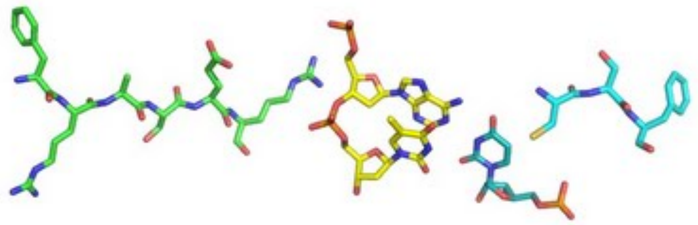
Why is it so hard to design new small molecule drugs?

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

Who am I?

- James (or Jaime, but not Jamie or Jim) Fraser - he/him pronouns
  - Background in Protein Biophysics and Evolutionary Biology
  - Ph.D. in Molecular and Cell Biology from UC Berkeley
  - I've run a lab at UCSF since 2011



- If you have additional questions:
  - email: **jfraser@fraserlab.com**
  - twitter: **@fraser_lab**
  - office hours by appointment: **Mission Bay GH S472E**

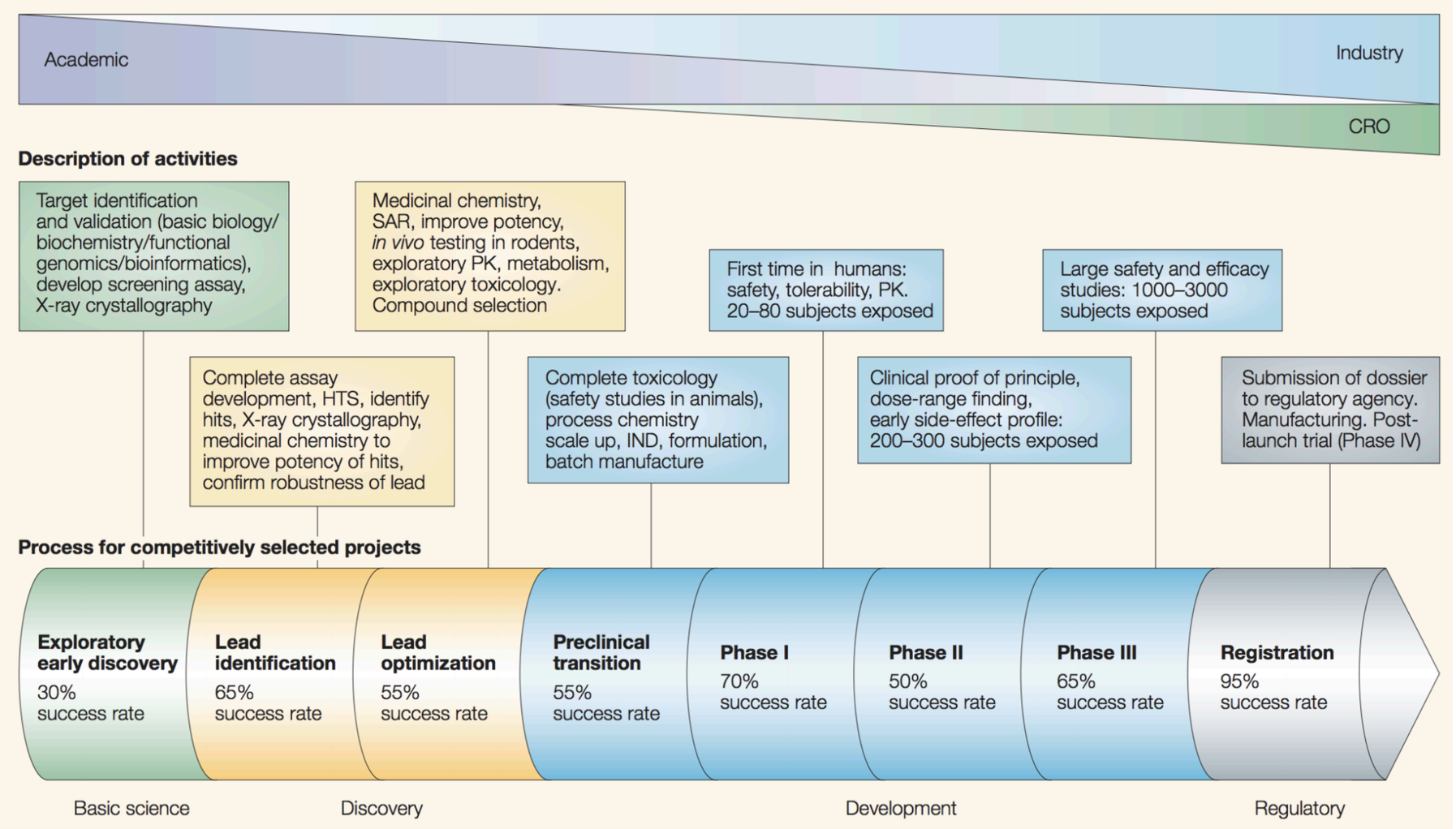
Who are you?

Class information - https://fraserlab.com/inquiry/

- Today Jan 6: Kinase and Phosphatase Drug Discovery
- Tuesday Jan 7: Crystallography 101, Practical in Crystallography Lab (Liam McKay)
- Wednesday Jan 8: ChimeraX and VR demos (Tom Goddard)
- Thursday Jan 9: Docking, what works and what doesn't (John Irwin)
- Tuesday Jan 14: Computational Protein Design/Rosetta and Biologics (Tanja Kortemme)
- Wednesday Jan 15: New topics, CryoEM lab tour (David Bulkley)

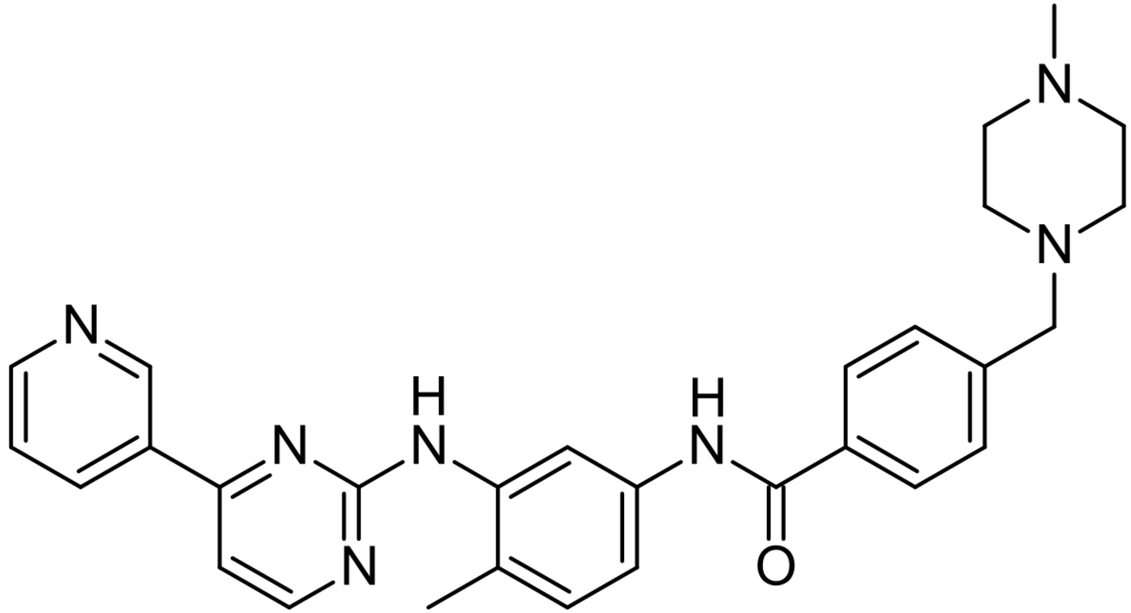
Forces and approximate affinities 101 - whiteboard

Why is it so hard to design new small molecule drugs?

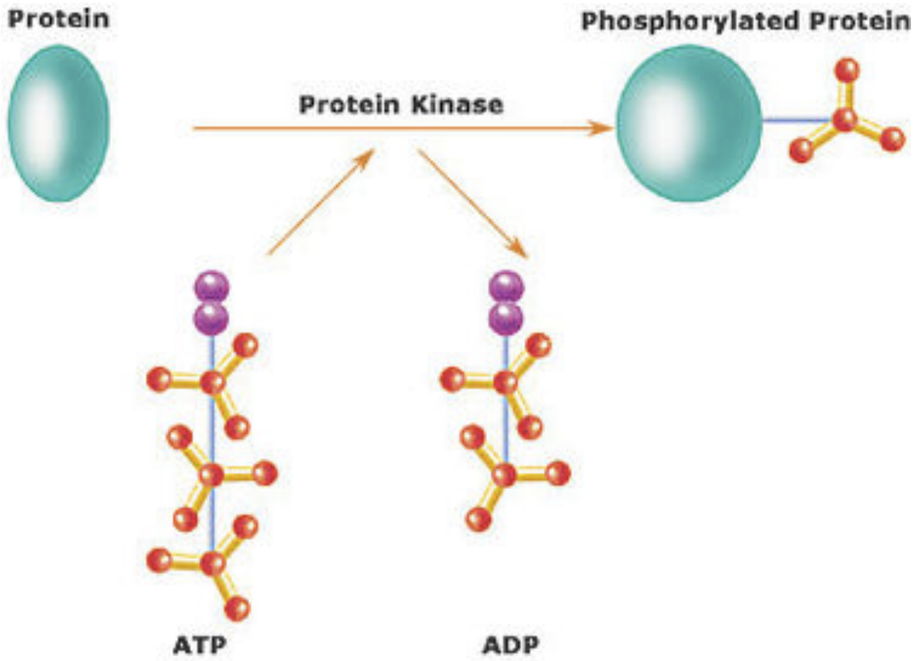


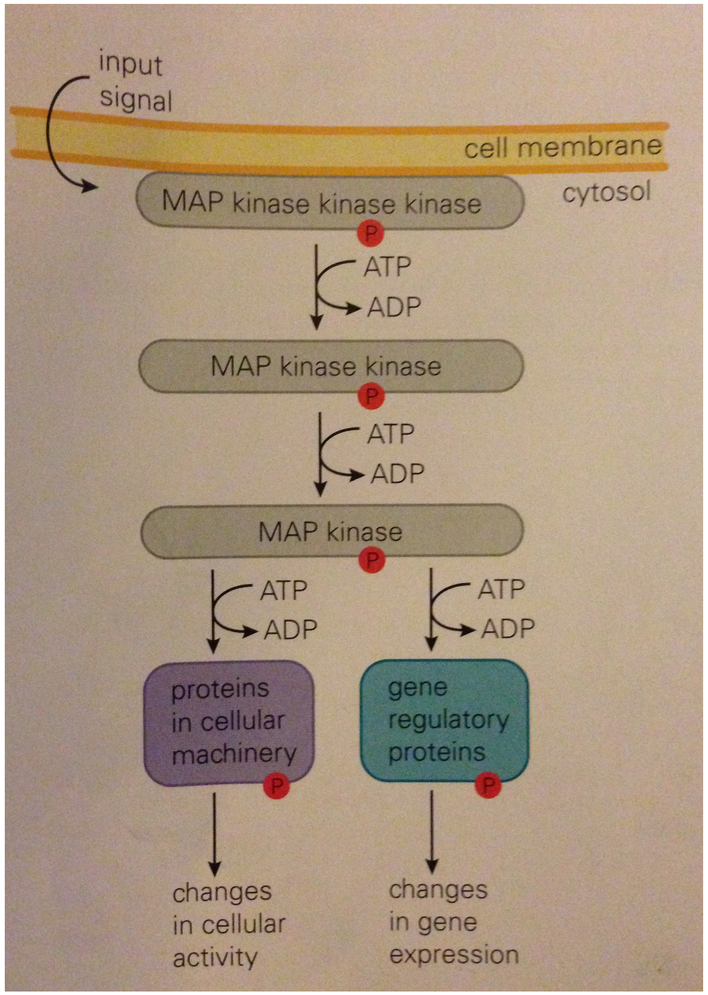
Nwaka S and Ridley RG *Nature Reviews Drug Discovery* 2, 919-928 (2003)

