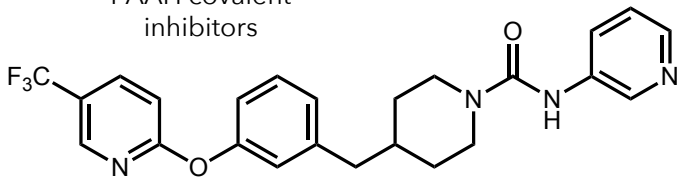

- Meta-analysis of idiosyncratic drug toxicity reveals most “structural alerts” in drug design are over exaggerated

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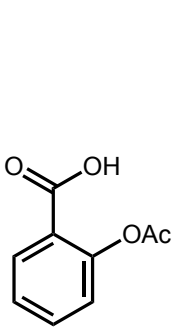

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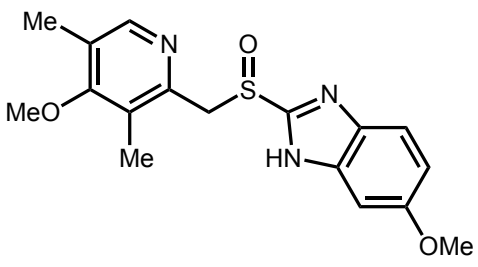

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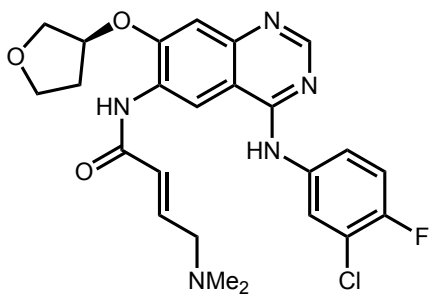

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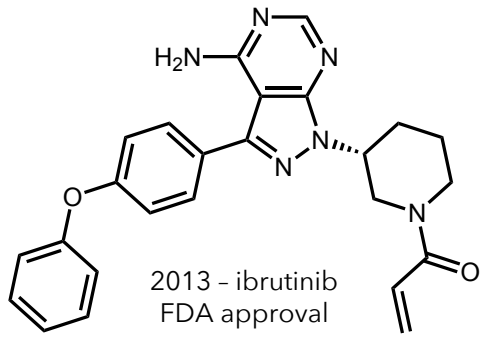

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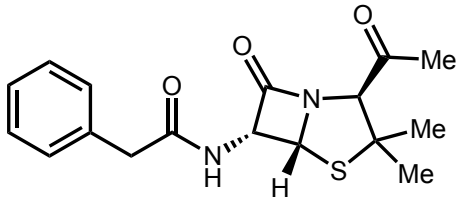

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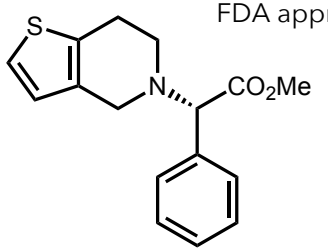

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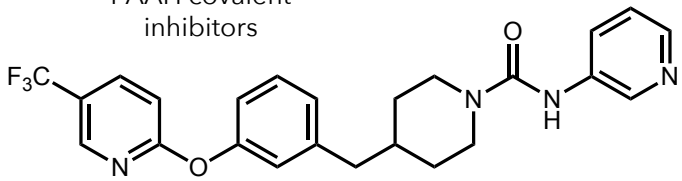

Pfizer

- Meta-analysis of idiosyncratic drug toxicity reveals most “structural alerts” in drug design are over exaggerated
- The single biggest link to toxicity were high dosages (several hundred mgs) rather than specific mechanisms or structures

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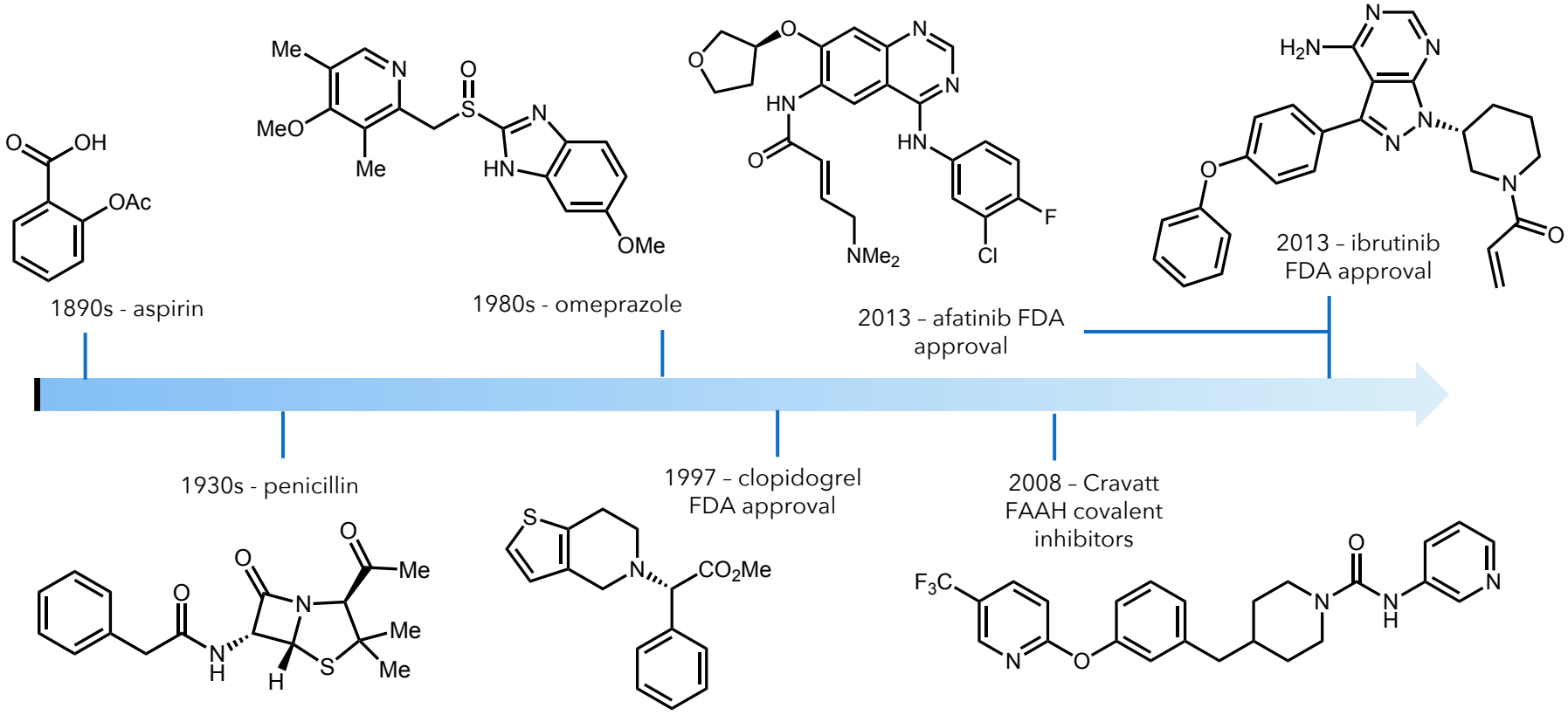

Prior to such methods [ABPP], the question of selectivity of covalent drug candidates was pure speculation, which led to excessive concern. Once covalent drug candidates were shown to modify proteins with very high selectivity by ABPP, it gave medicinal chemists more confidence that this modality could be safe.

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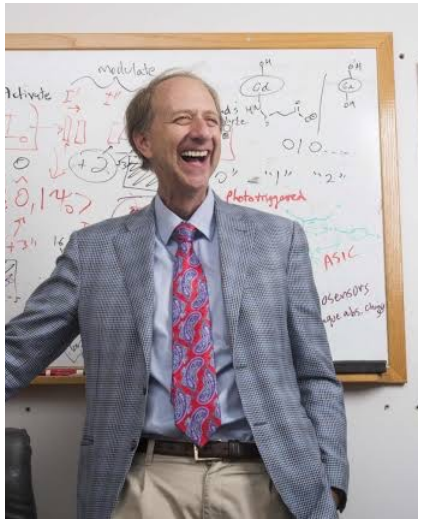
Outline

- Introduction to undruggable proteins
  - What makes a protein "undruggable"?
  - Attempts to drug K-Ras mutations
- Activity-based approaches to finding "druggable" sites
- Success stories in covalent drugs
  - Ibrutinib and Bruton's tyrosine kinase
  - Sotorasib and K-Ras G12C
- Conclusions
  - "Yet to be drugged" instead of "undruggable"

Everything is bigger in Texas



Prof. Jonathan Sessler
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Known for: synthesis of
"texaphyrins",
supramolecular chemistry

Acc. Chem. Res. **1994**, 27, 2, 43–50

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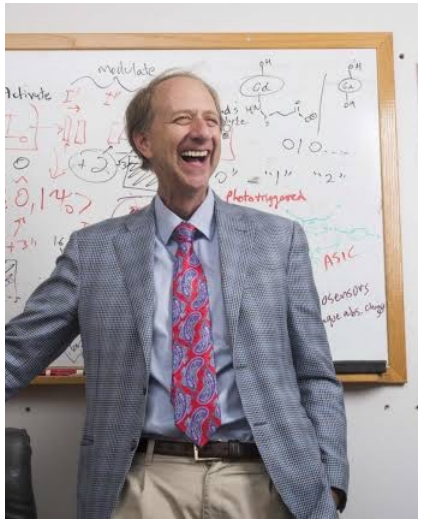

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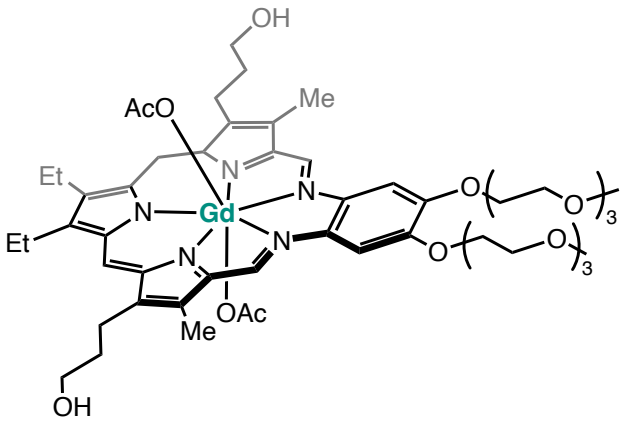

Xcytrin (motexafin gadolinium)

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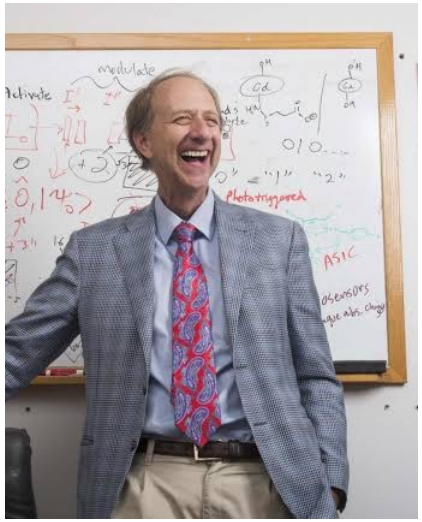

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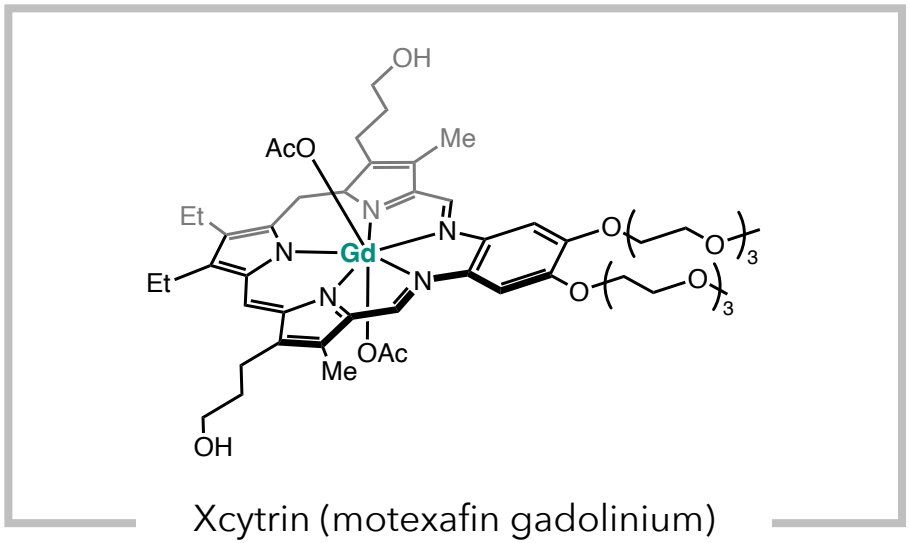

Xcytrin (motexafin gadolinium)

Localization in tumors for more efficient radiation treatment

Attempt to develop treatment for brain metastases for lung cancer

pharmacyclics

Acc. Chem. Res. **1994**, 27, 2, 43-50

Pharmacyclics acquisition of Celera Genomics

- In 2006, Pharmacyclics had acquired several small molecule probes from now defunct Celera Genomics, mostly focusing on **histone deacetylase inhibitors (HDACs)**

Nathan Vardi, *For Blood and Money,* Ch. 2, p. 25

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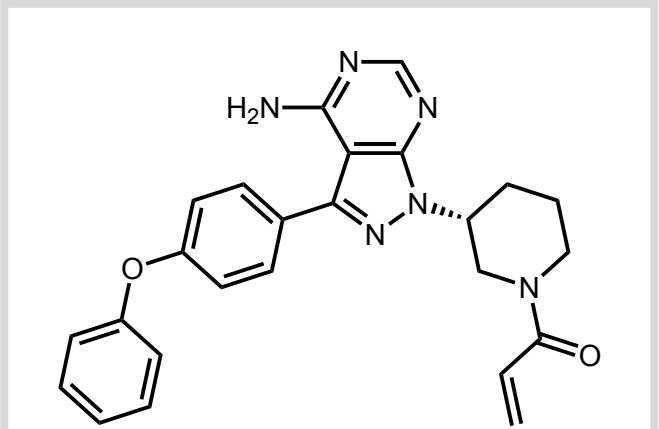

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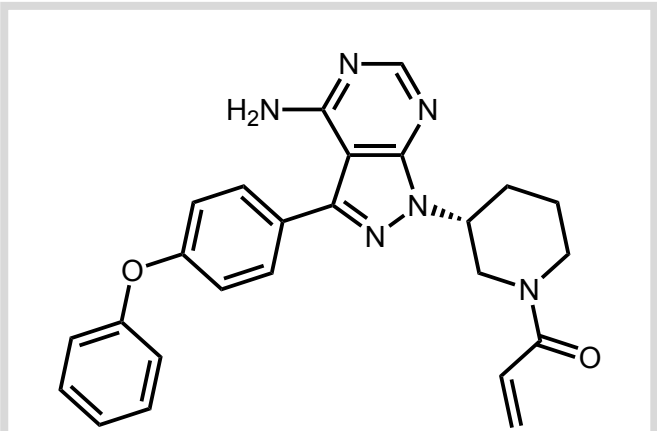
In the $6.6 million transaction, the Celera team ascribed **next to no value to the BTK inhibitors**… *Celera didn't even bother to secure future milestone payments… **The company essentially include CRA-032765 in the deal for nothing.***



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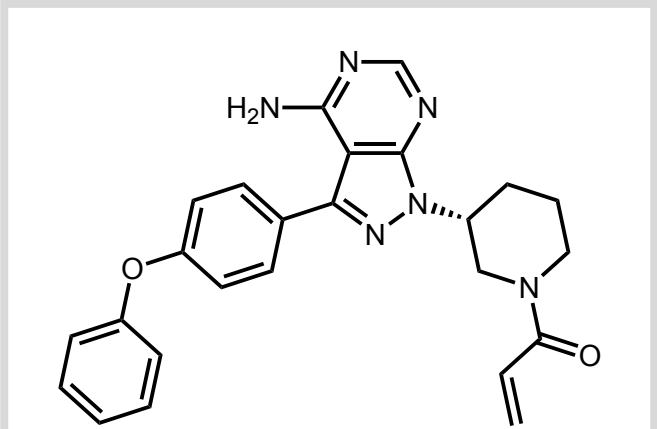
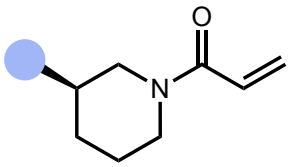


CRA-032765

- Early studies showed CRA-032765 effectively inhibited BTK, but concerns about covalent binding mechanism led to it being cast aside



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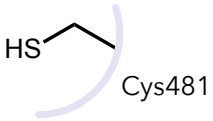
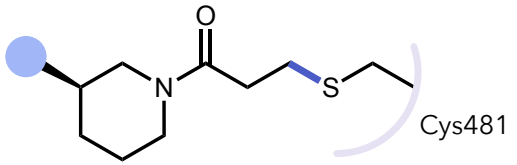


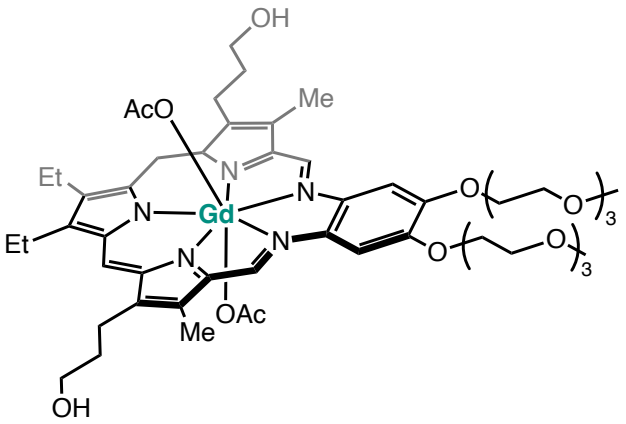
Bruton's tyrosine kinase (BTK)



covalent inhibition of BTK prevent B-cell proliferation

Nathan Vardi, *For Blood and Money,* Ch. 2, p. 25

Pharmacyclics and Xcytrin



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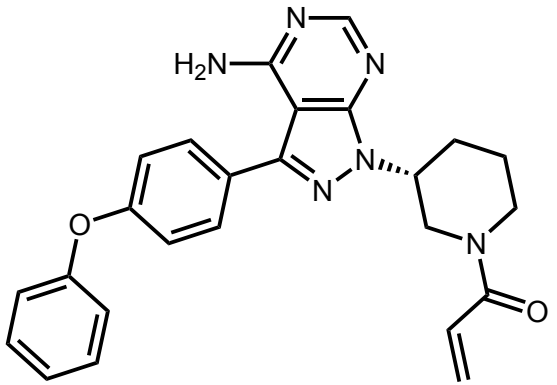

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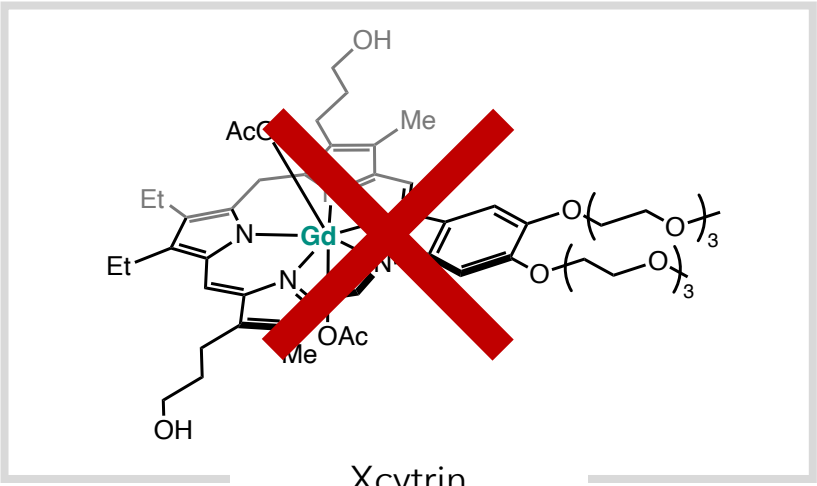

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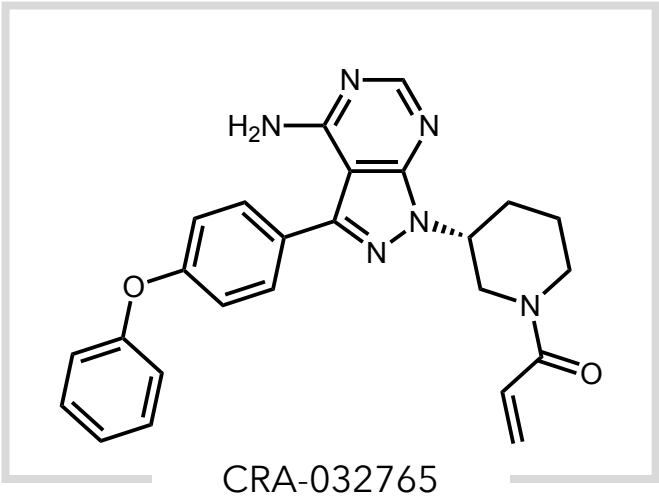

CRA-032765

- After years of attempts, Pharmacyclics received a non-approvable letter from the FDA in 2007

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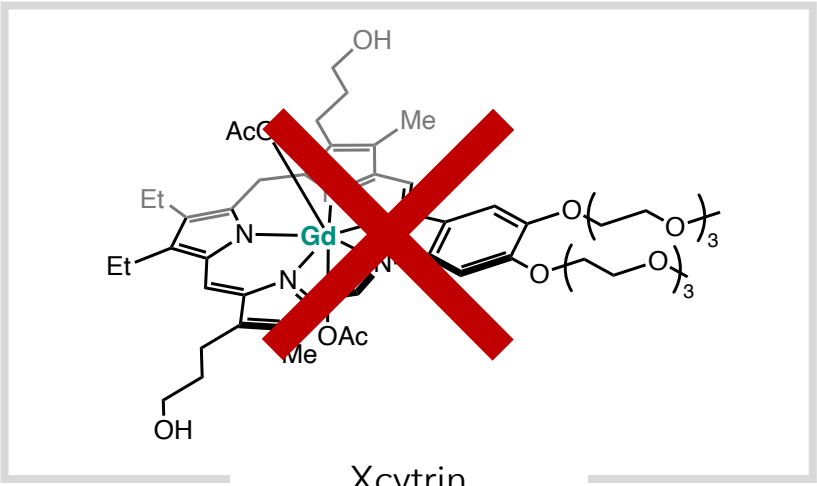

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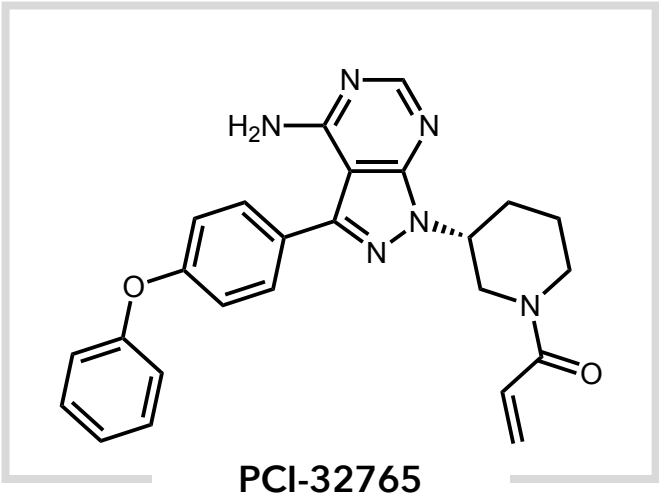

PCI-32765

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- Scrambling during a financial crisis, Pharmacyclics invested some effort into studying CRA-032765, now given codename **PCI-32765**

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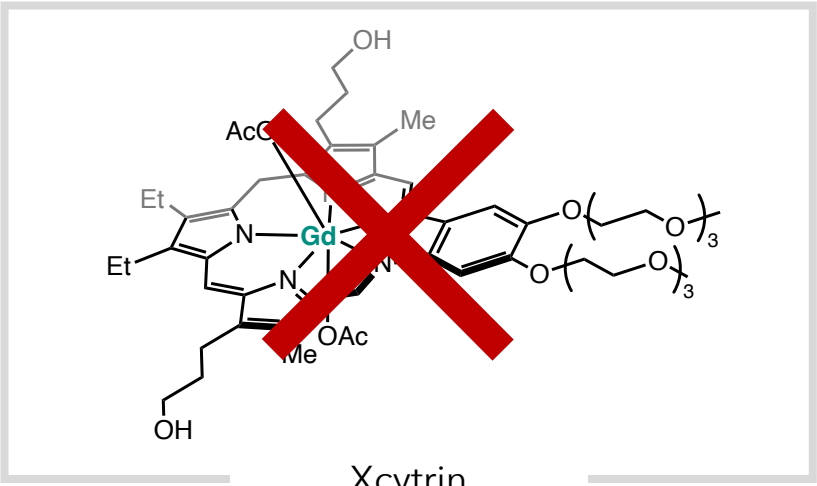
Pharmacyclics and Xcytrin



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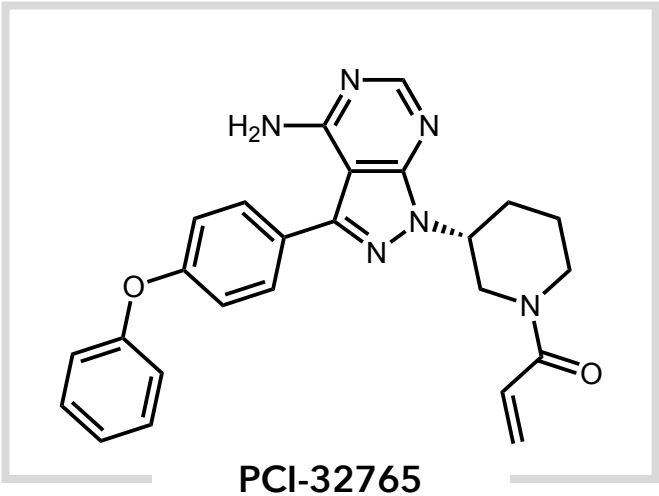

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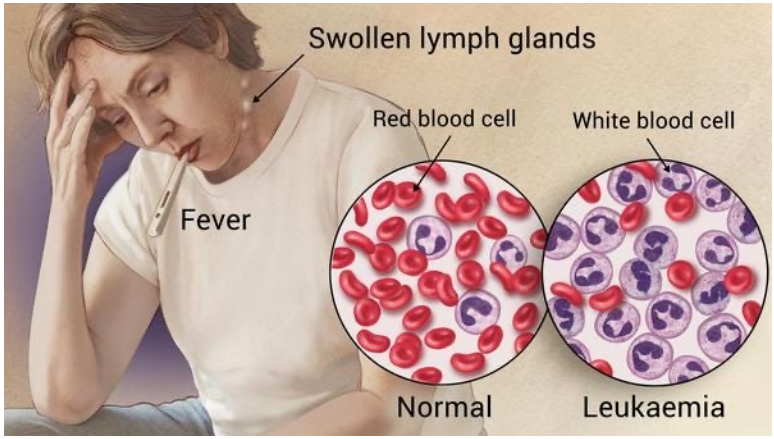

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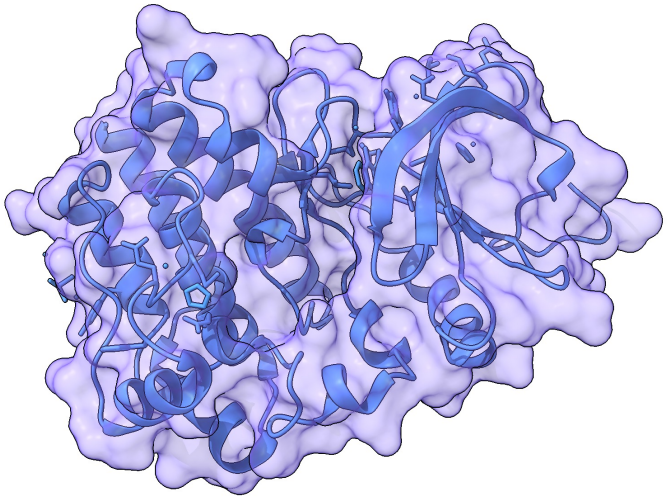
- Scrambling during a financial crisis, Pharmacyclics invested some effort into studying CRA-032765, now given codename **PCI-32765**
- Early clinical trials showed reduction of tumors in patients with **chronic lymphocytic leukemia (CLL),** the most common form of adult leukemia

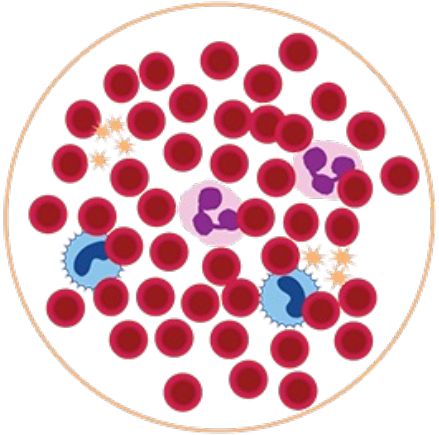
Acc. Chem. Res. **1994**, 27, 2, 43–50

Bruton's tyrosine kinase (BTK) and B-cell cancers

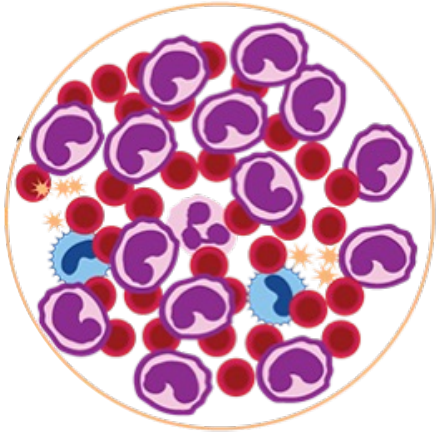


Bruton's tyrosine kinase

- Mutations cause various B-cell malignancies
- Invoked in non-Hodgkin lymphomas, chronic lymphocytic leukemia, multiple myeloma,
- Traditional treatments include harsh chemotherapies