Kinases have become one of the major drug target classes over the past 20 years



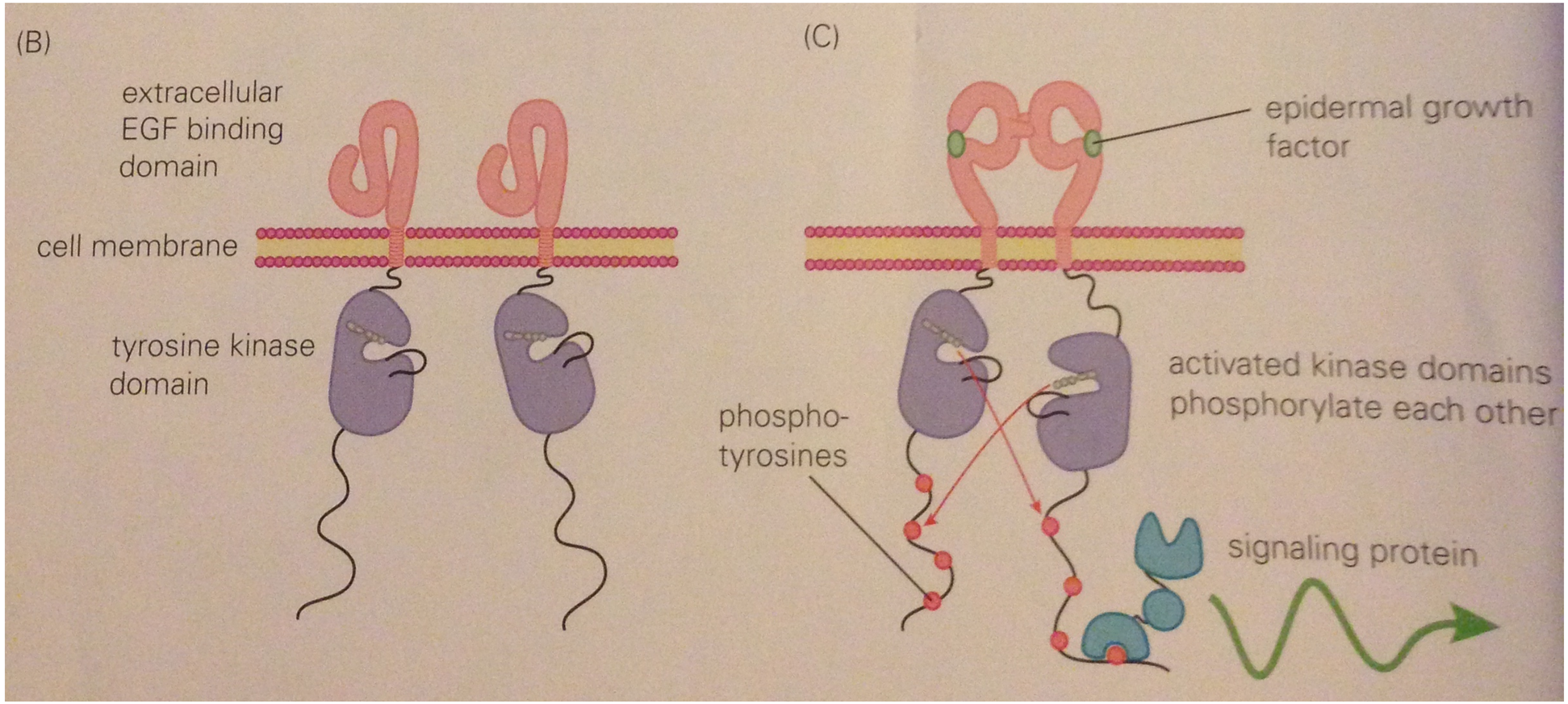
Kinases are enzymes that control cellular information flow



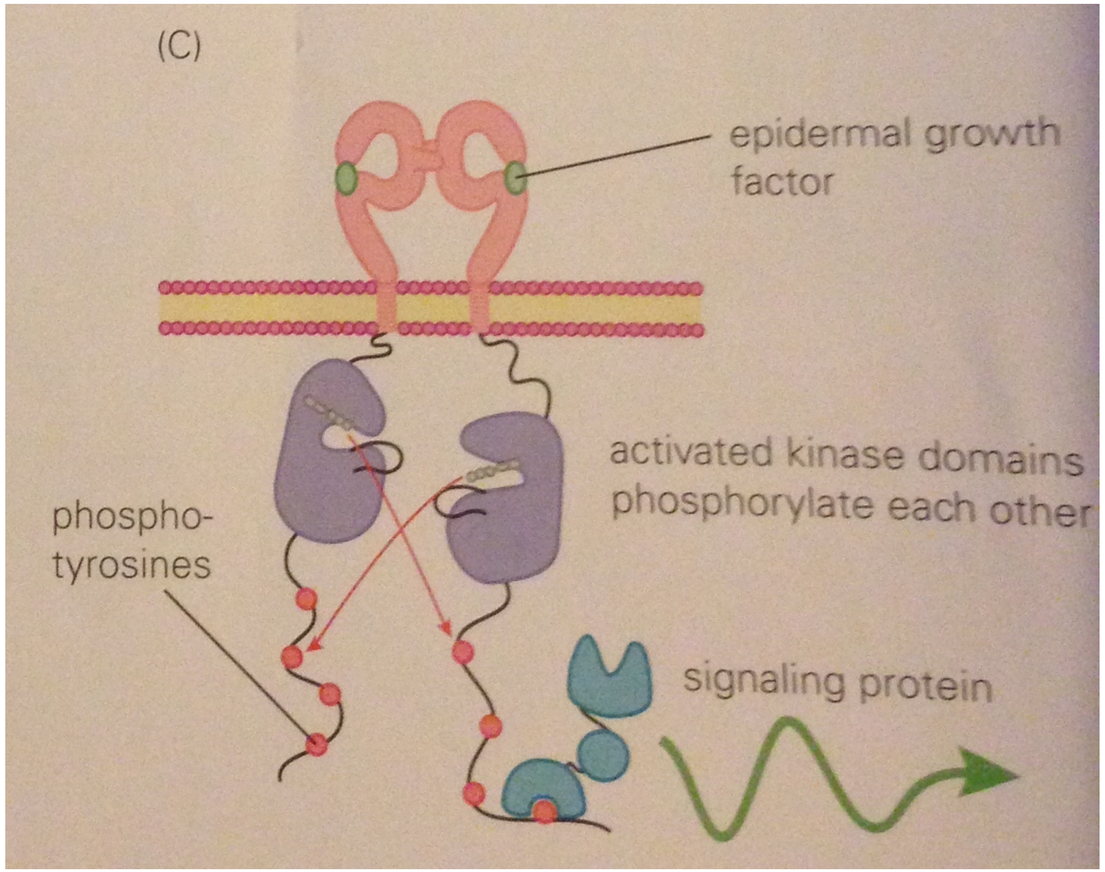


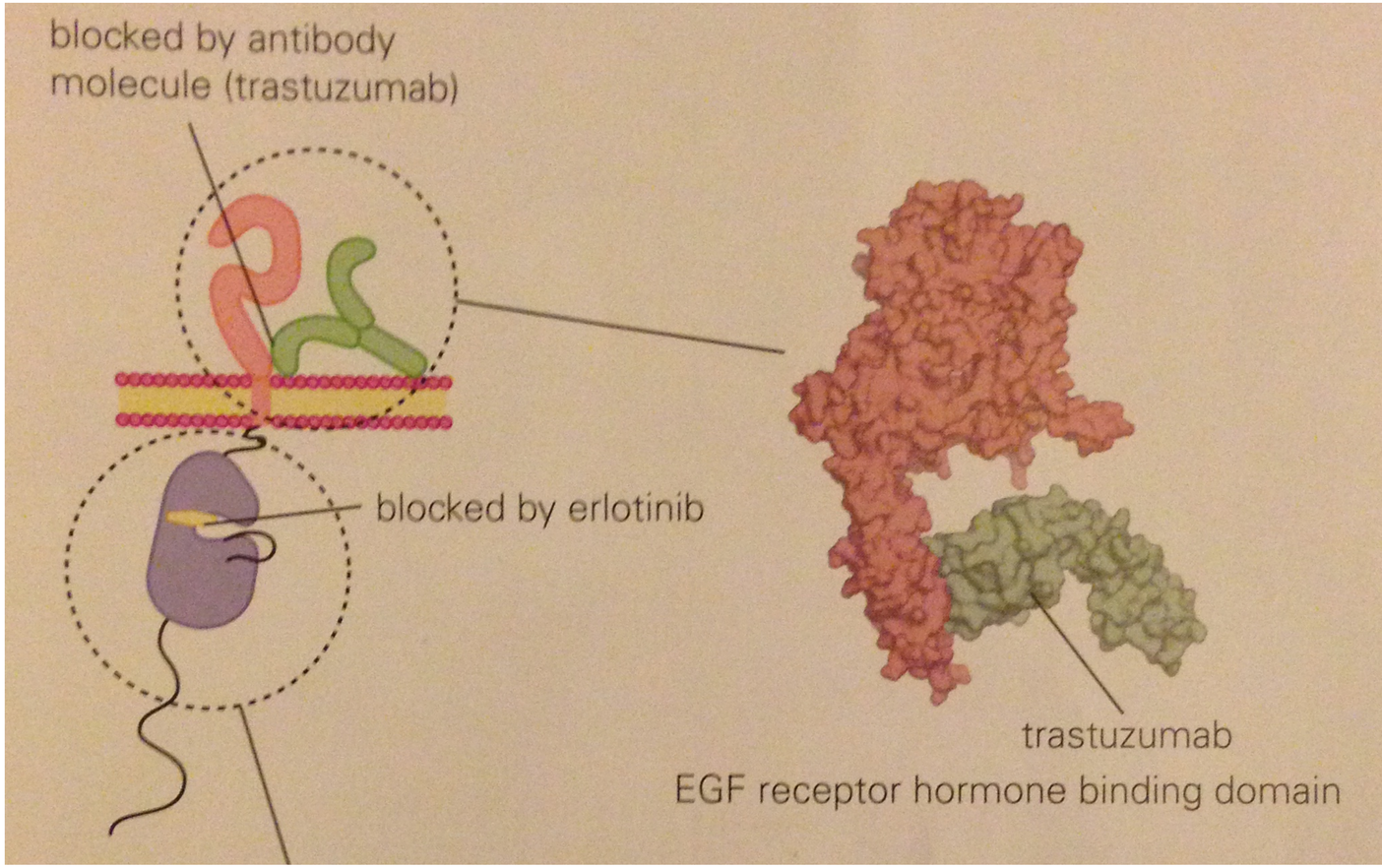
Control many **growth**/cell cycle signals
Antagonized by **phosphatases**
(to which there are no inhibitors in the clinic)