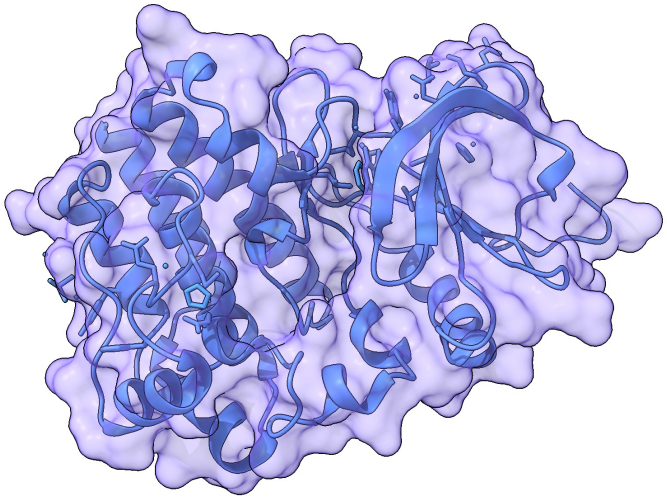
Normal Blood



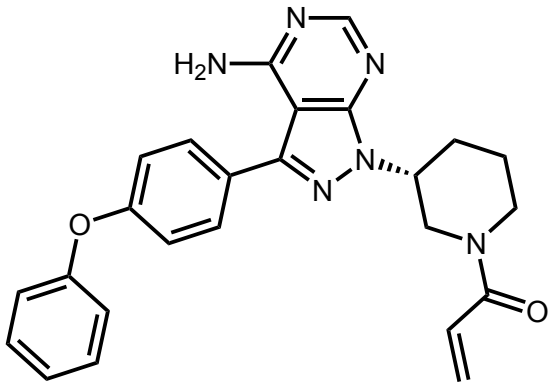
Leukemia

Leukemia **2021** 35, 312–332

Bruton's tyrosine kinase (BTK) and B-cell cancers

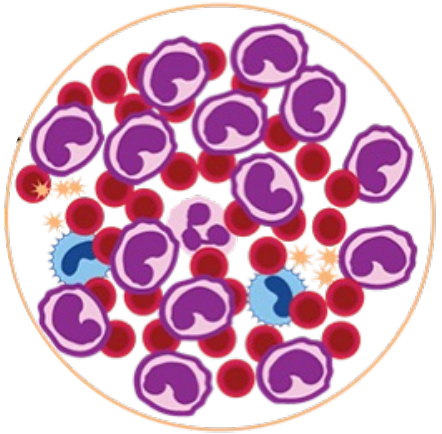


Bruton's tyrosine kinase



PCI-32765

- Mutations cause various B-cell malignancies
- Invoked in non-Hodgkin lymphomas, chronic lymphocytic leukemia, multiple myeloma,
- Traditional treatments include harsh chemotherapies
- **PCI-32765 shows remarkable selectivity for BTK inhibition**

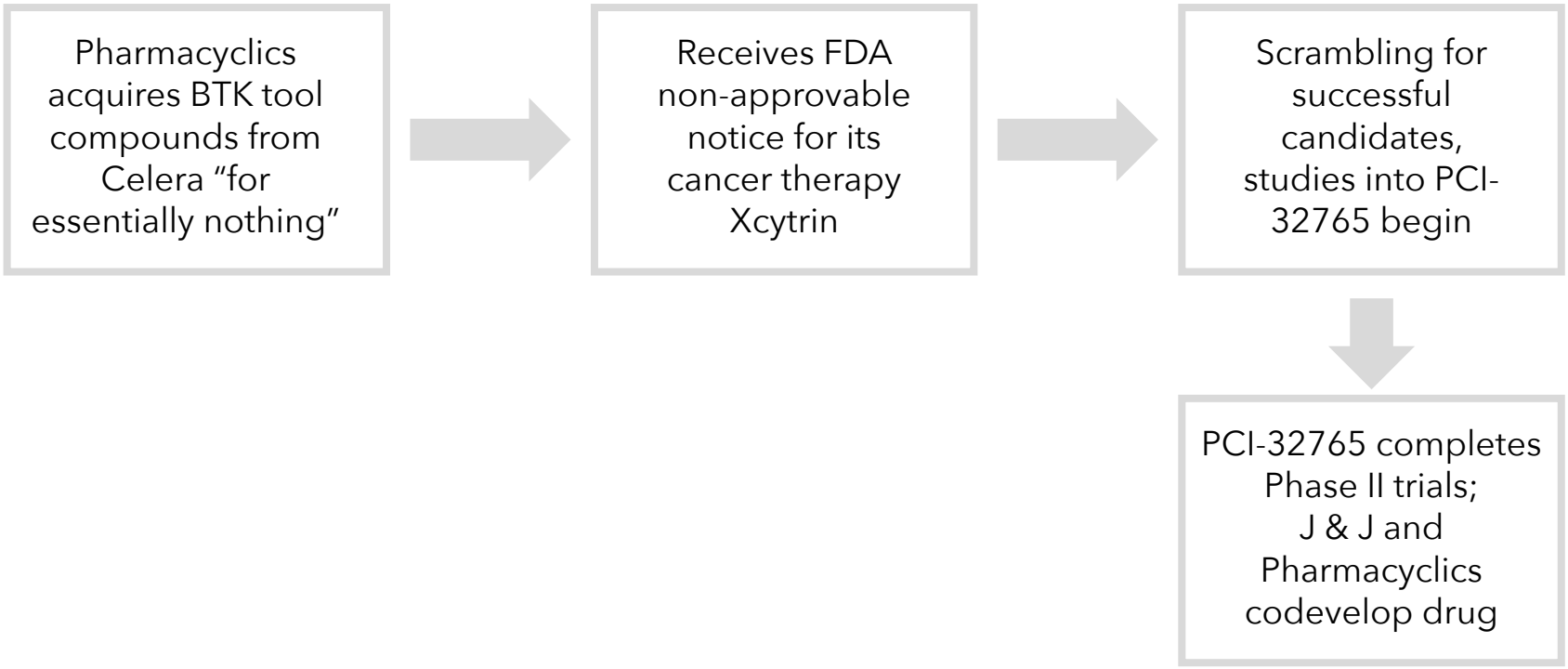


Leukemia

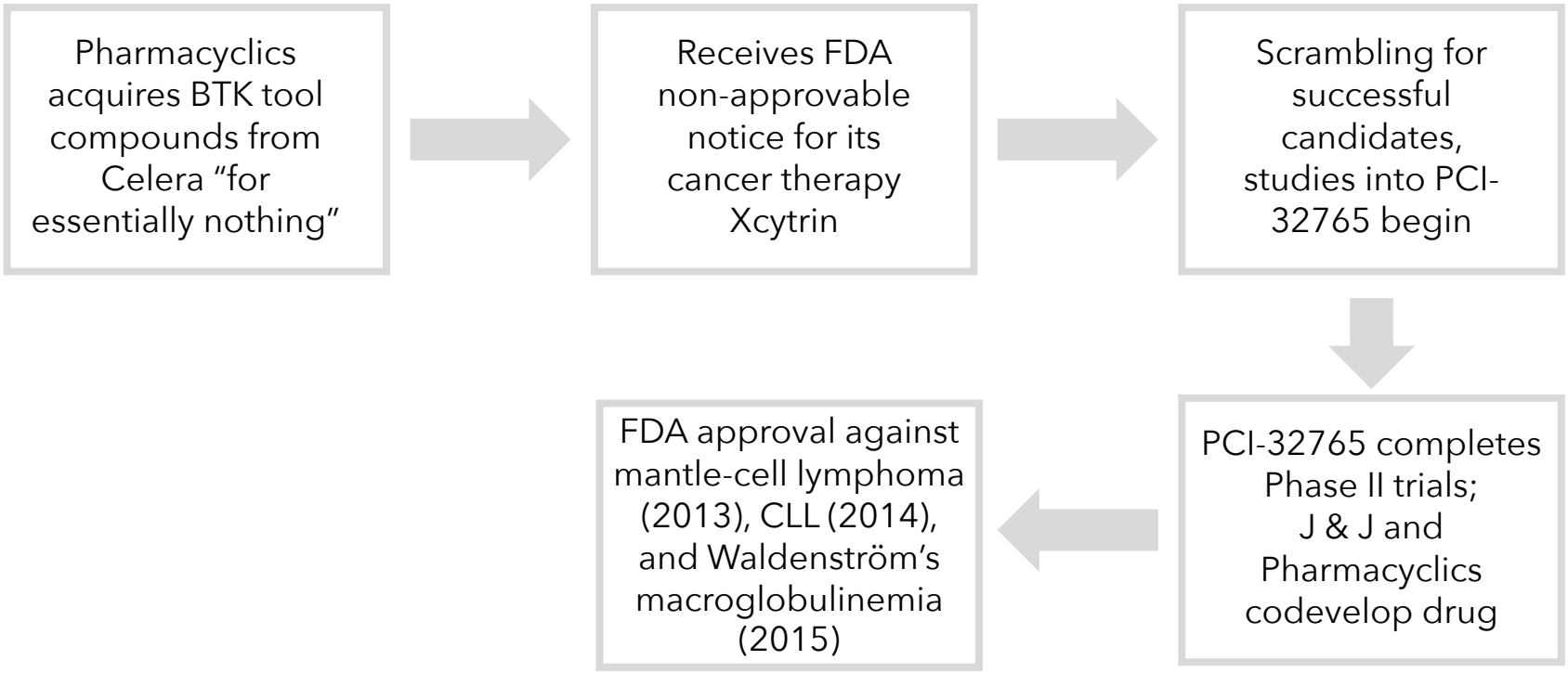
Leukemia **2021** 35, 312–332

The remarkable story of ibrutinib

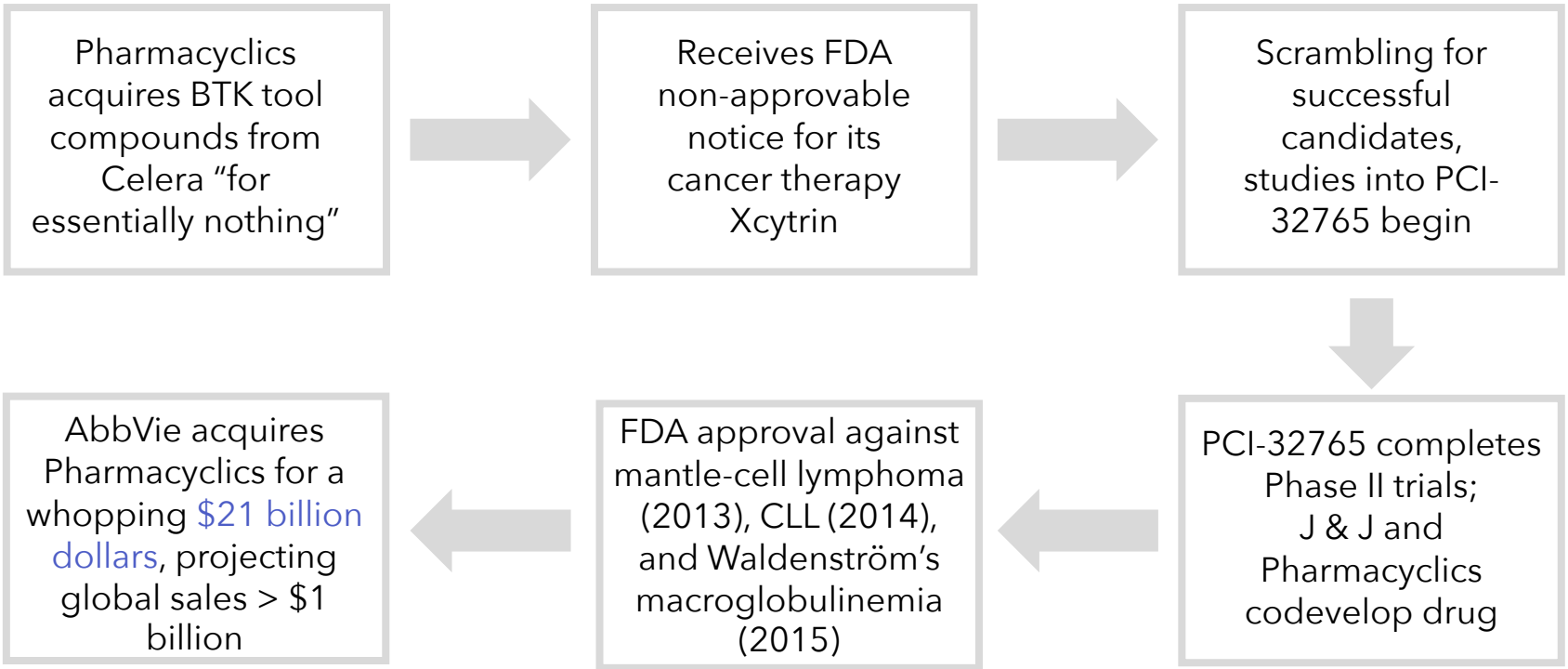
The remarkable story of ibrutinib



The remarkable story of ibrutinib



The remarkable story of ibrutinib



The remarkable story of ibrutinib

Pharmacyclics acquires BTK tool compounds from Celera "for essentially nothing"

→

Receives FDA non-approvable notice for its cancer therapy Xcytrin

→

Scrambling for successful candidates, studies into PCI-32765 begin

↓

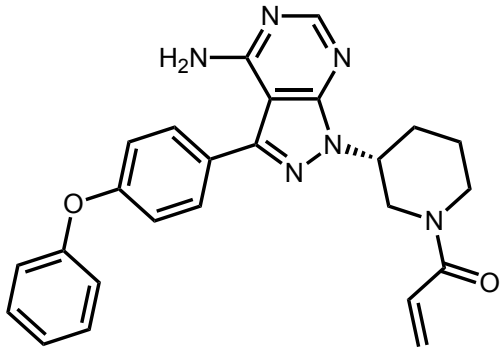
PCI-32765 completes Phase II trials; J & J and Pharmacyclics codevelop drug

←

FDA approval against mantle-cell lymphoma (2013), CLL (2014), and Waldenström's macroglobulinemia (2015)

←

AbbVie acquires Pharmacyclics for a whopping $21 billion dollars, projecting global sales > $1 billion



Imbruvica® (ibrutinib)

- Irreversibly binds Bruton's tyrosine kinase, inhibiting the B-cell receptor pathway
- Proves covalent inhibition is a viable strategy for drug design
- In 2022, global Imbruvica net revenues were $1.115 billion

Happy Cinco de Mayo!

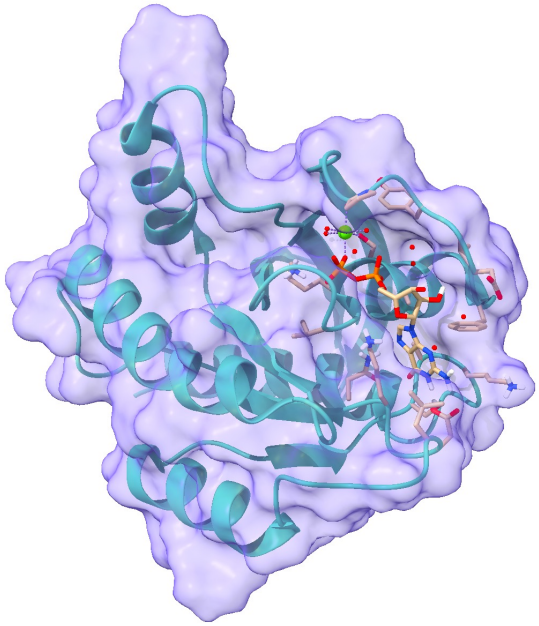
…and why most people in Mexico don't celebrate

- Cinco de Mayo does not celebrate Mexico's independence!
- Mexican independence is September 16th (Huge holiday in Mexico!
- Instead corresponds to Battle of Puebla against France in 1862
  - *"…the victory was short-lived—the French later captured Mexico City and took over the country…,"*

- Honestly, Cinco de Mayo is not really a thing in Mexico…
- Has become a much bigger thing in the US as a celebration of Mexican-American heritage
- Enjoy a margarita or some tacos today!