Receptor Kinases transmit signals from outside the cell, often through ligand-induced dimerization



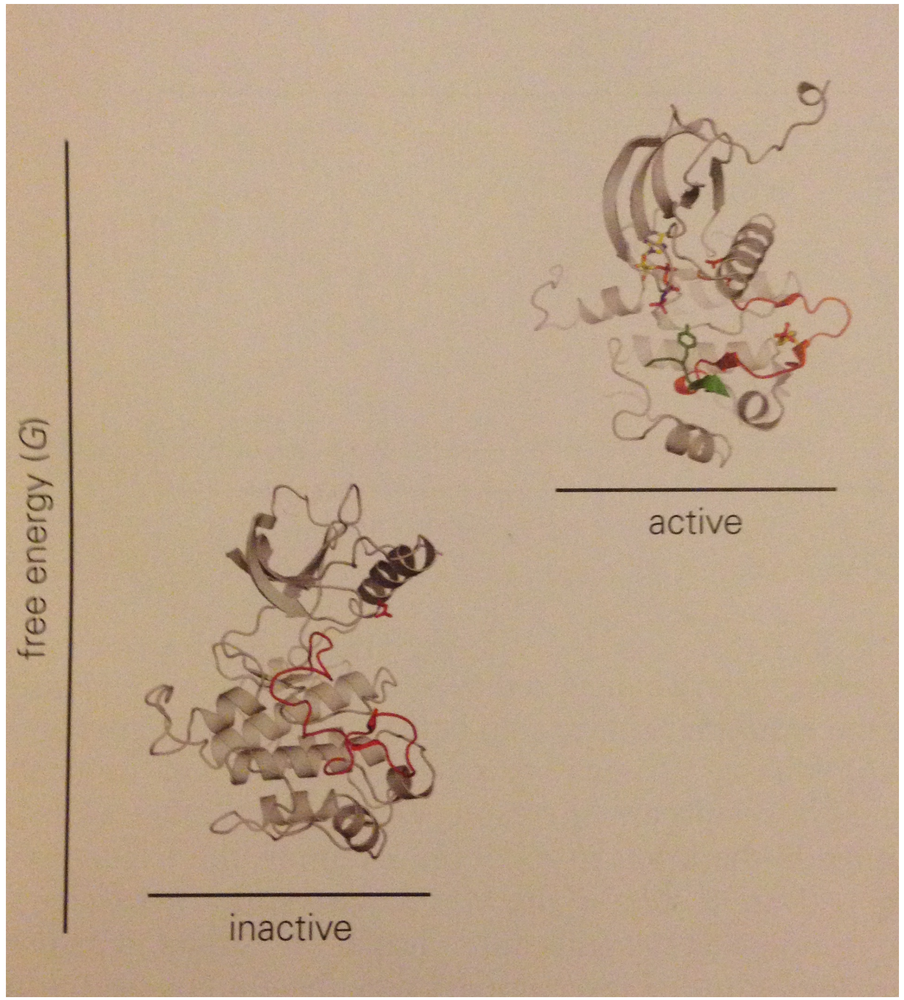
Therapeutic antibodies block extracellular dimerization, often using a distinct set of interactions

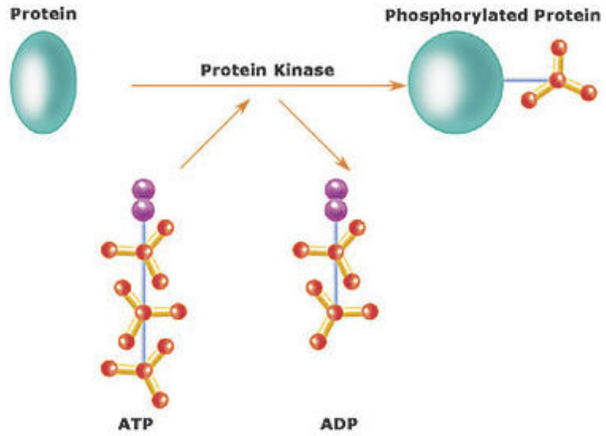




More on antibodies from Prof. Kortemme (next Tuesday)

Kinases switch between active and inactive conformations

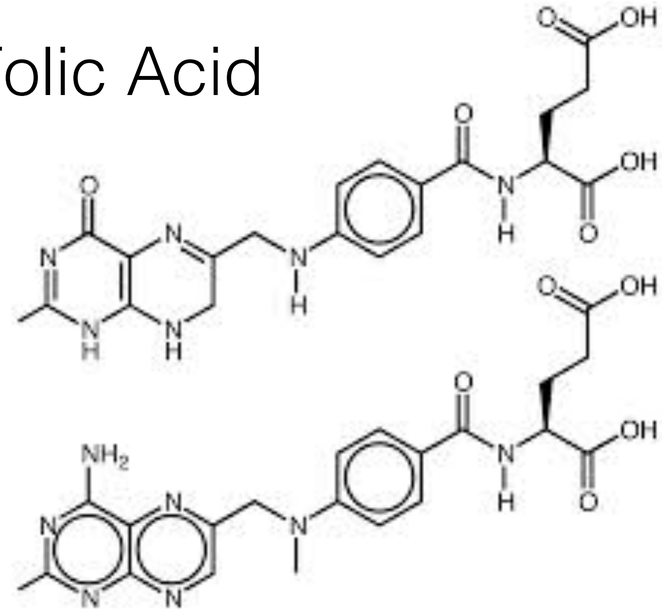




Hyperactive kinases are a common cause of cancer

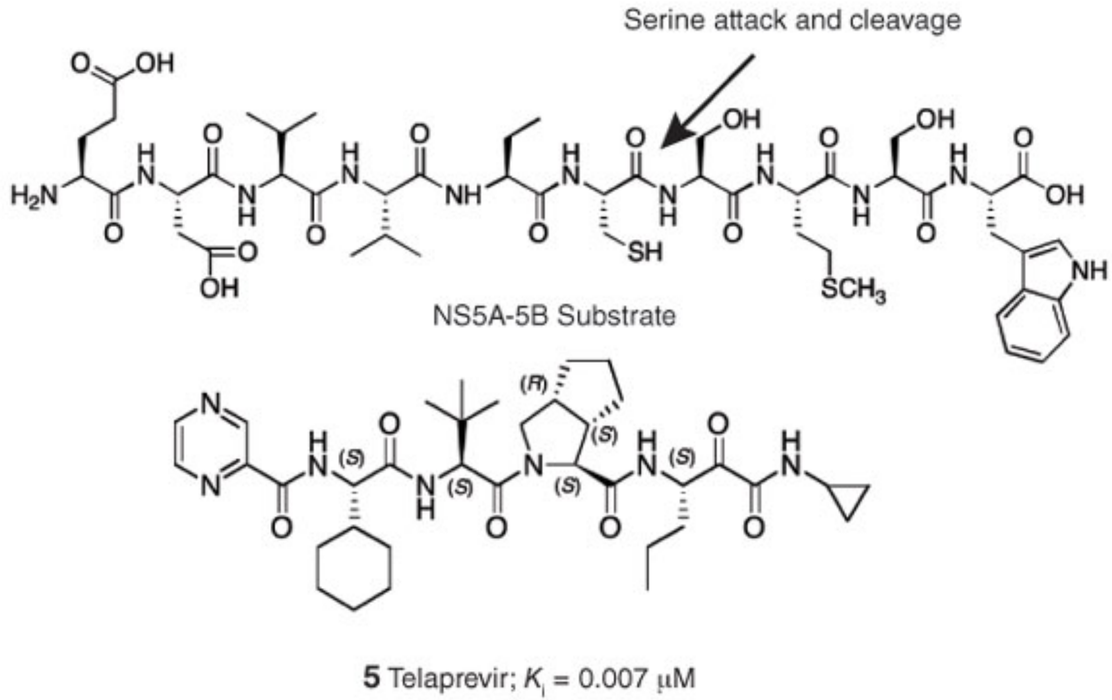
Drugs targeting enzymes
(like kinase intracellular domain)
tend to look like natural substrates (like ATP)

Folic Acid



Methotrexate

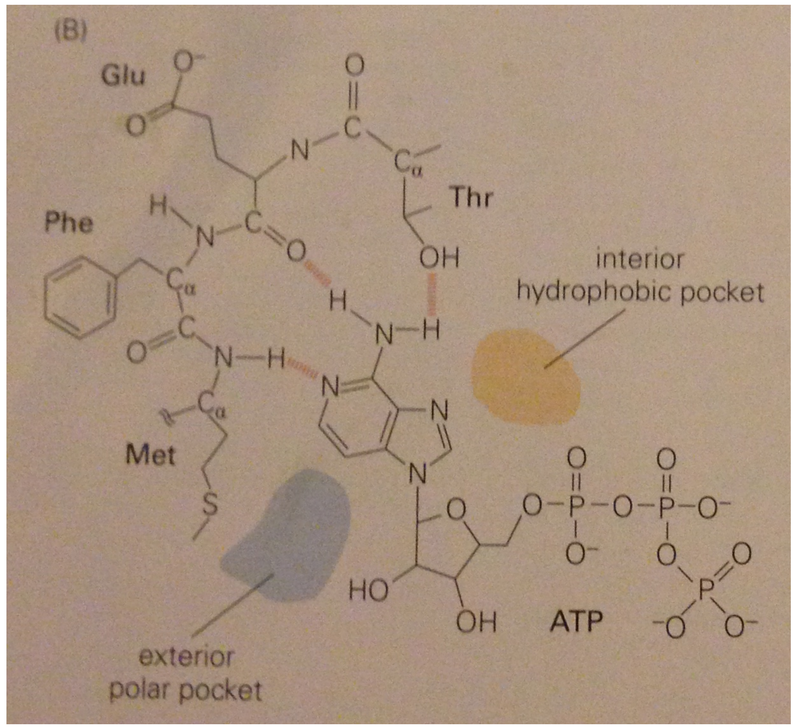
Target: DHFR

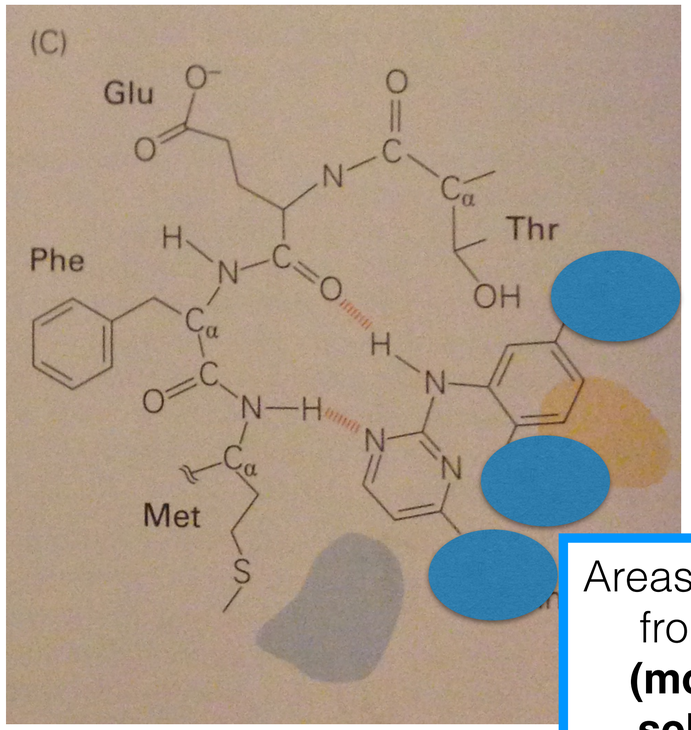


5 Telaprevir; K_i = 0.007 μM

Target: HCV Protease

Kinase inhibitors mimic ATP and compete for the same binding site





Areas that can be optimized from common scaffold **(more on scaffold and selectivity tomorrow)**