Revisiting K-Ras and the undruggable proteome

Prof. Kevan Shokat
UCSF



K-Ras (GDP bound)

Revisiting K-Ras and the undruggable proteome

Prof. Kevan Shokat
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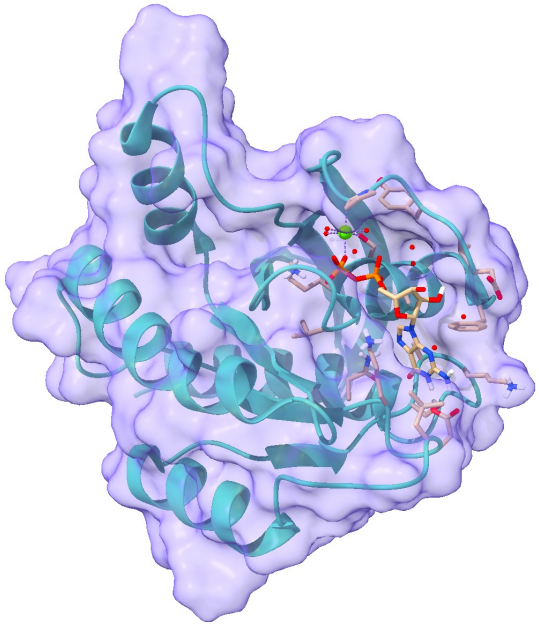
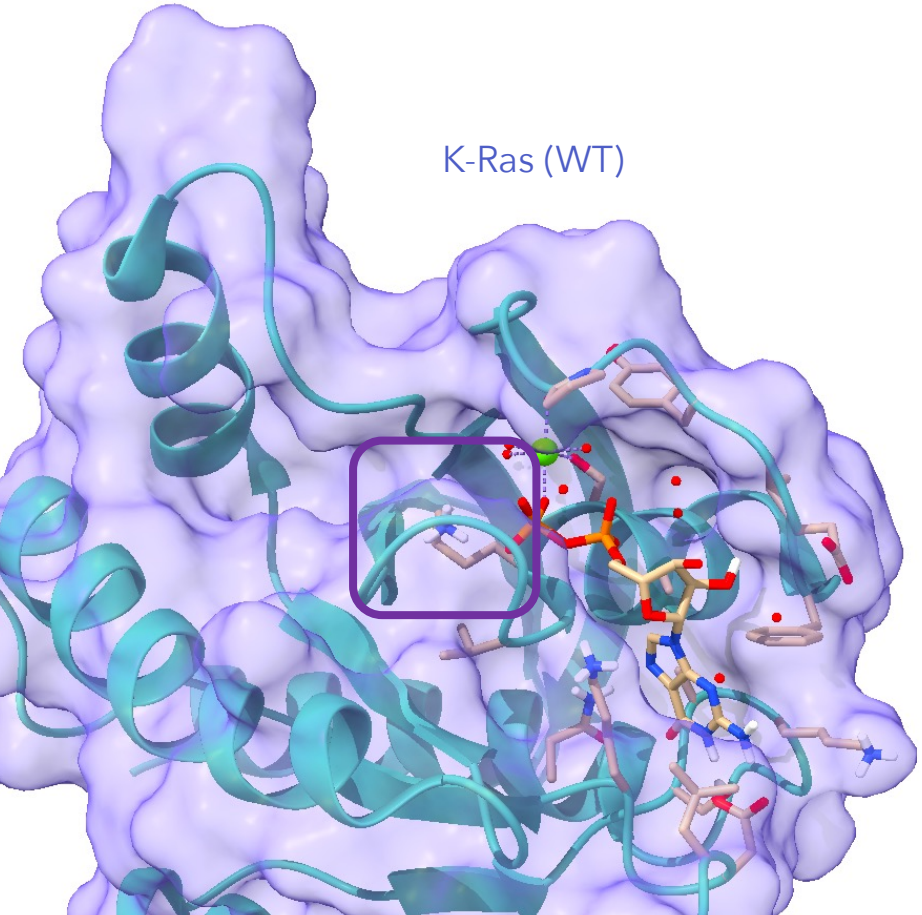


K-Ras (GDP bound)

Reasons K-Ras was deemed "undruggable"

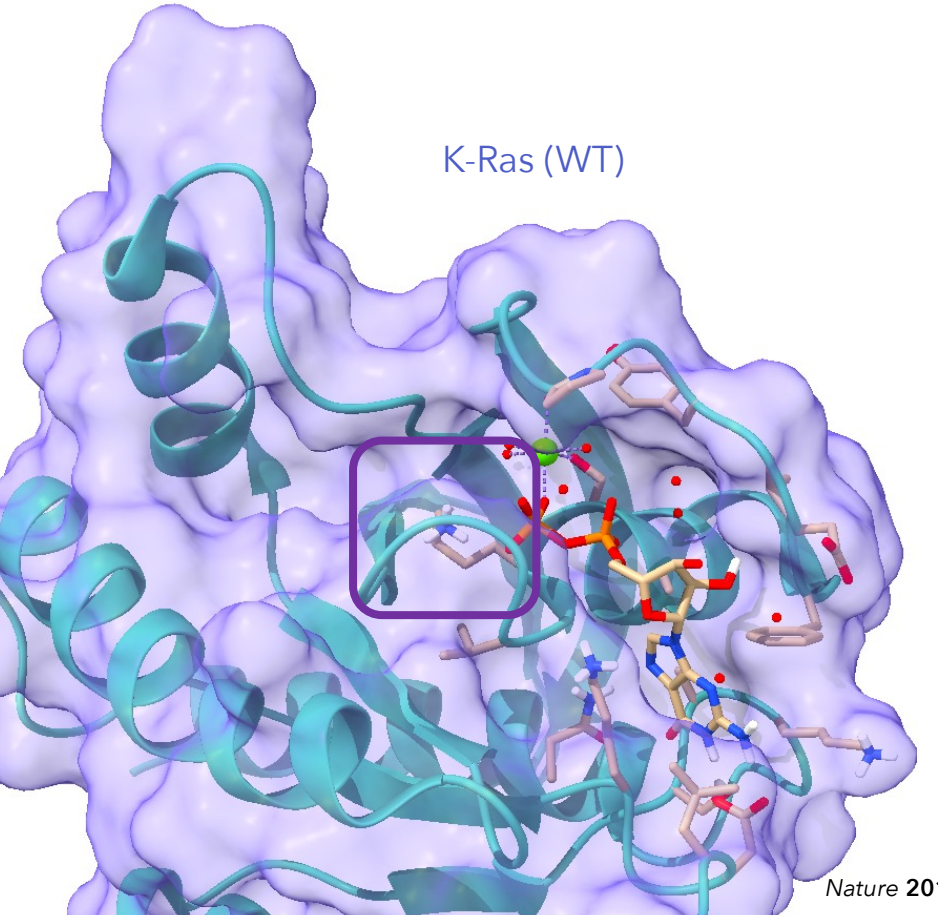
- High affinity for native GTP substrate
- Inhibition of membrane localization is ineffective
- K-Ras involved in highly complex signaling pathway – difficult to understand how knocking out one protein affects downstream effects!

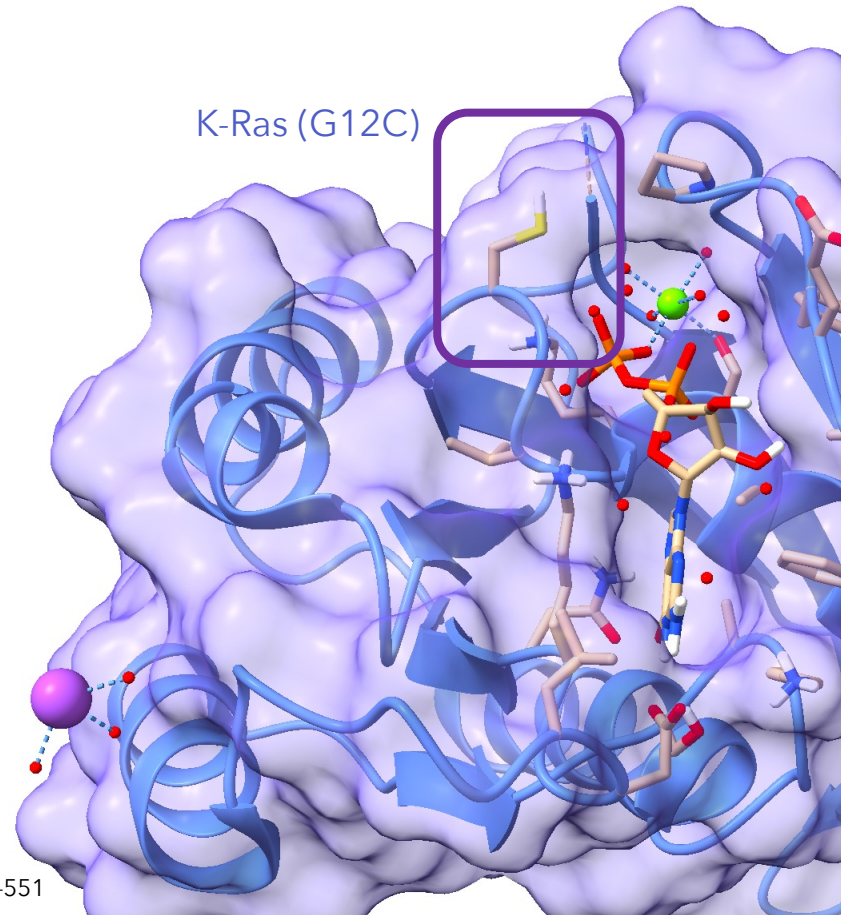
Chemical strategies to drug K-Ras



Nature **2013**, 503, 548–551

Chemical strategies to drug K-Ras



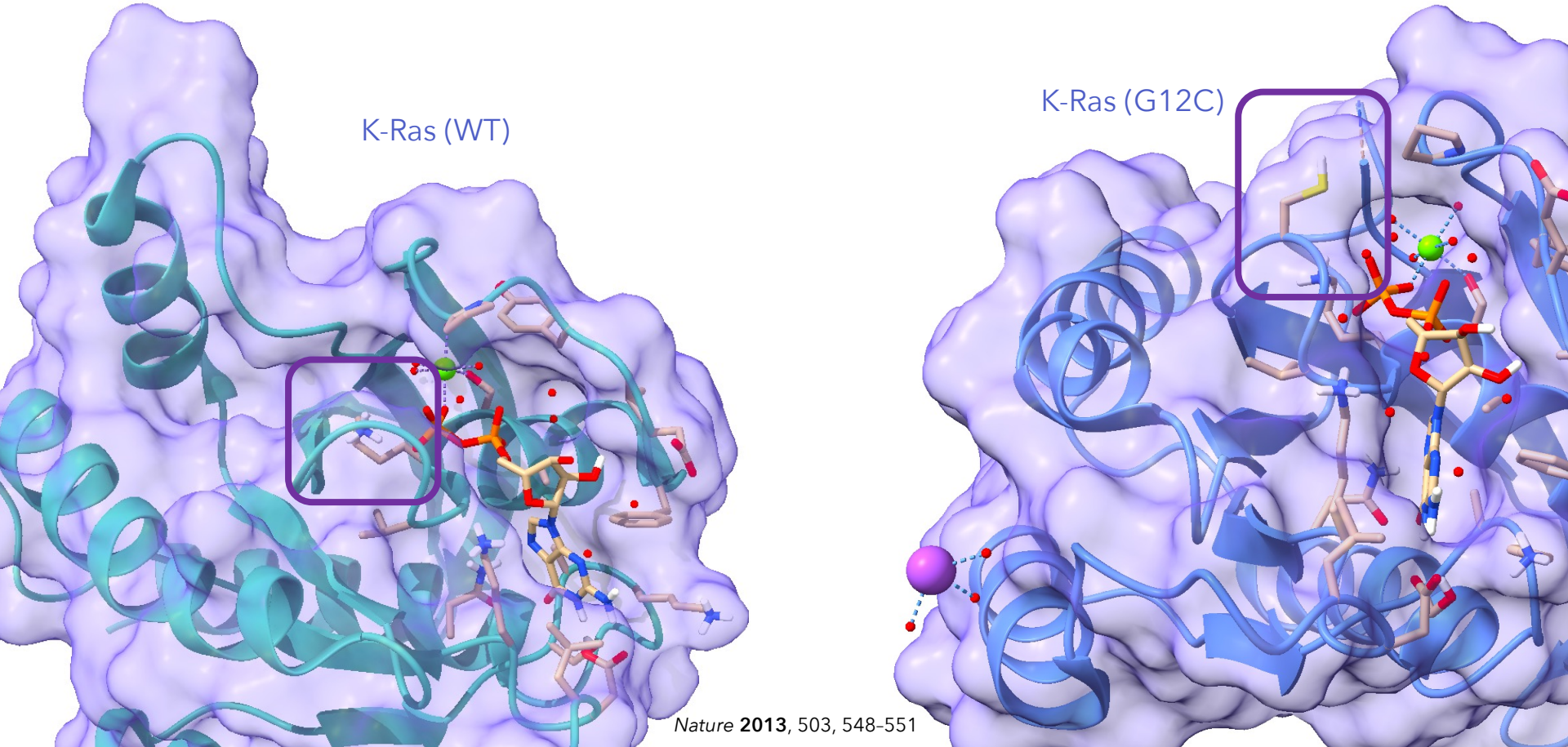


Nature **2013**, 503, 548–551

Chemical strategies to drug K-Ras

Strategy: use cysteine-reactive functional groups in an ABPP-type approach to determine what ligands bind to K-Ras

Determine allosteric sites while simultaneously only targeting mutated Ras owing to Cys12 reactivity



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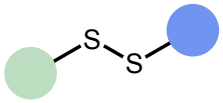
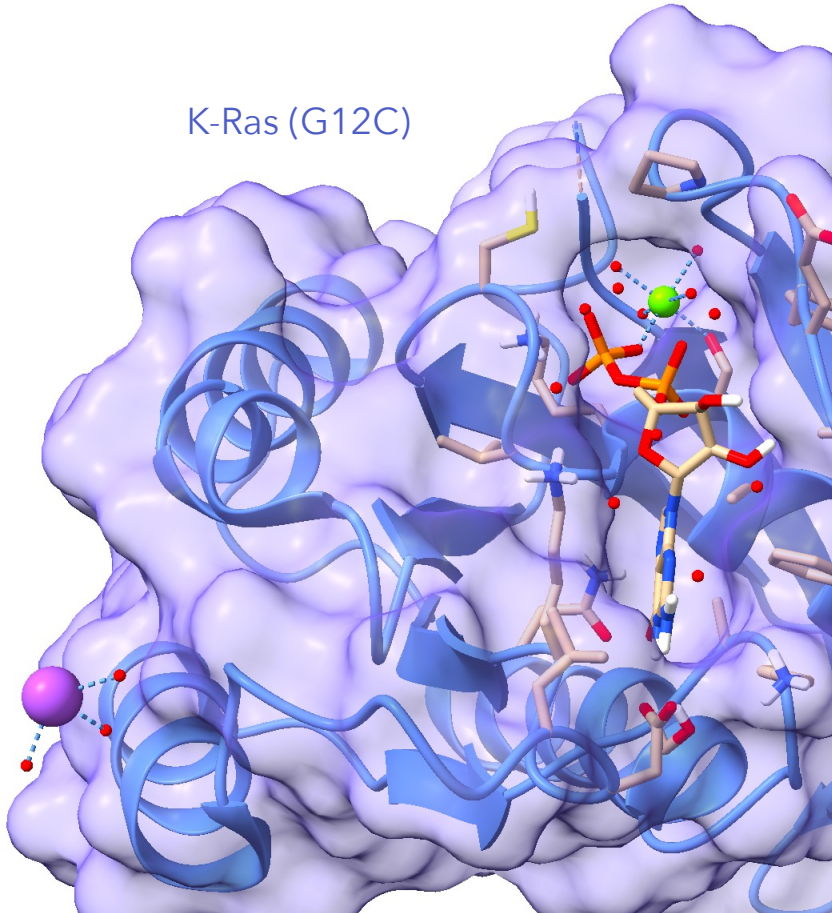
Chemical strategies to drug K-Ras