Large medicinal chemistry efforts to “tune” selectivity for an individual kinases’ ATP binding site

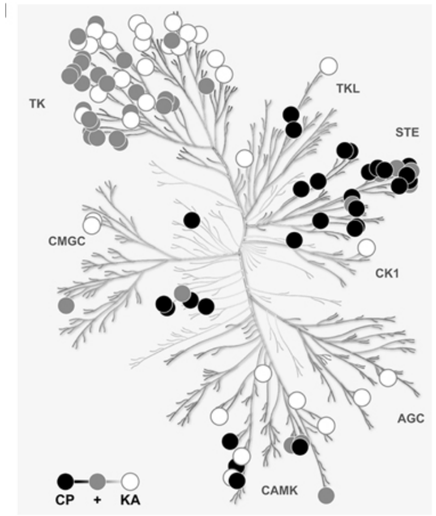
Keep in mind - nucleosides (base and ribose) are relatively hydrophobic

The kinase active site is highly conserved and optimized for ATP binding

Description	CHK1	CDK2	SRC	ABL	EGFR	RAF	MEK
Ribose/hydrophobic pocket	L15 G16	I10 G11	L273 G274	L248 G249	L718 G719	I463 G464	L74 G75
"Roof" of adenine pocket	V23	V18	V281	V256	V726	V471	V82
Glu-Lys ion pair	K38 E55	K33 E51	K295 E310	K271 E286	K745 E762	K483 E501	K97 E114
Gatekeeper residue	L84	F80	T338	T315	T790	T529	M143

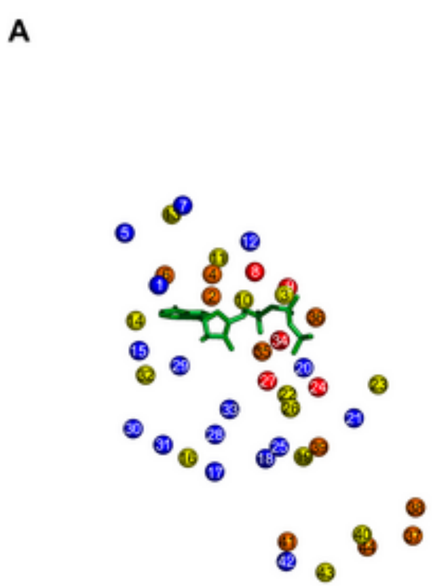
Only the "gatekeeper" residue is variable

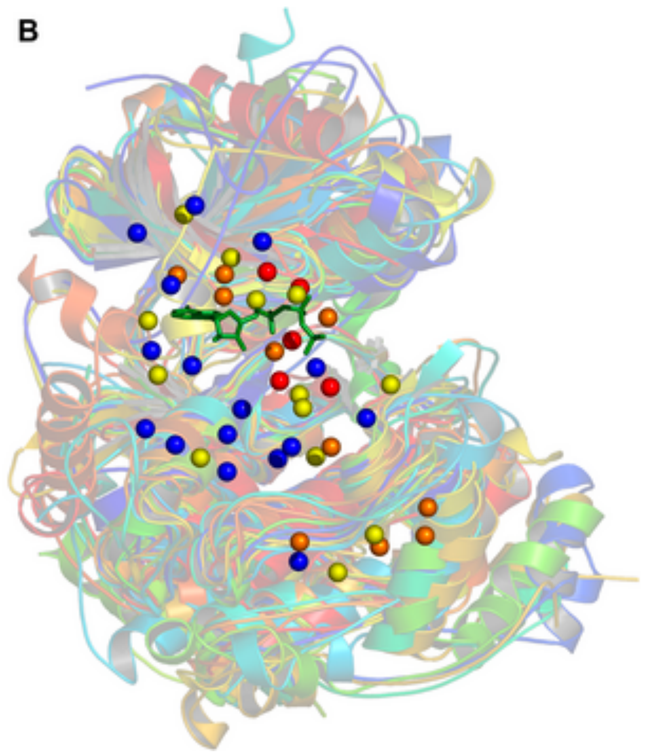
Catalytic aspartate	D130	D127	D386	D363	D837	D576	D190
Phosphate binding region	N135	N132	N391	N368	N842	N581	N195
"Floor" of adenine pocket	L137	L134	L393	L370	L844	F583	L197

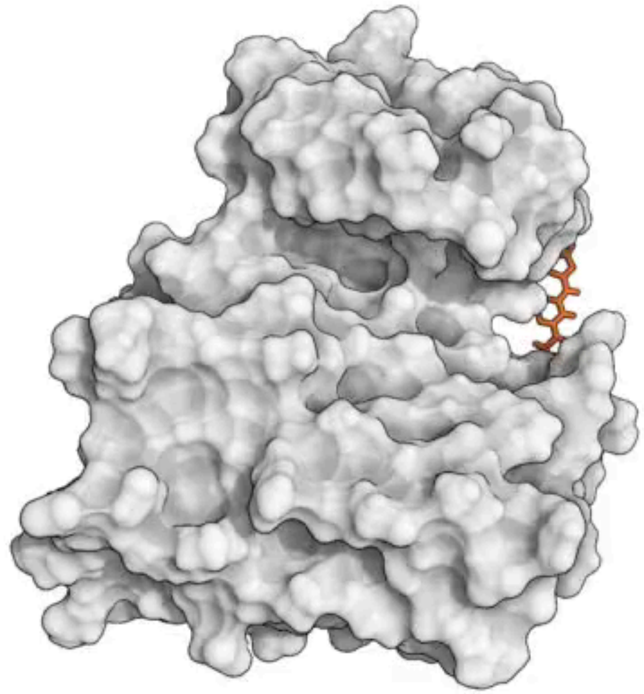


…because of this kinases were considered "undruggable"

Fortunately two things help : 1) conservation is reduced away from the binding site, 2) kinases are structurally plastic







Conservation, Variability and the Modeling of Active Protein Kinases

James D. R. Knight, Bin Qian, David Baker, Rashmi Kothary

Published: October 3, 2007 • https://doi.org/10.1371/journal.pone.0000982

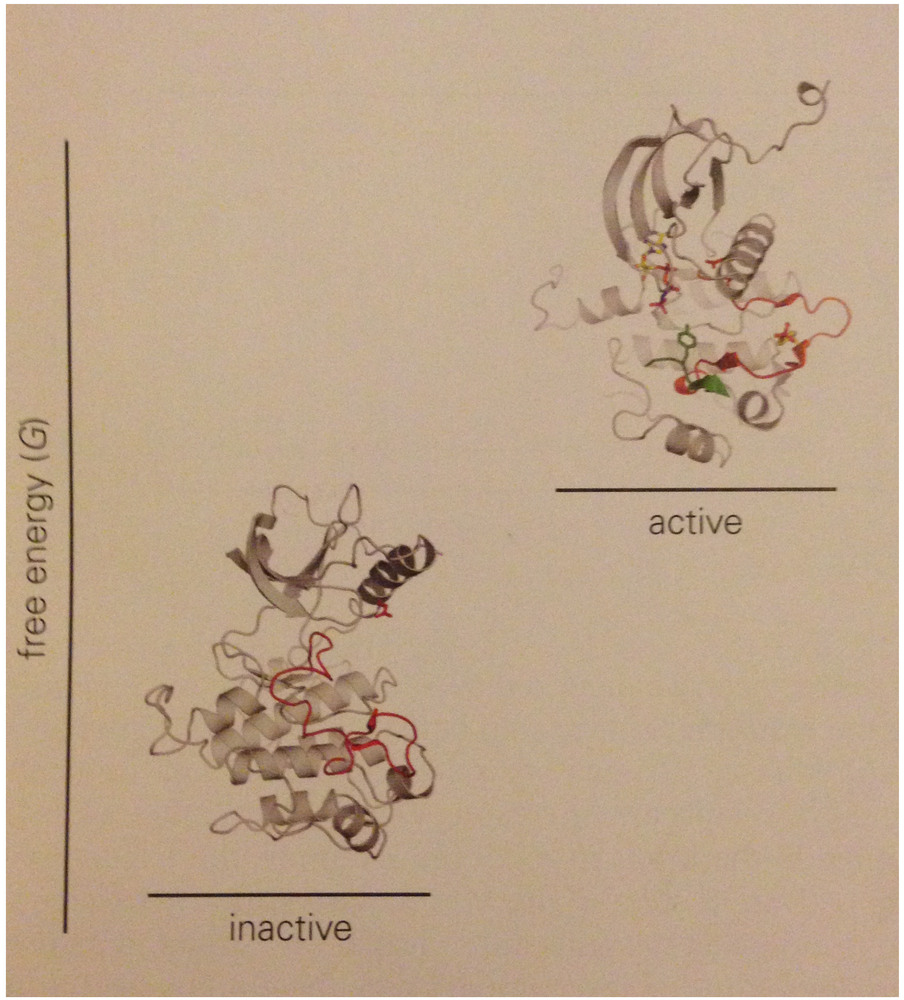
How Does a Drug Molecule Find Its Target Binding Site?

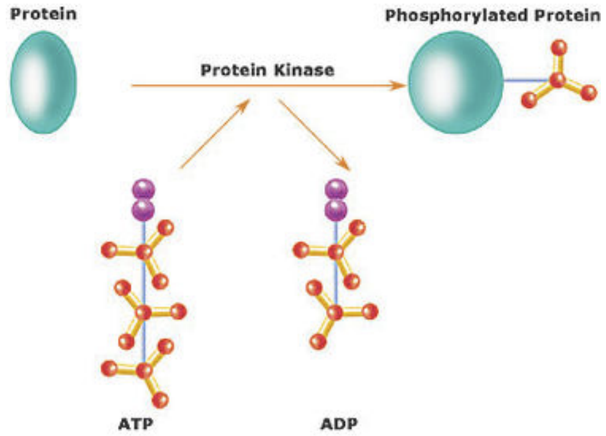
Yibing Shan[†], Eric T. Kim[†], Michael P. Eastwood[†], Ron O. Dror[†], Markus A. Seeliger[§] and David E. Shaw[*†‡]

View Author Information

Cite this: *J. Am. Chem. Soc.* 2011, 133, 24, 9181-9183

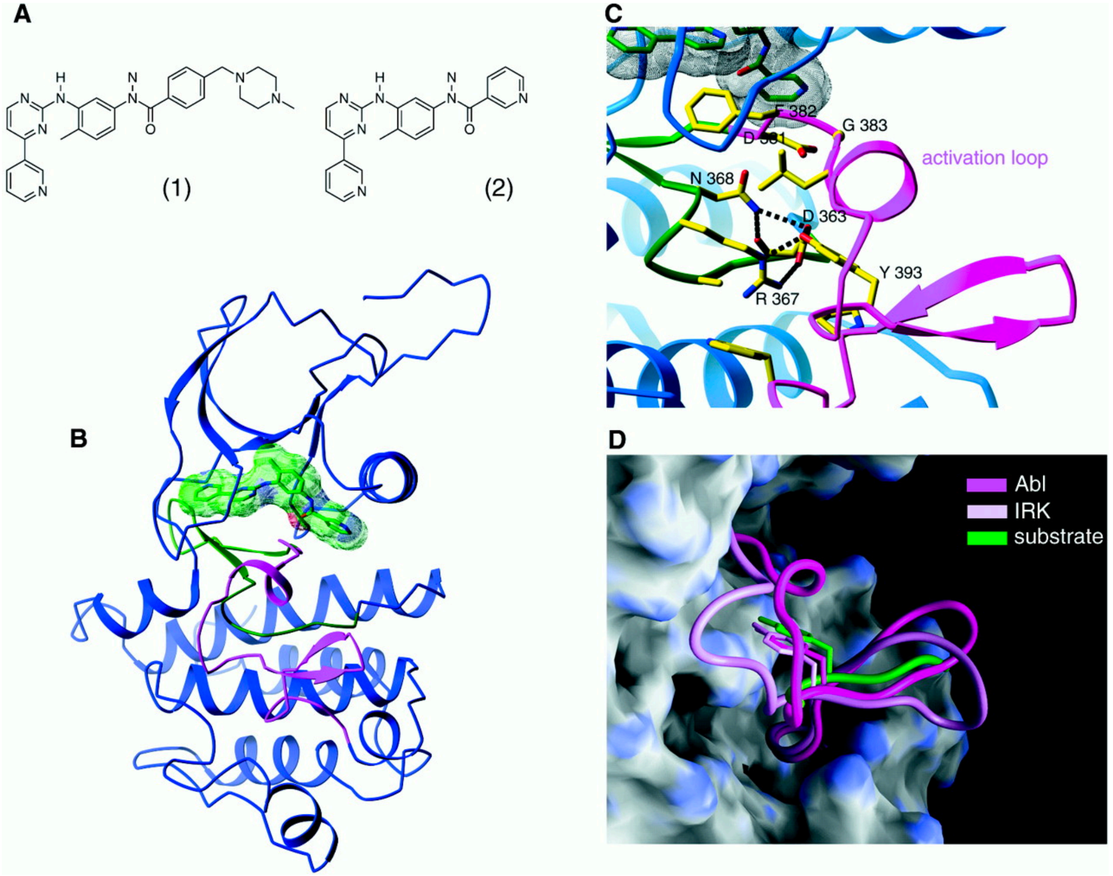
Kinases switch between active and inactive conformations

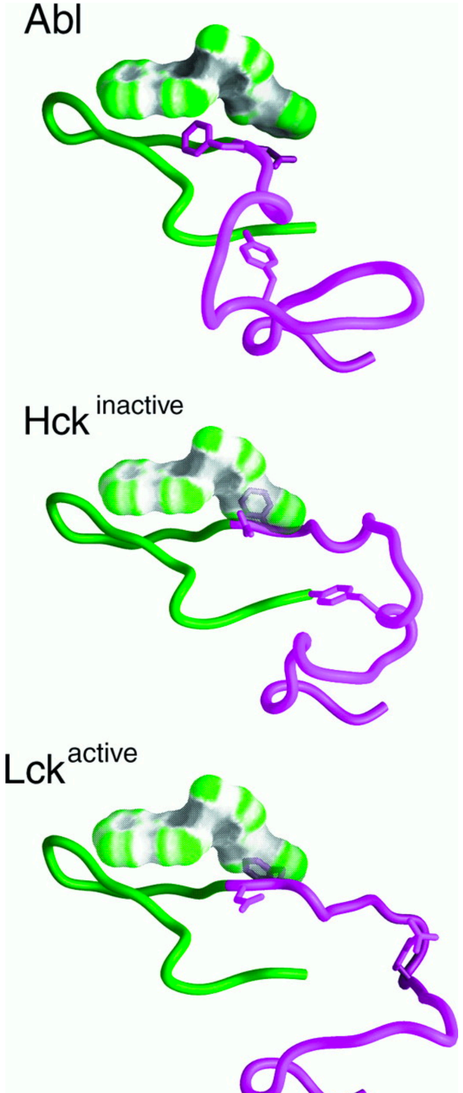




Hyperactive kinases are a common cause of cancer

Binding of Gleevec to Abl exploits the active-inactive equilibrium



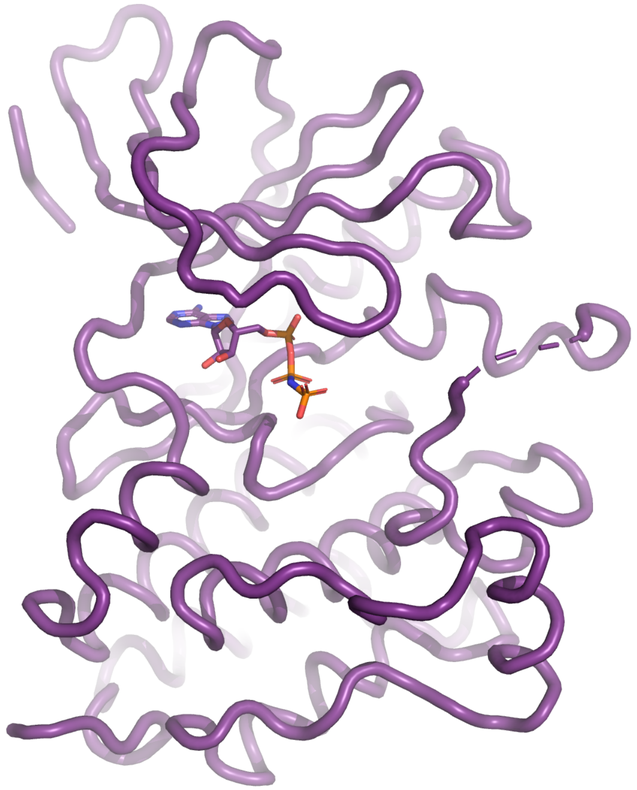


Structural Mechanism for STI-571 Inhibition of Abelson Tyrosine Kinase

Thomas Schindler[1], William Bornmann[3], Patricia Pellicena[4], W. Todd Miller[4], Bayard Clarkson[3], John Kuriyan[1,2,*]
+ See all authors and affiliations

Science 15 Sep 2000:
Vol. 289, Issue 5486, pp. 1938-1942
DOI: 10.1126/science.289.5486.1938

While kinase inhibitors maintain overlap with the adenine ring of ATP, the search for specificity goes elsewhere



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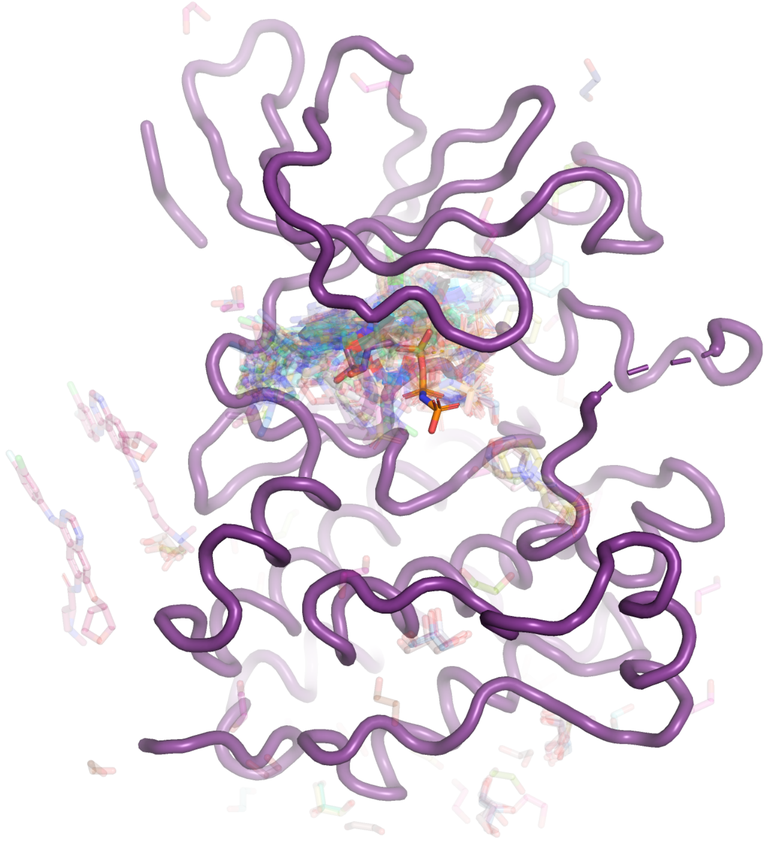
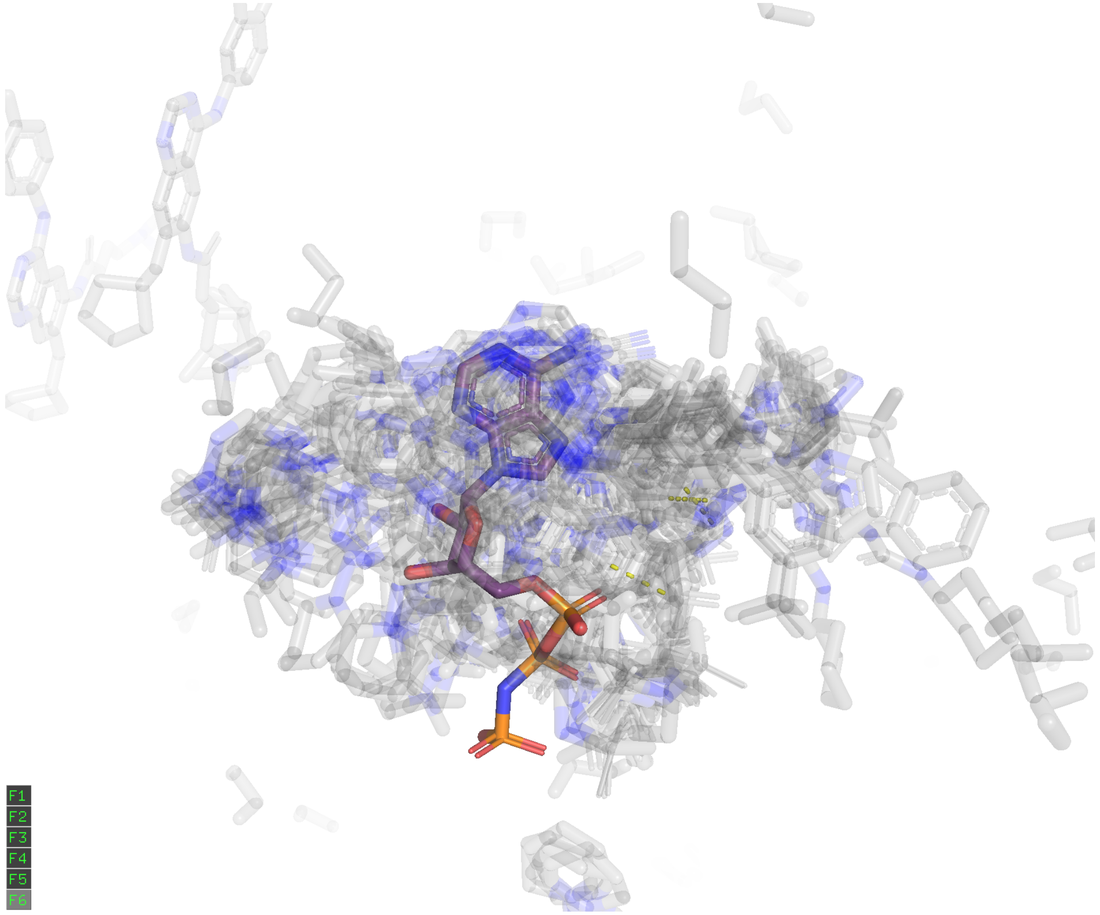
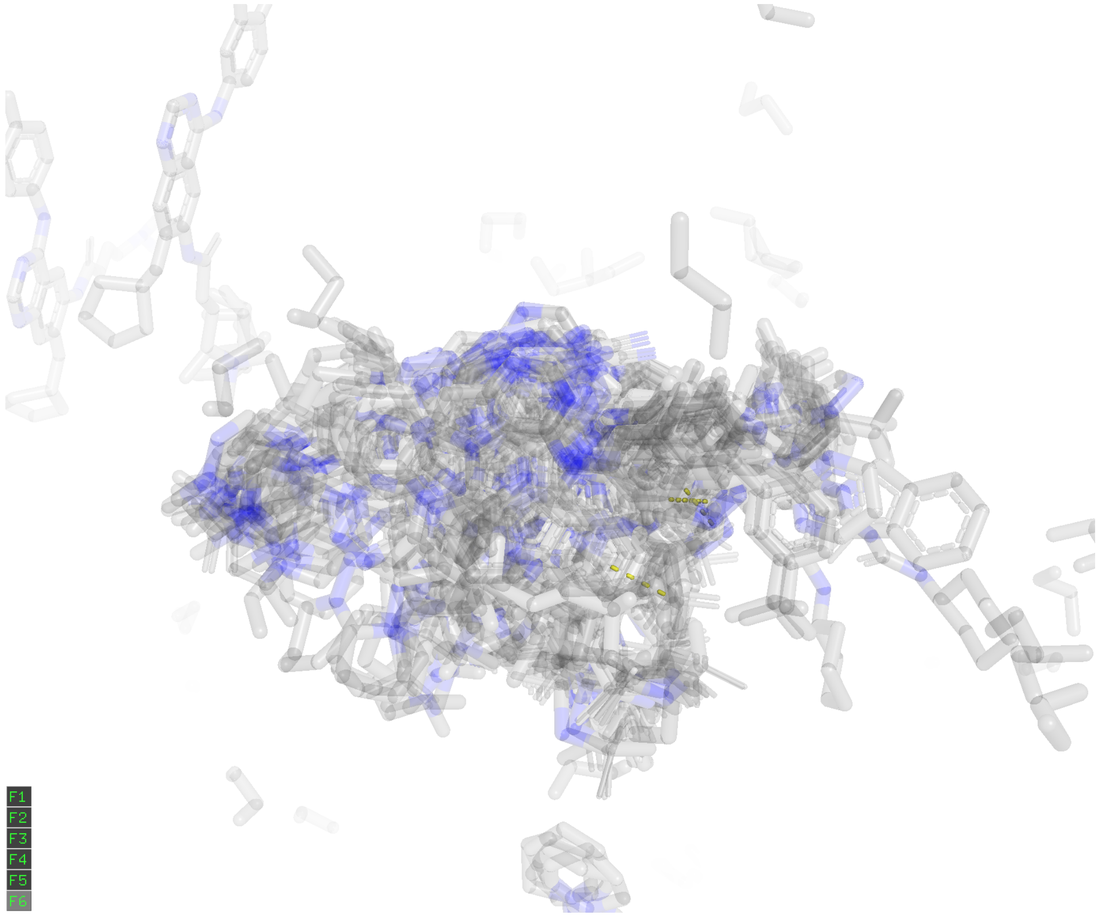