Disulfide “tethering”



Determine allosteric sites while simultaneously only targeting mutated Ras owing to Cys12 reactivity



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Chemical strategies to drug K-Ras

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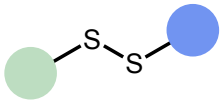
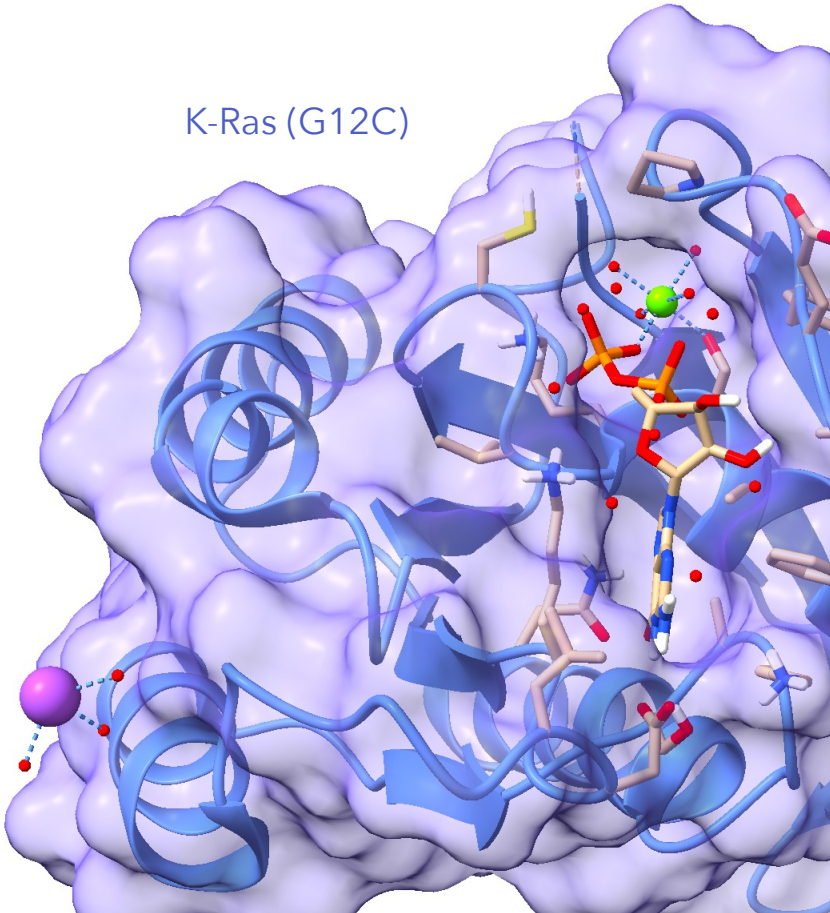


480 compounds screened

Determine allosteric sites while simultaneously only targeting mutated Ras owing to Cys12 reactivity



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Chemical strategies to drug K-Ras

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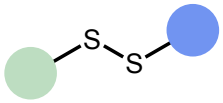

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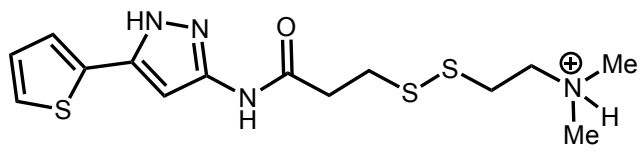

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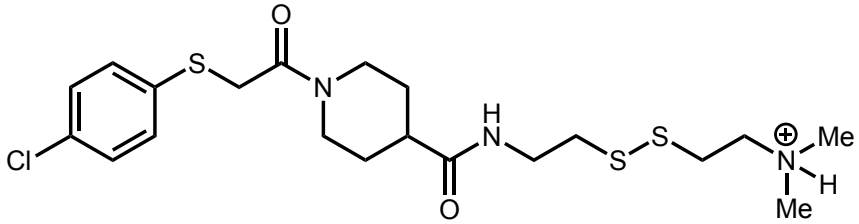
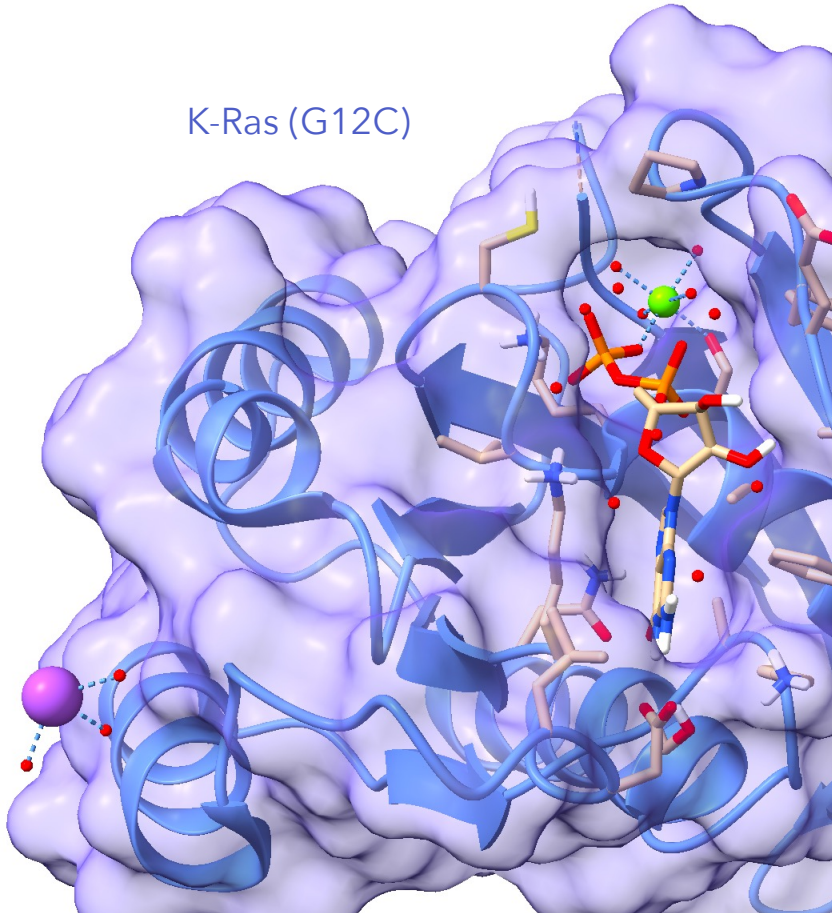


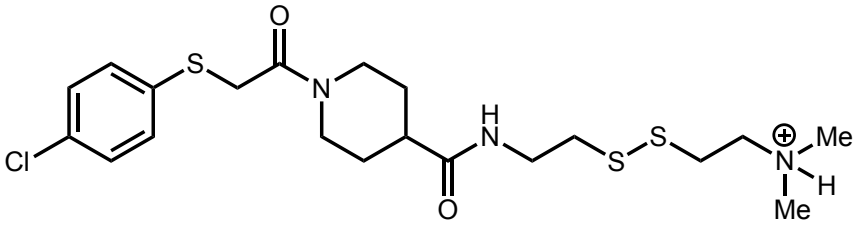
6H05

Determine allosteric sites while simultaneously only targeting mutated Ras owing to Cys12 reactivity

K-Ras (G12C)



A structure-activity campaign in understanding K-Ras inhibition



6H05

Nature **2013**, 503, 548–551

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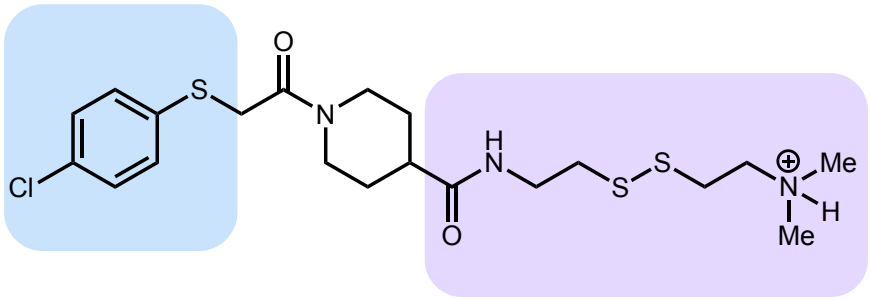

6H05

fragment A	fragment B	relative potency

Nature **2013**, 503, 548–551

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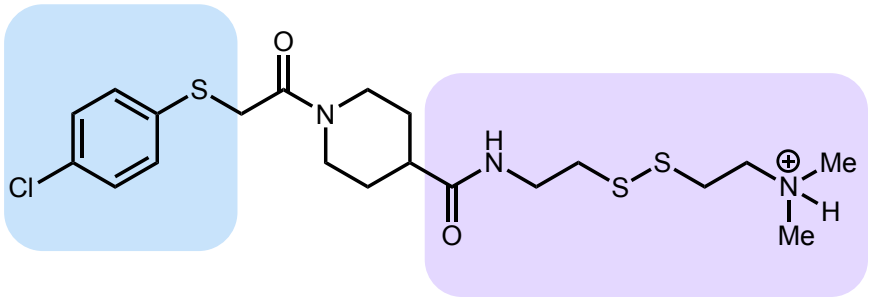
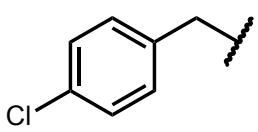
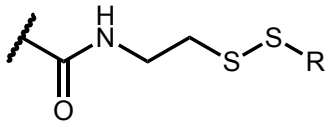
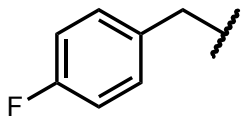
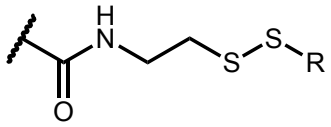


6H05

fragment A	fragment B	relative potency
		<0.1
		0.34

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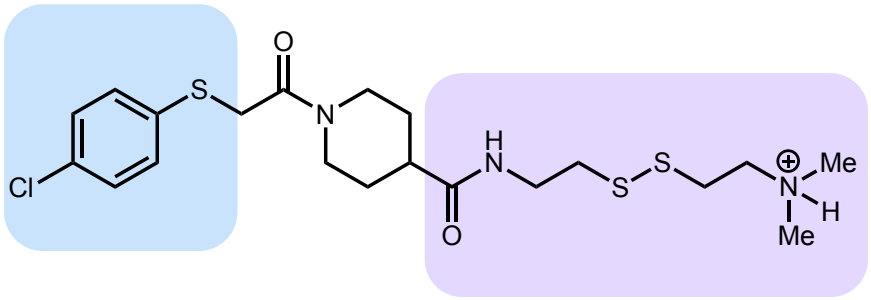
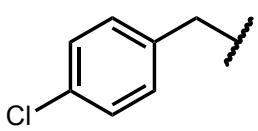
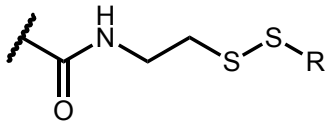
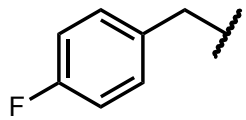
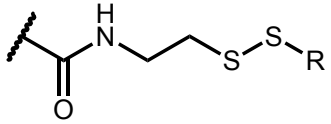
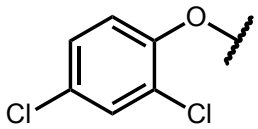
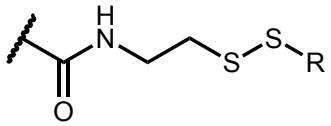
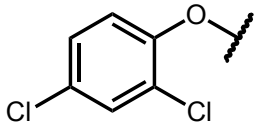
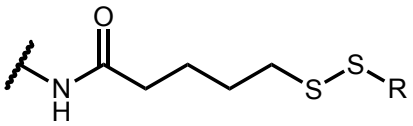


6H05

fragment A	fragment B	relative potency
		<0.1
		0.34
		1.2
		4.2

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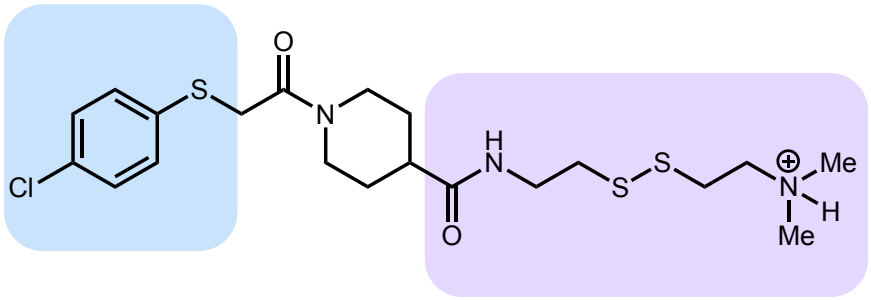