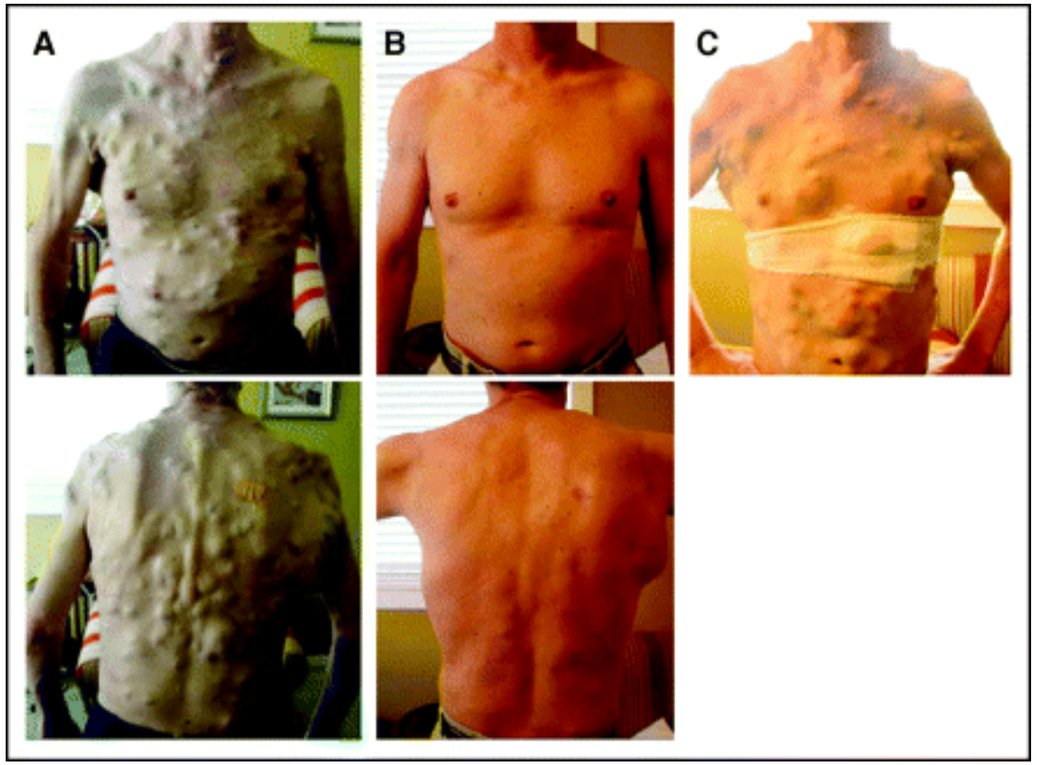
All EGFR ligands

Key “hinge” hydrogen bonds are a major design element in kinase inhibitors, but other areas provide specificity



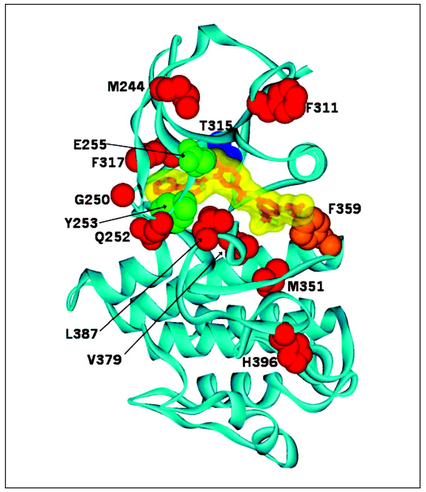


Clinical introduction of potent kinase inhibitors is closely followed by resistance

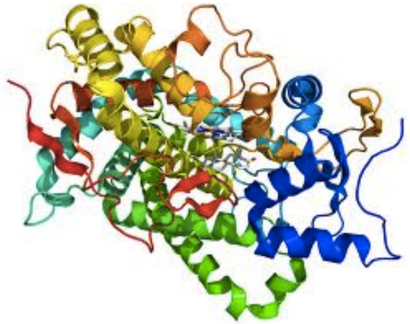


A 38-year-old man with BRAF-mutant melanoma and subcutaneous metastatic deposits. Photographs were taken (A) before initiation of PLX4032, (B) after 15 weeks of therapy with PLX4032, and (C) after relapse, after 23 weeks of therapy.

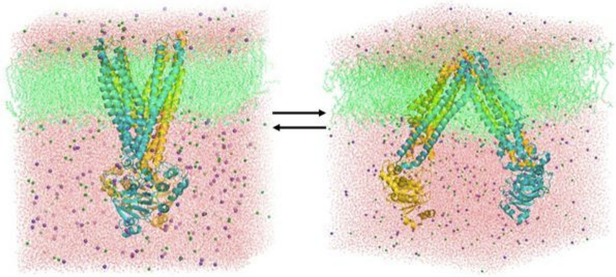
The common resistance mechanisms for small molecules



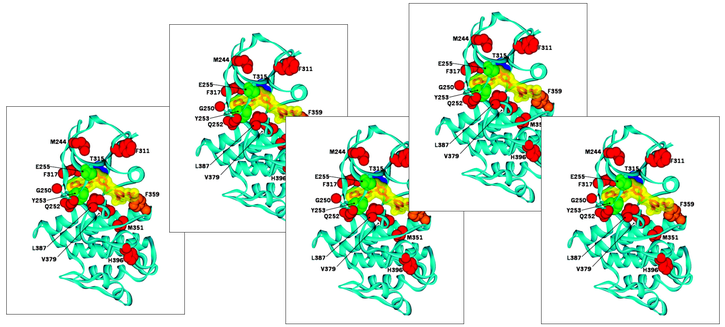
active site mutations



degradation of inhibitor

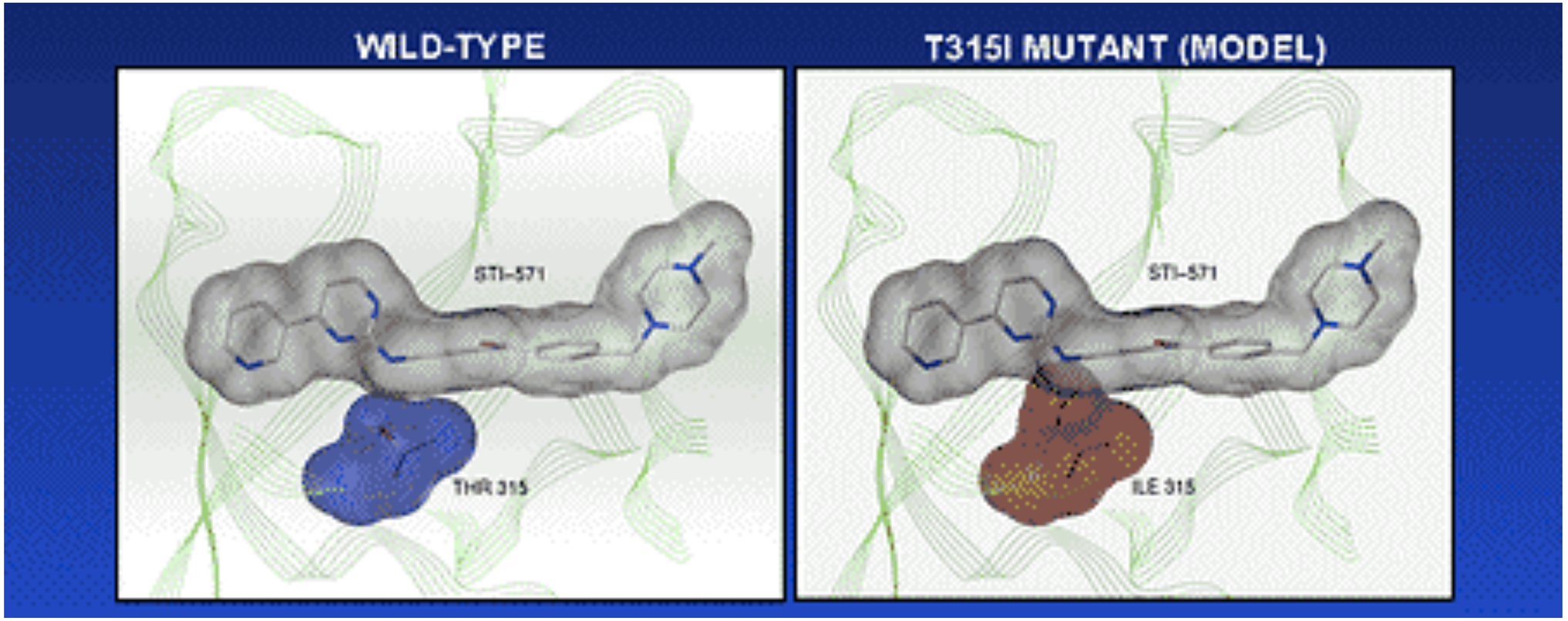


efflux



over-expression+other signaling

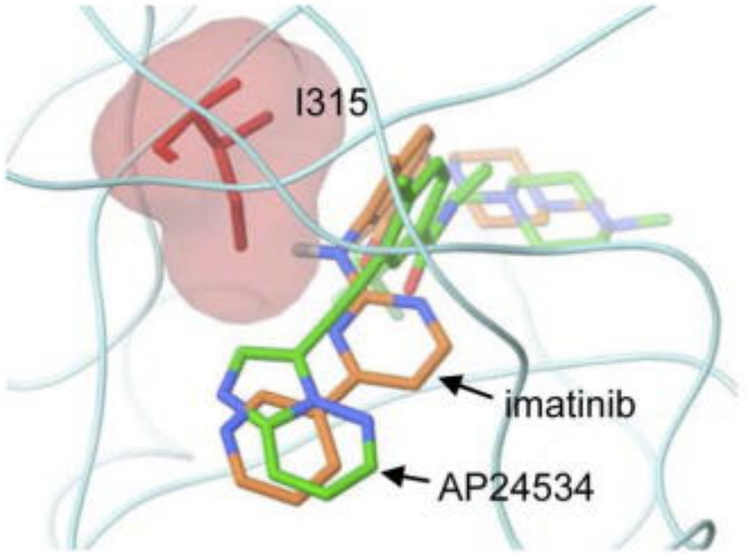
Active site mutations directly alter interactions with drugs



Mutation at variable “gatekeeper” residue

Protein modeling and structural biology play a large role in combating resistance

- X-ray crystallography of mutant proteins
- Trimming the molecule to avoid clashes caused by Small-to-Large mutations
- Conformational changes are difficult to predict (molecular dynamics simulations can help)



O'Hare…Clackson
Cancer Cell, 2009

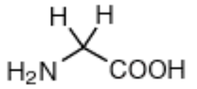
VOLUME 29 · NUMBER 22 · AUGUST 1 2011
JOURNAL OF CLINICAL ONCOLOGY

Table 2. Exemplary Mechanisms of Acquired Resistance to Kinase Inhibitors

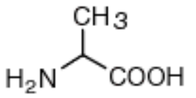
Targeted Agent	Target Gene	Acquired Resistance via Secondary Mutation, Amplification, or Activation of Target	Acquired Resistance via Bypass	Acquired Resistance via Downstream Mutation
Imatinib				
	ABL	T315I	*IGF1R* amplification	
		Y253F/H	AXL overexpression*†	
		E255K/V		
		ABL amplification		
		T670I		
		V654A		
		D816A/G/H/V		
		D820A/E/G/Y		
	KIT	Y823D		
		KIT amplification		
	PDGFRA	T674I		
Gefitinib or erlotinib	*EGFR*	T790M	*MET* amplification	
		D761Y	HGF overexpression*†	
		L747S	IGFBP3 loss*†	
		T854A		
		EGFR amplification*		
Trastuzumab	*HER2*			
Lapatinib	*HER2/EGFR*			
PKC412	*FLT3*	N676K		
	FGFR			
AZD6044	*MEK1*	MEK1 P124L		
		BRAF amplification*		
PLX4032	*BRAF*	NRAS Q61K	COT overexpression†	MEK1 C121S
			PDGFRβ overexpression†	
			CRAF overexpression*†	
			AXL overexpression*†	
			HER2 overexpression*†	
Crizotinib	*ALK/MET*	L1196M		
		C1156Y		
		F1174L		

Abbreviations: IGF1R, insulin-like growth factor 1 receptor; HGF, hepatocyte growth factor; IGFBP3, insulin-like growth factor receptor binding protein-3; PDGFRβ, platelet-derived growth factor β; HER2, human epidermal growth factor receptor 2.
*Mechanisms that have been described in vitro.
†Nongenetic mechanisms.

Small

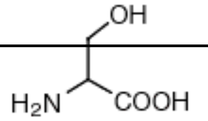


Glycine (Gly, G)
MW: 57.05

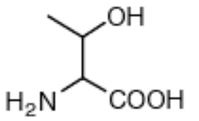


Alanine (Ala, A)
MW: 71.09

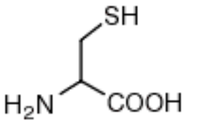
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

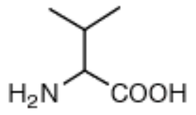


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

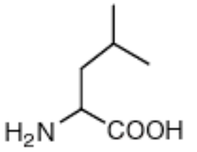


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

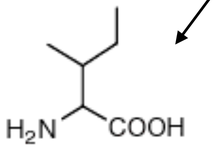
Hydrophobic



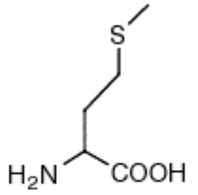
Valine (Val, V)
MW: 99.14



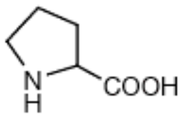
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

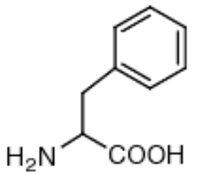


Methionine (Met, M)
MW: 131.19

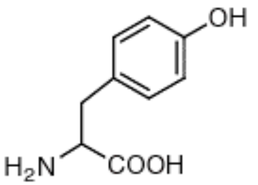


Proline (Pro, P)
MW: 97.12

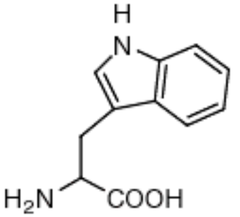
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

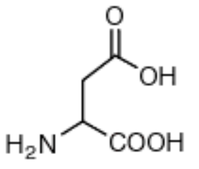


Tyrosine (Tyr, Y)
MW: 163.18

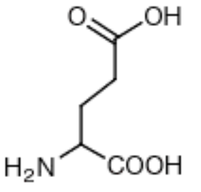


Tryptophan (Trp, W)
MW: 186.21

Acidic

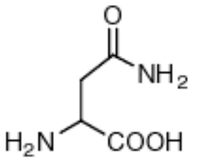


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

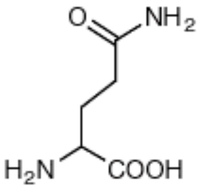


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

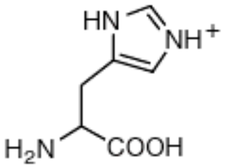


Asparagine (Asn, N)
MW: 114.11

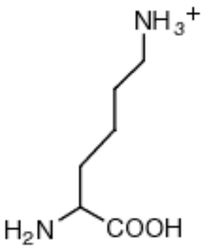


Glutamine (Gln, Q)
MW: 128.14

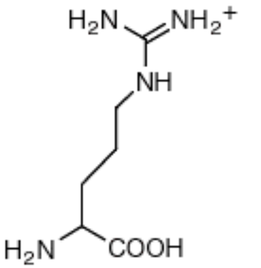
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



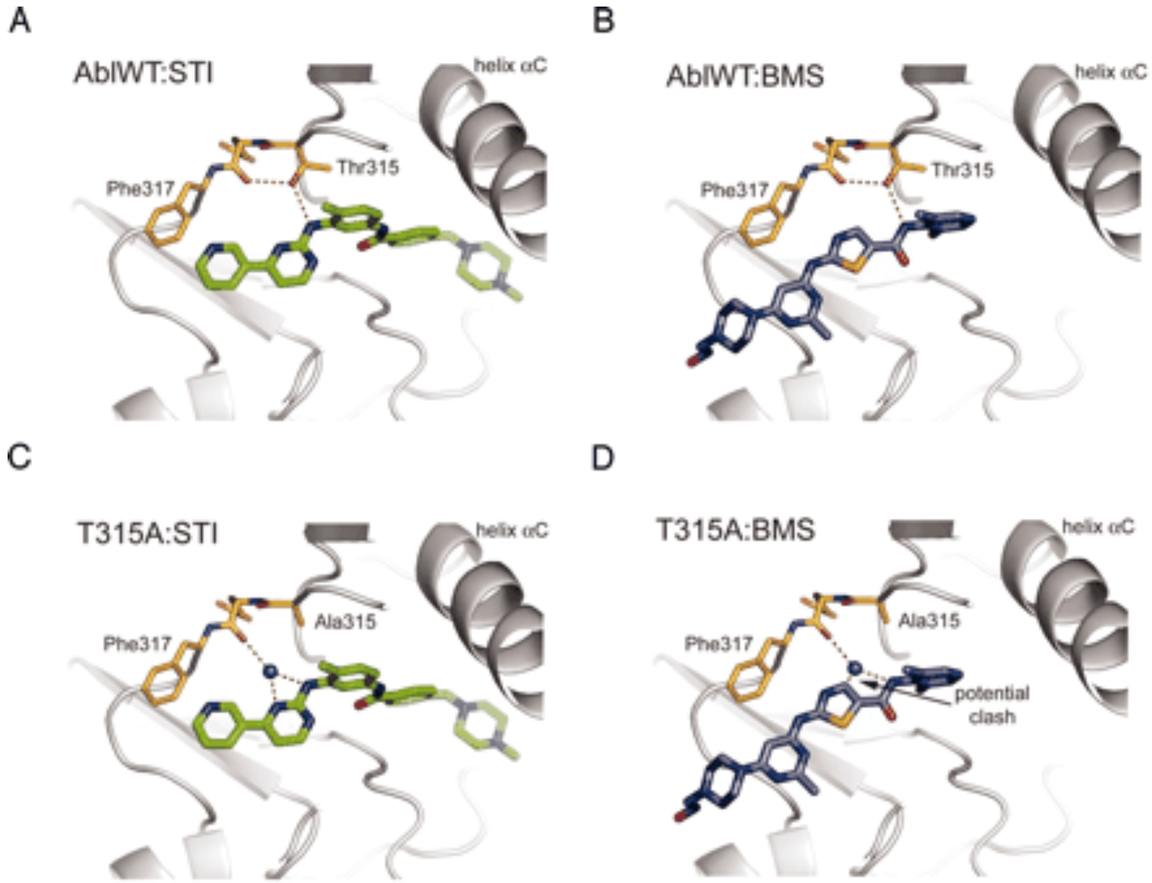
Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