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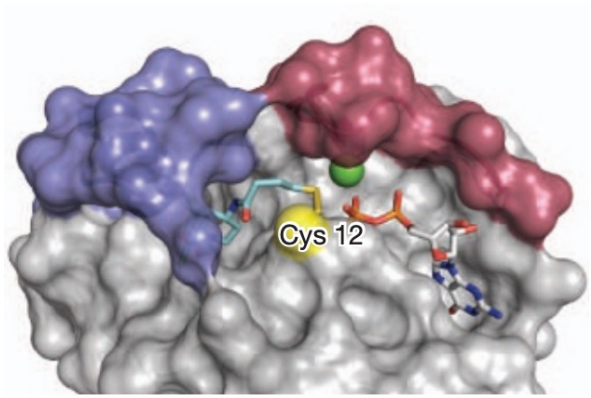
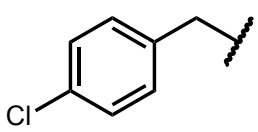
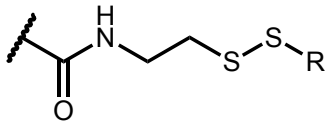
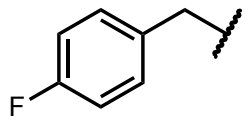
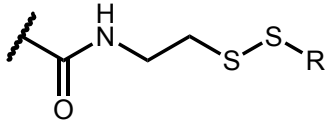
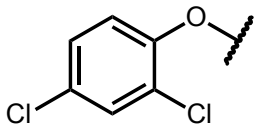
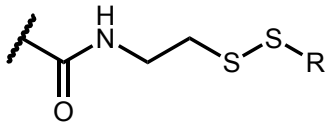
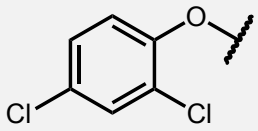
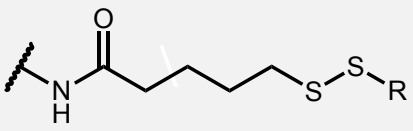
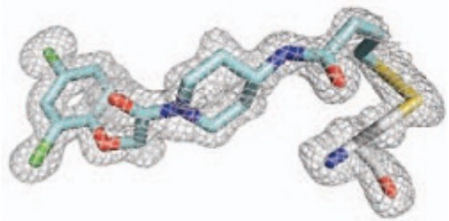
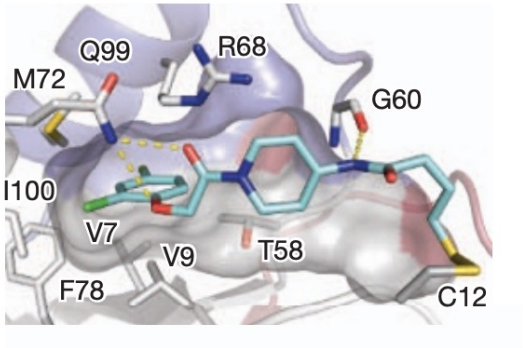


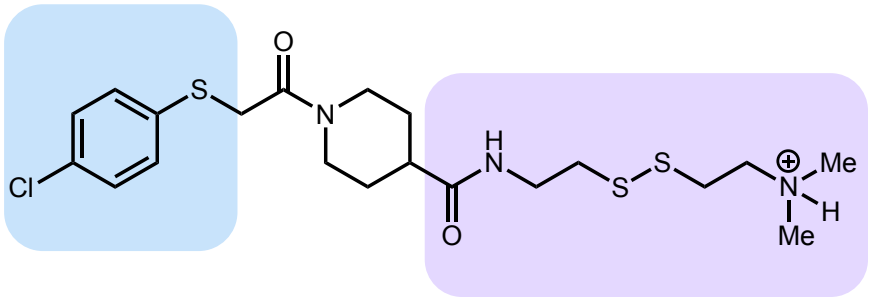
fragment A	fragment B	relative potency
		<0.1
		0.34
		1.2
		4.2



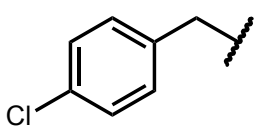
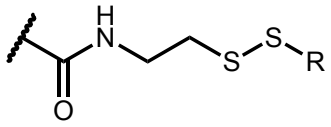
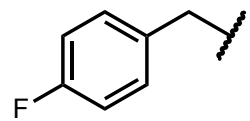
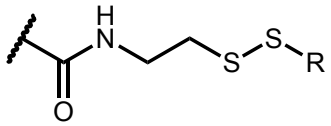
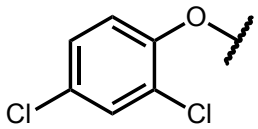
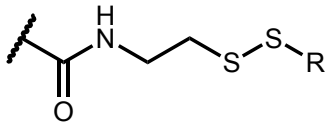
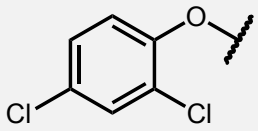
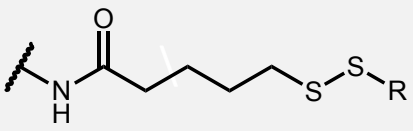


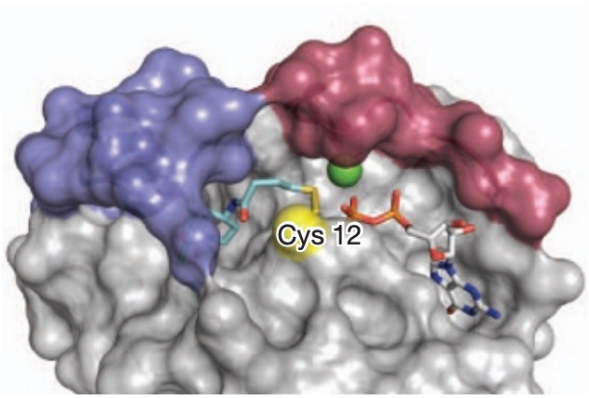
Nature **2013**, 503, 548–551

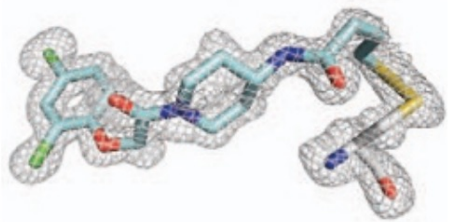
A structure-activity campaign in understanding K-Ras inhibition

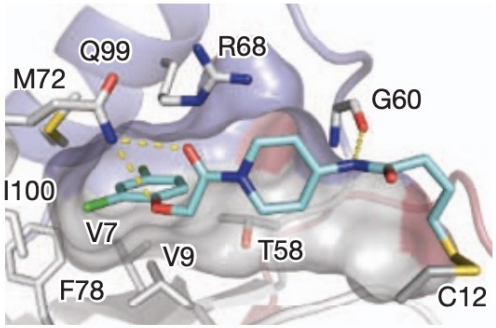


6H05

fragment A	fragment B	relative potency
		<0.1
		0.34
		1.2
		4.2



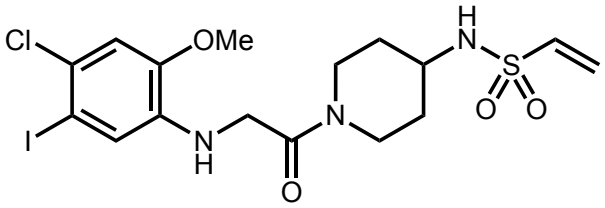




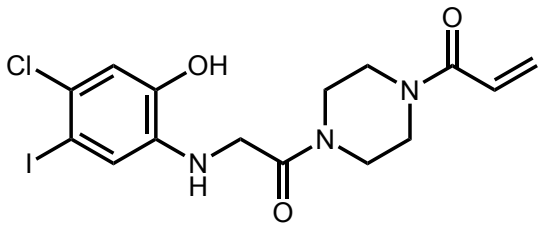
Activity-based approach reveals allosteric "Switch-II" pocket

Nature **2013**, 503, 548–551

Switching to irreversible activity-based probes



compound 9



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Nature **2013**, 503, 548–551

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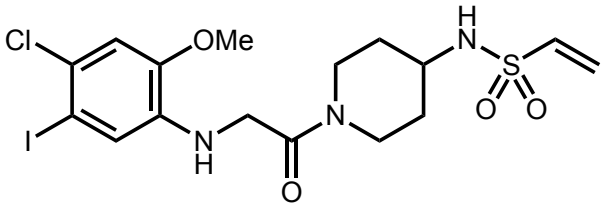

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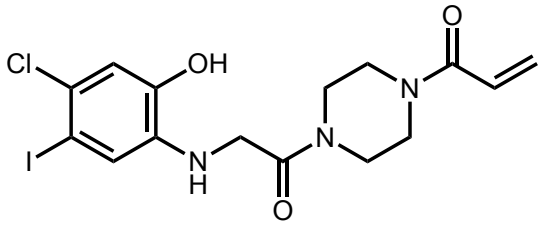

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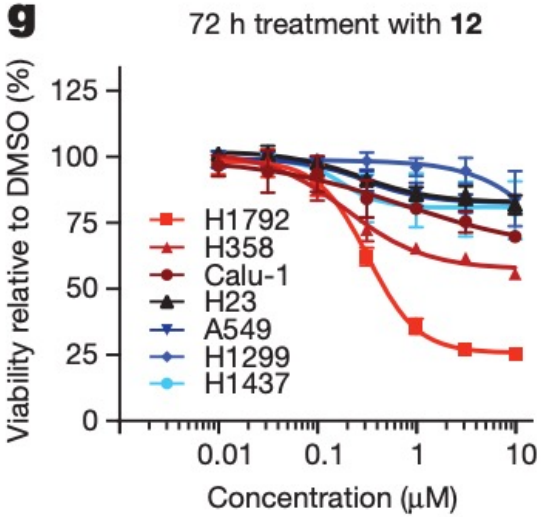
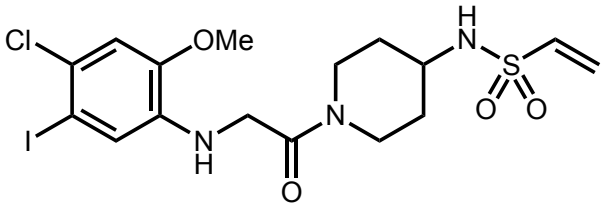


Nature **2013**, 503, 548–551

Switching to irreversible activity-based probes



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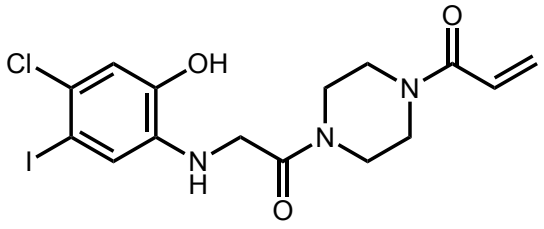

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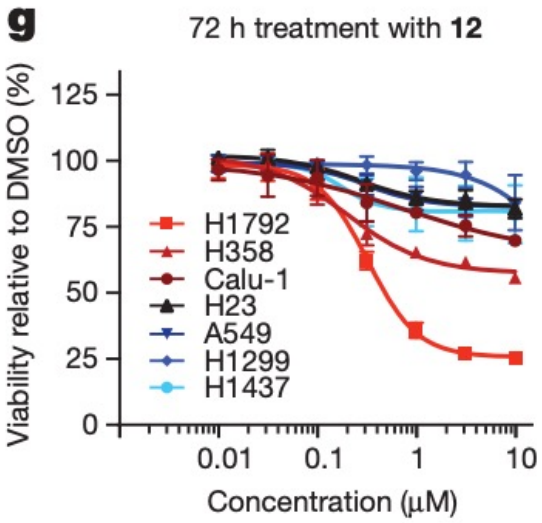
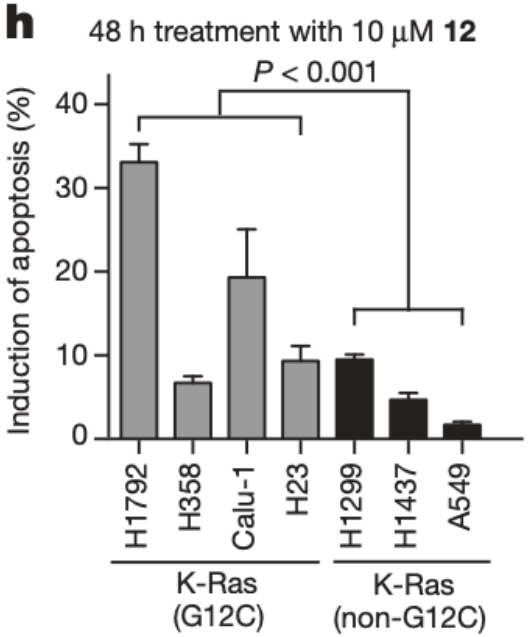
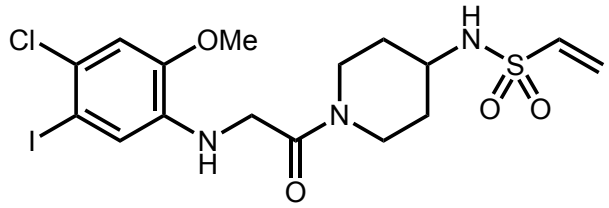




Nature **2013**, 503, 548–551

Switching to irreversible activity-based probes



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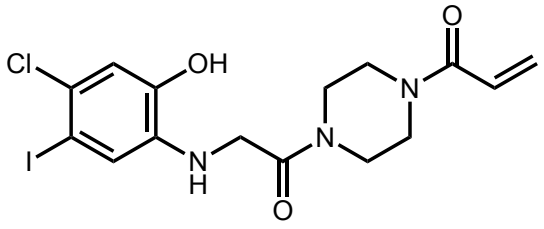

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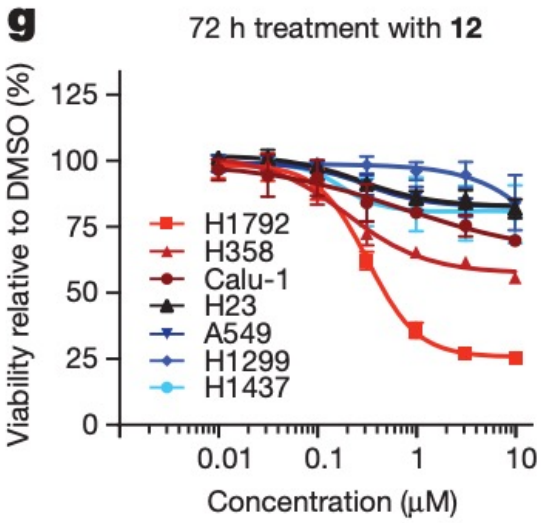
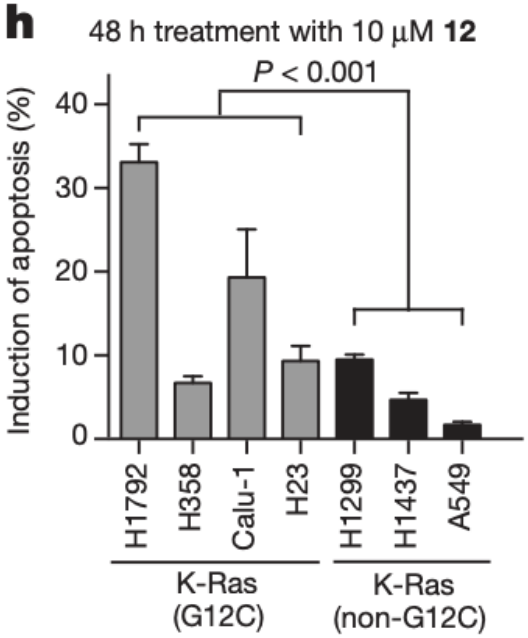


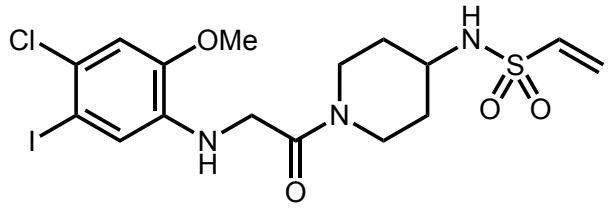


Compound 12 served as an efficient covalent, irreversible inhibitor of K-Ras G12C

Insights gained from activity-based profiling

Nature **2013**, 503, 548–551

Switching to irreversible activity-based probes



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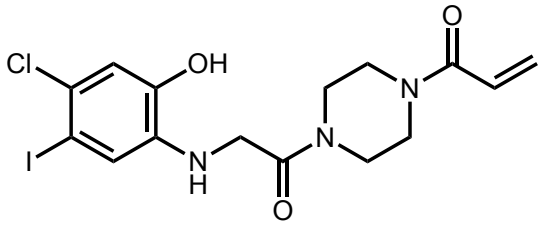
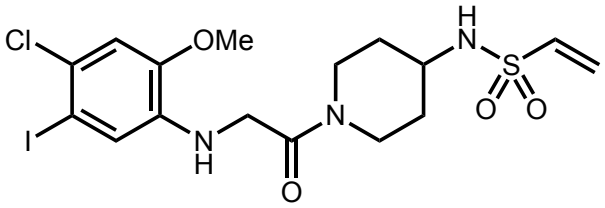


compound 12

AMGEN

Journal of Hematology & Oncology **2022**, 15, 152

Switching to irreversible activity-based probes



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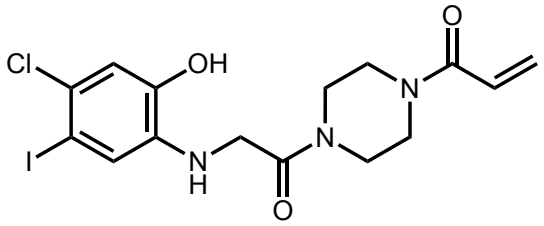
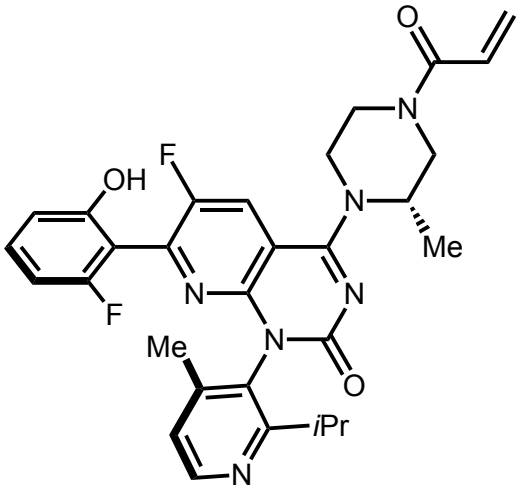


compound 12

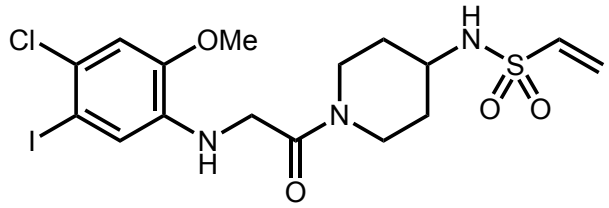
Sotorasib
FDA approval in 2021

Treatment of K-Ras G12C mutations



Journal of Hematology & Oncology **2022**, 15, 152

Switching to irreversible activity-based probes



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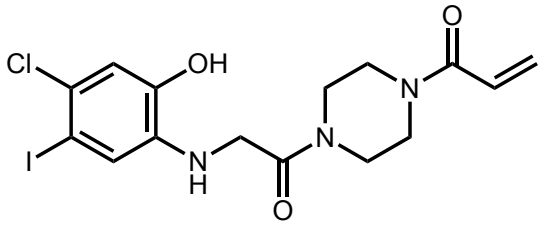

compound 12

AMGEN

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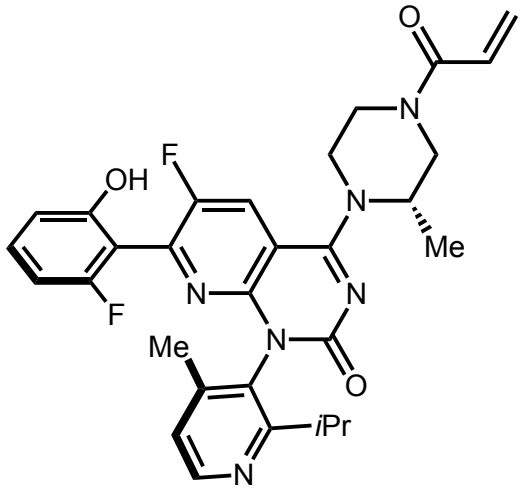
FDA approval in 2021

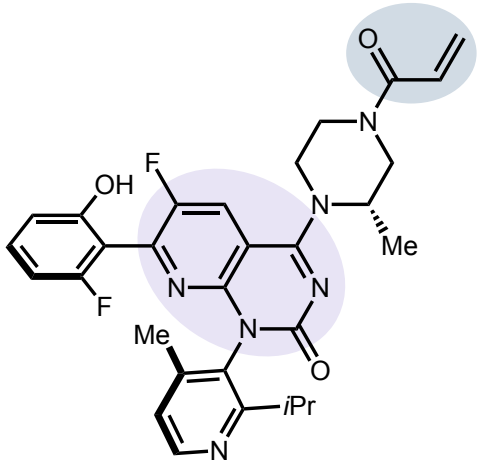
Treatment of K-Ras G12C mutations

Successful drugging on an "undruggable" oncogene!



Journal of Hematology & Oncology **2022**, 15, 152

Drugging the “undruggable” K-Ras G12 mutations



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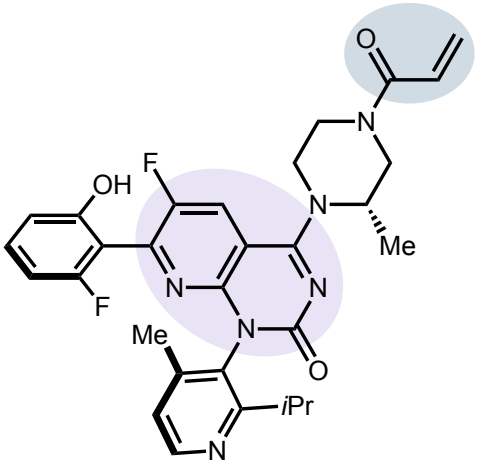
N. Engl. J. Med. **2021**; 384:2371-2381; *Nature* **2013**, 503, 548–551; *Nature Chem. Bio.* **2022**, 18, 1177–1183; *J. Am. Chem. Soc.* **2022**, 144, 35, 15916–15921

Drugging the “undruggable” K-Ras G12 mutations



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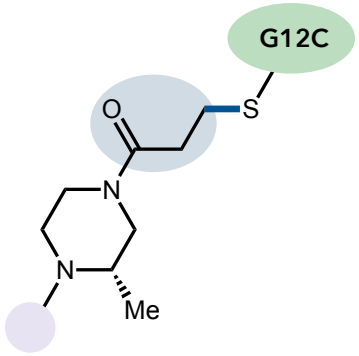
FDA approval in 2021



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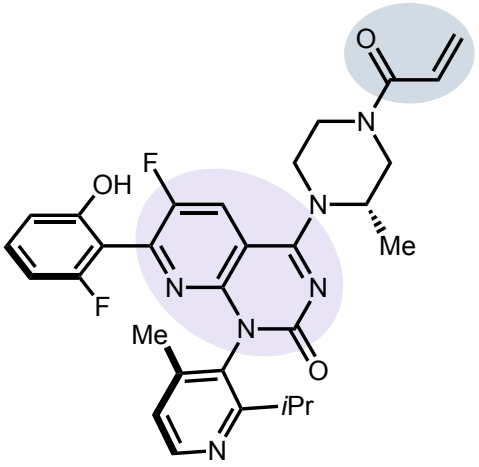
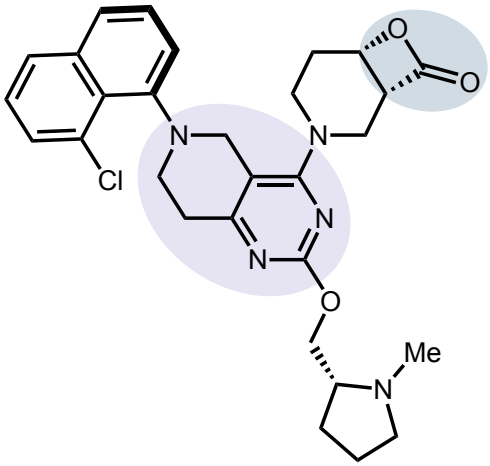
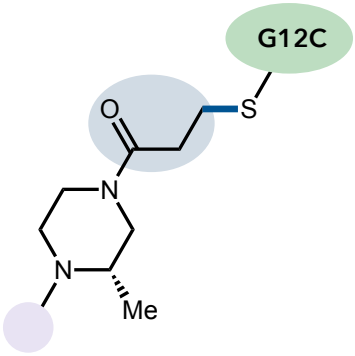
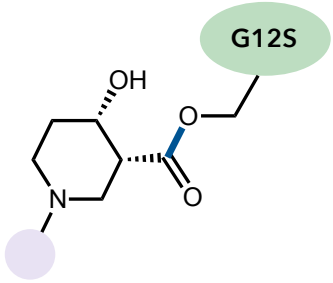


Sotorasib
FDA approval in 2021



Shokat (2022)





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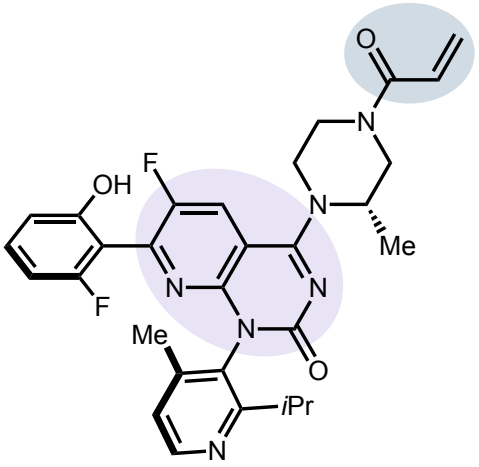
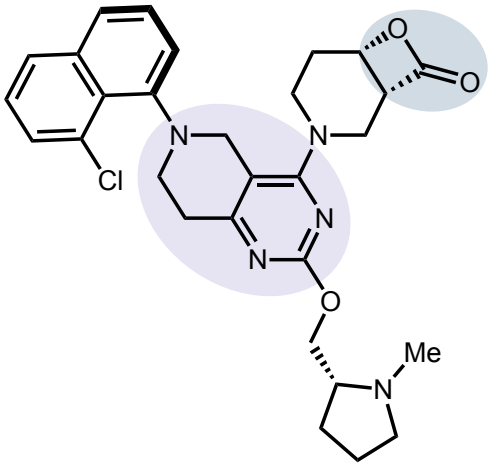
Drugging the “undruggable” K-Ras G12 mutations



Sotorasib
FDA approval in 2021



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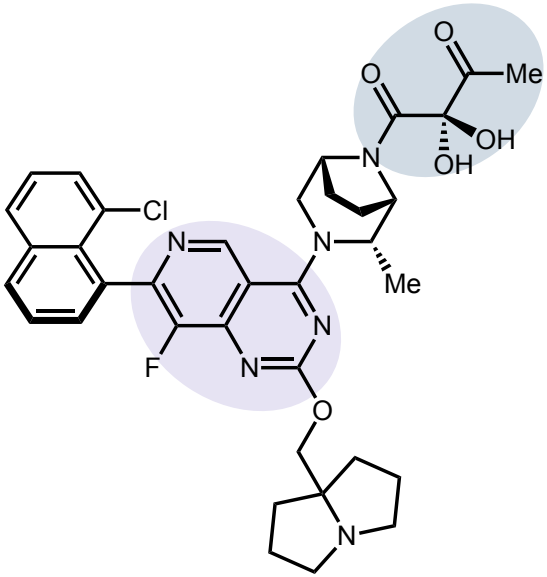

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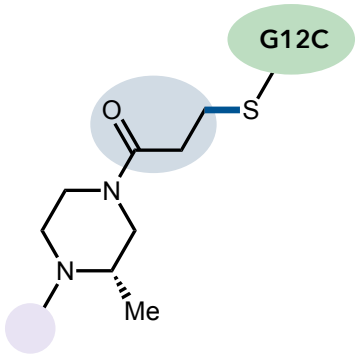

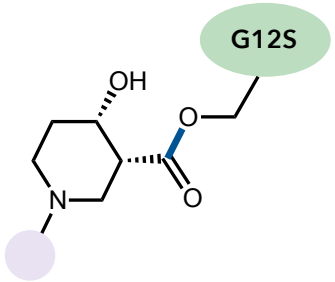

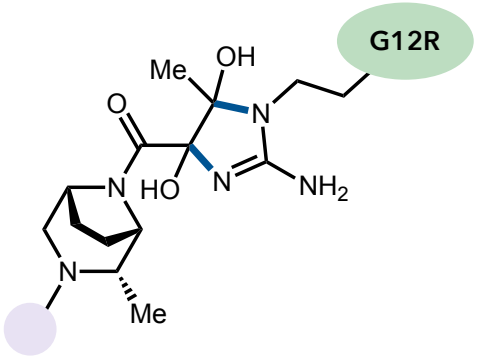

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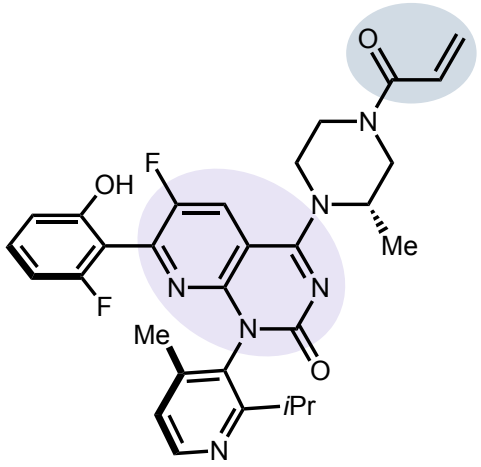
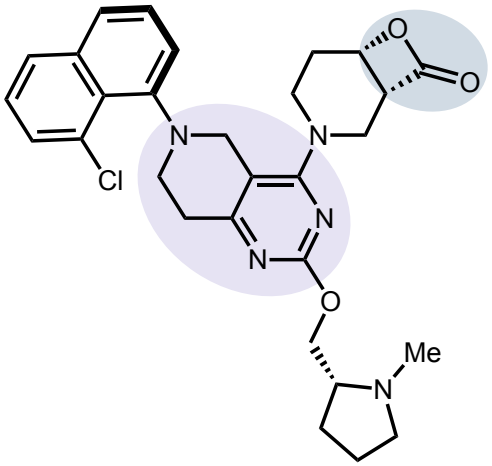
Drugging the "undruggable" K-Ras G12 mutations



Sotorasib
FDA approval in 2021



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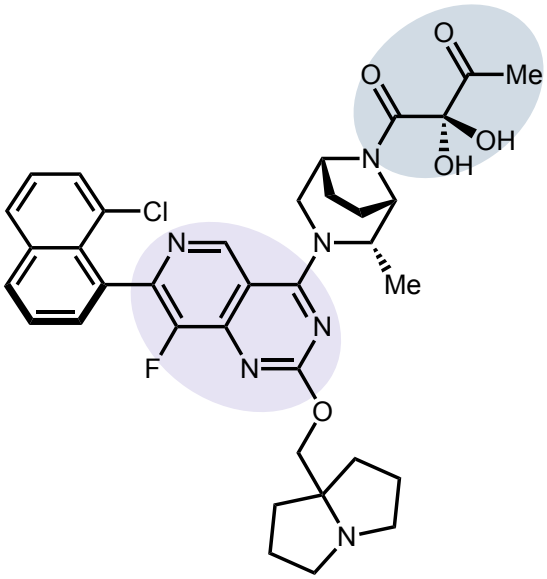
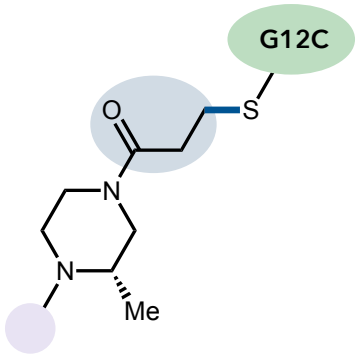
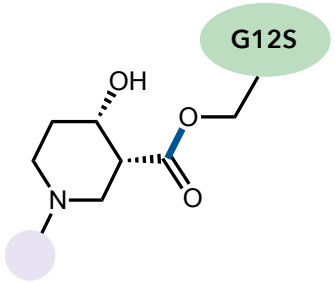
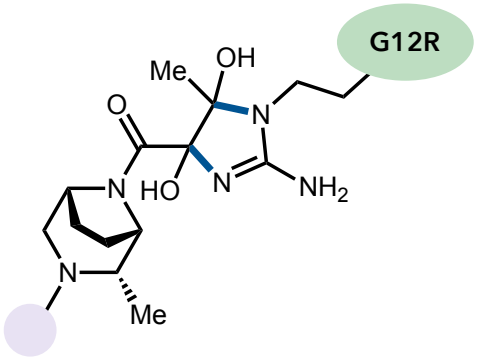


Shokat (2022)

Successful K-Ras inhibitors dependent on covalent inhibition

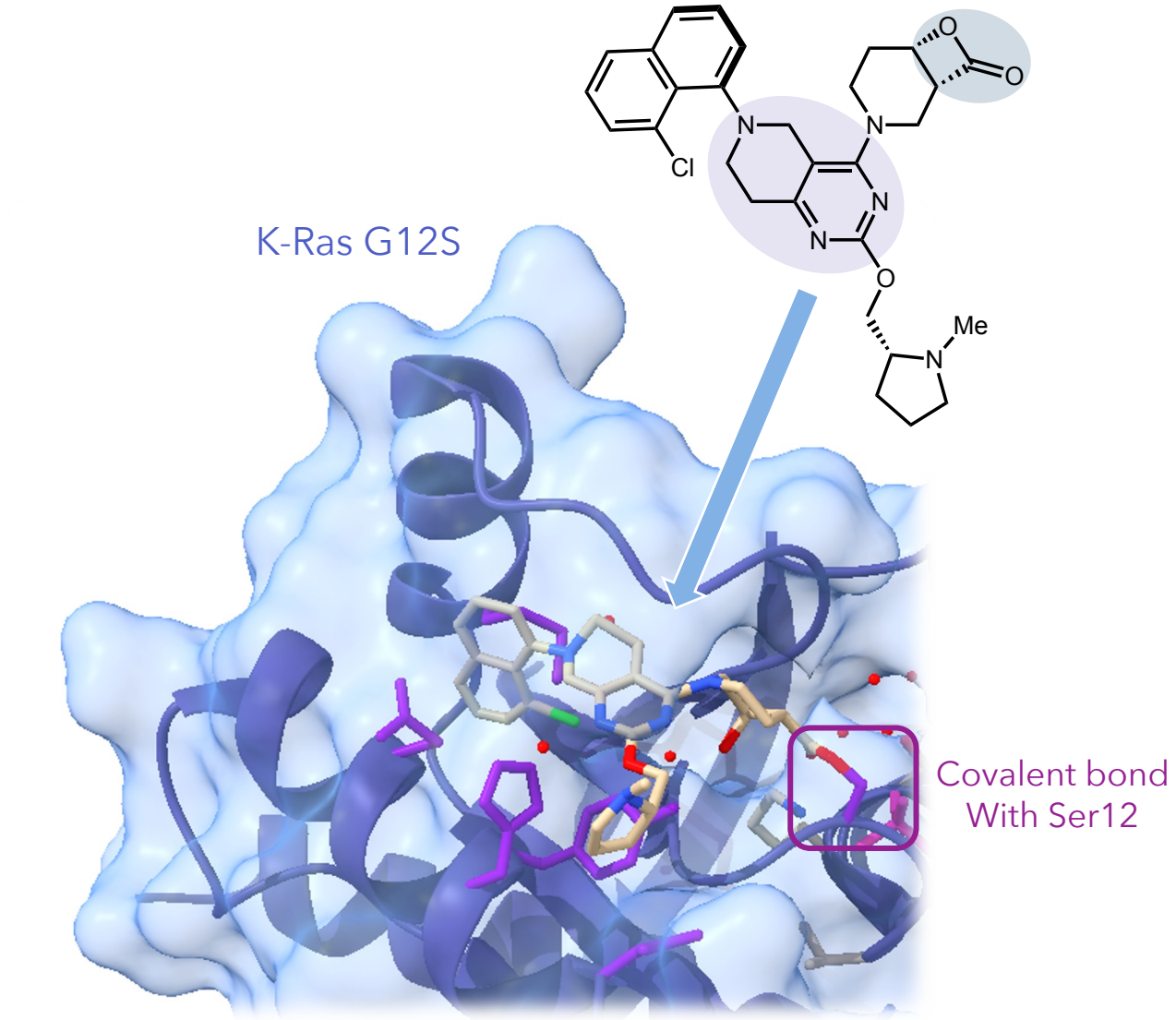






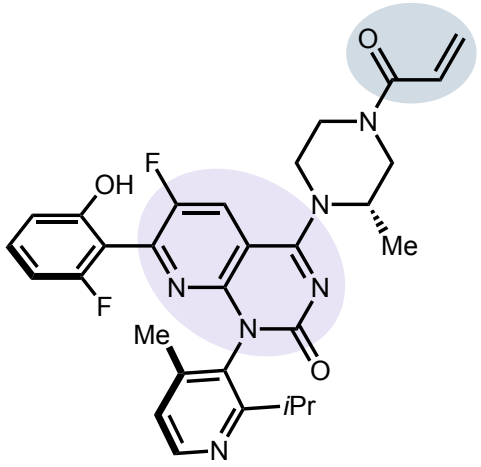
N. Engl. J. Med. **2021**; 384:2371-2381; *Nature* **2013**, 503, 548–551; *Nature Chem. Bio.* **2022**, 18, 1177–1183; *J. Am. Chem. Soc.* **2022**, 144, 35, 15916–15921

The switch-II pocket is flexible and inhibitors dock and covalently bind

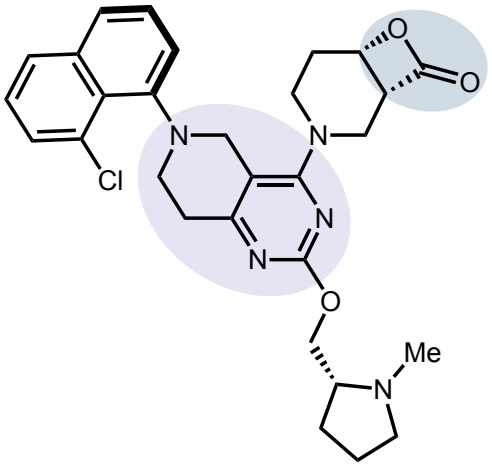


N. Engl. J. Med. **2021**; 384:2371-2381; *Nature* **2013**, 503, 548–551; *Nature Chem. Bio.* **2022**, 18, 1177–1183; *J. Am. Chem. Soc.* **2022**, 144, 35, 15916–15921; (PBD 7TLE)

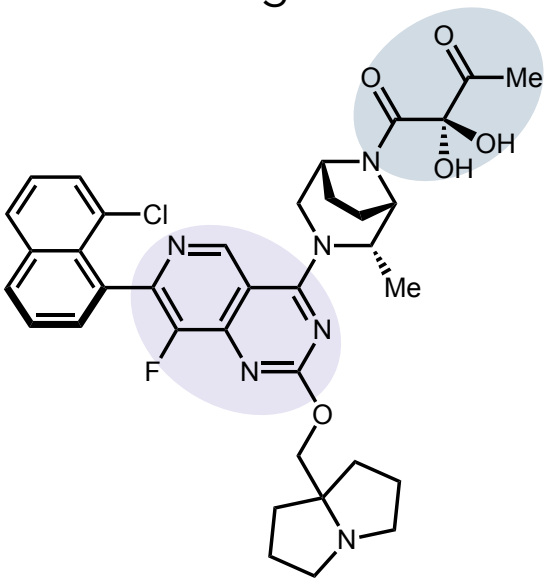
Expanding beyond G12C with different covalent strategies



Sotorasib
FDA approval in 2021

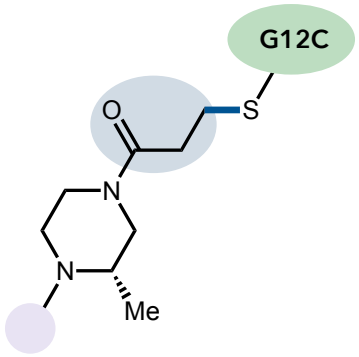


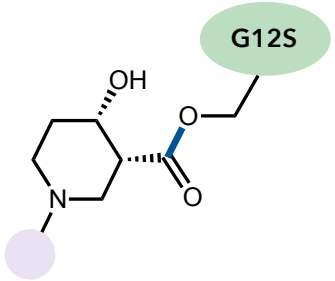
Shokat (2022)

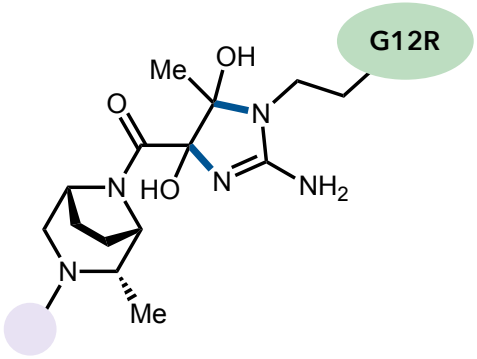


Shokat (2022)

Successful K-Ras inhibitors dependent on covalent inhibition







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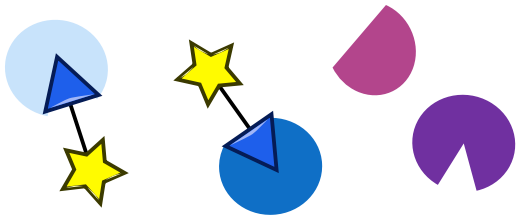
Outline

- Introduction to undruggable proteins
  - What makes a protein “undruggable”?
  - Attempts to drug K-Ras mutations
- Activity-based approaches to finding “druggable” sites
- Success stories in covalent drugs
  - Ibrutinib and Bruton’s tyrosine kinase
  - Sotorasib and K-Ras G12C
- Conclusions
  - ”Yet to be drugged” instead of “undruggable”

Activity-based protein profiling approaches and the advancement of proteomics have allowed for identification of variety of new covalent candidates and druggable sites



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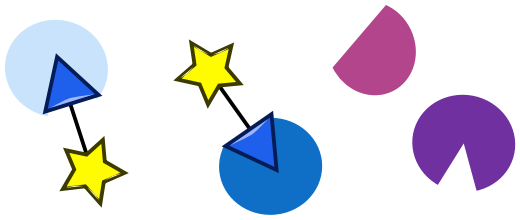

Analysis of **drug toxicity** concerns reveals that toxicity is mostly correlated to dosage, not mechanism of binding or structural alerts

Pfizer

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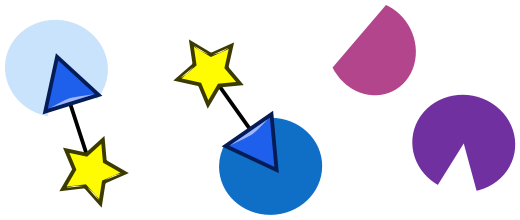

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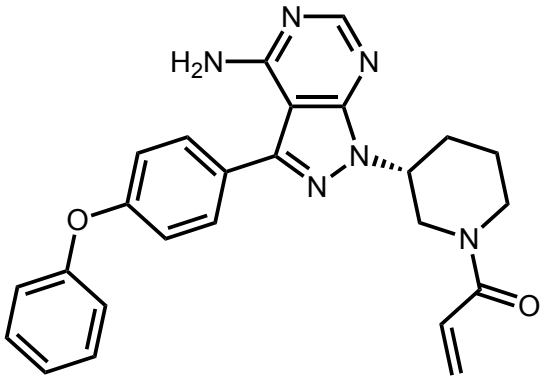
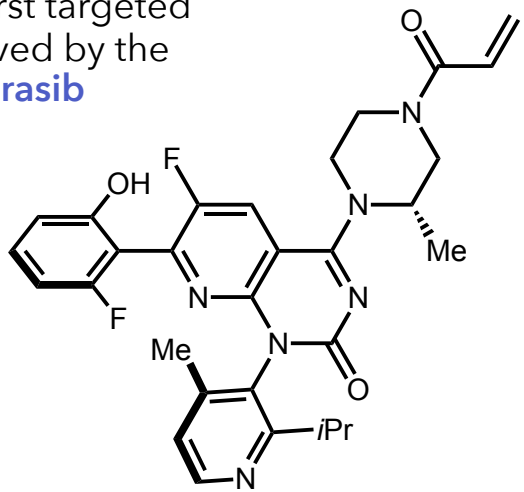
Ibrutinib represented a "behind-the-scenes" development of a covalent inhibitor that made its way into the market



Activity-based approaches allowed for identification of a druggable site on K-Ras, leading to the first targeted therapy approved by the FDA, **sotorasib**



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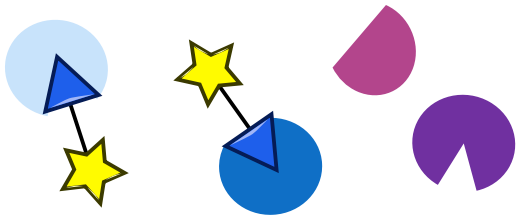

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Chemical strategies to drug "undruggable" proteins

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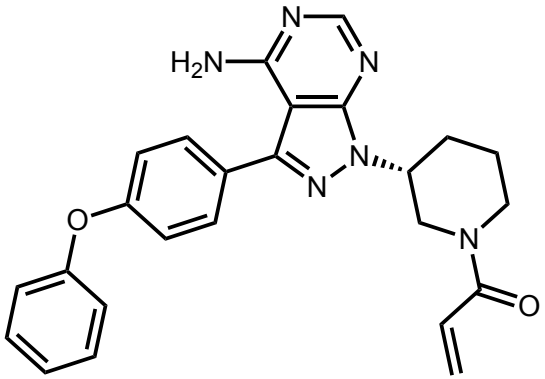

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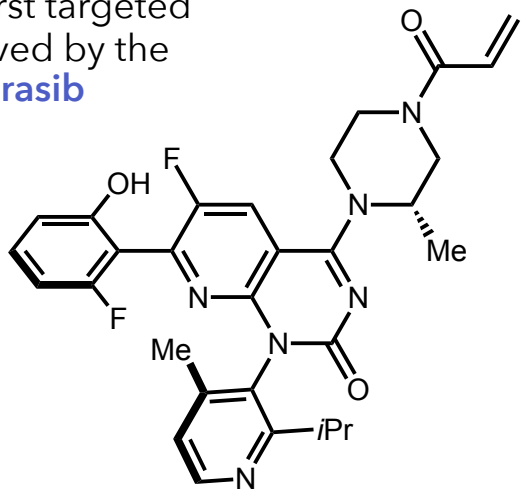

Thank you!