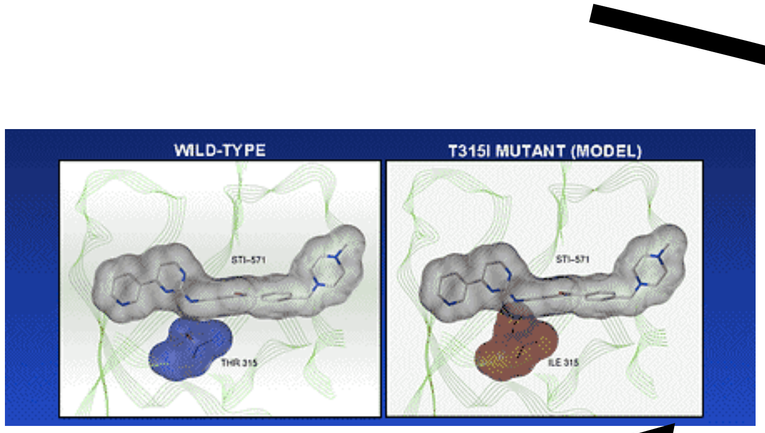
Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

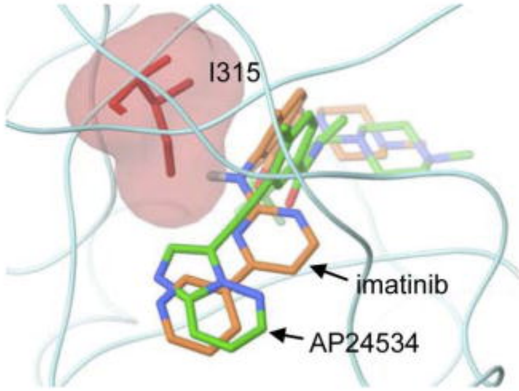
Compensatory chemical changes in drugs can target resistance mutations

- Filling the new holes created by Large-to-Small mutations
- or exploiting solvent interactions

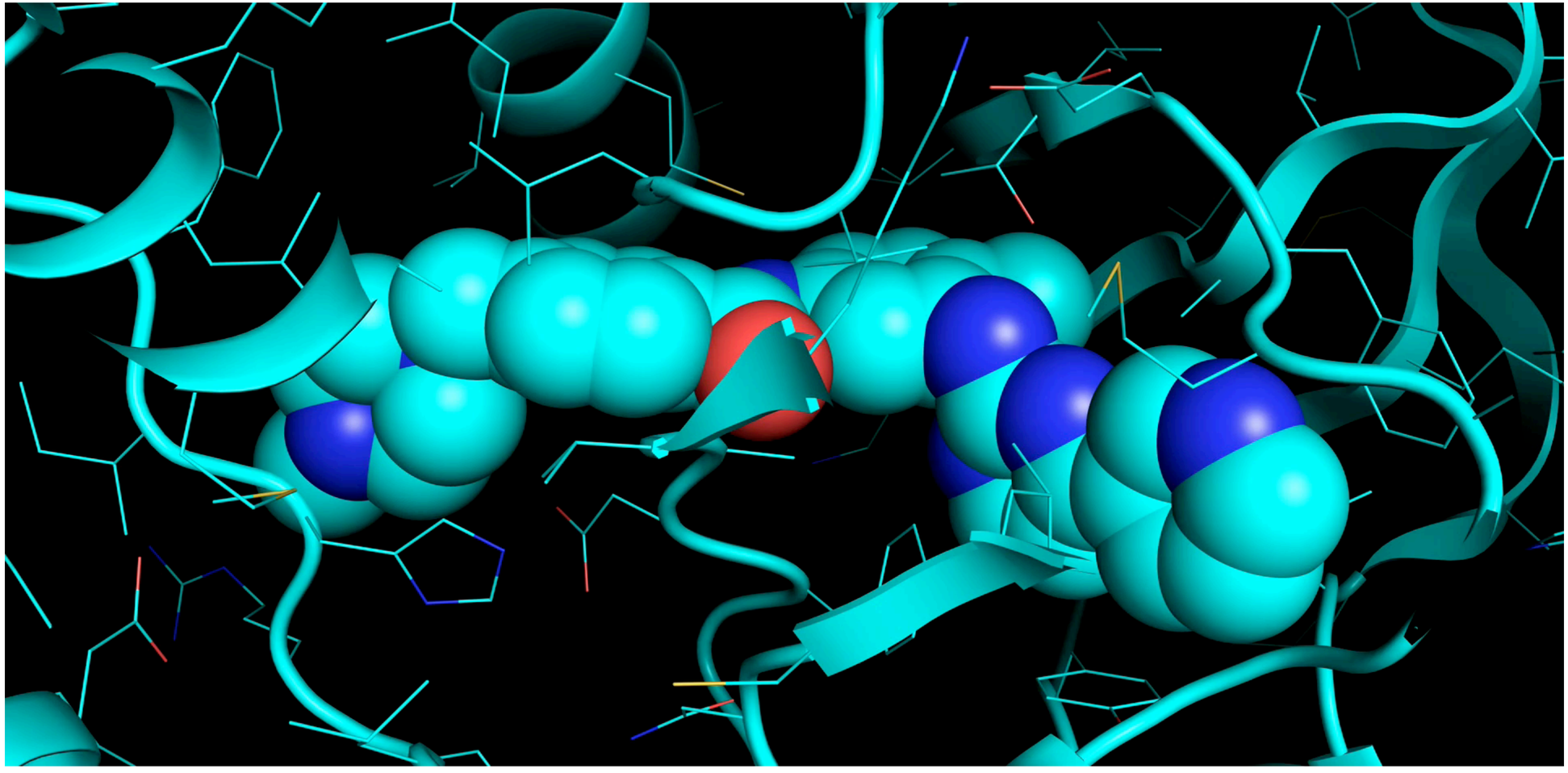


The cycle of compensatory changes - an evolutionary arms race!

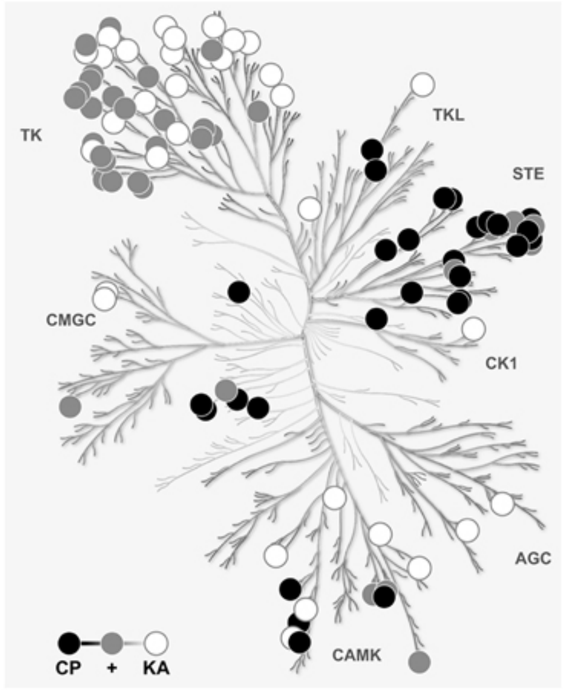




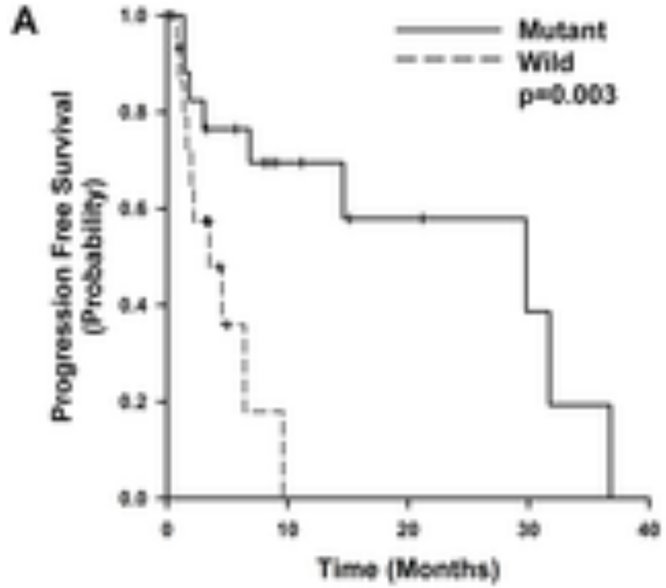
More common to have many cycles of this race for anti-virals and anti-bacterials than anti-cancer



Mutant kinase profiling and sequencing studies will enable rapid feedback between drugs



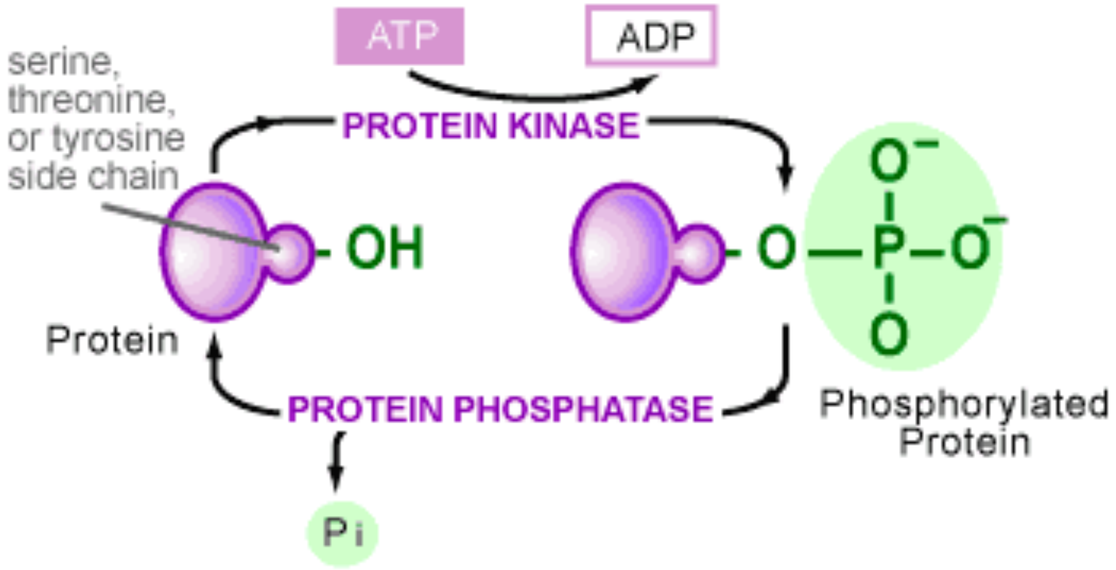
Kinase profiling to expand from WT to include mutants



Drugs targeting emerging resistance will be more effective

Keep in mind - nucleosides (base and ribose) are relatively hydrophobic

>200 small molecules tested in humans
>30 approved inhibitors



…but none against phosphatases
(a phosphopeptide is very charged!)

The disease biology of **phosphatases** is, perhaps, no less compelling than kinases

Table 3. Disease-related protein phosphatases. Each row shows a human protein phosphatase gene, its classification, disease(s), and whether it is a cancer gene.

Gene symbol	Fold	Family	Subfamily	Disease(s)	Cancer gene
CDKN3	CC1	DSP	CDKN3	Hepatocellular carcinoma	Yes
DUSP6	CC1	DSP	DSP6	Hypogonadotropic hypogonadism	
DUSP16	CC1	DSP	DSP8	Tumor suppressor	Yes
Laforin	CC1	DSP	Laforin	Lafora disease	
MTM1	CC1	Myotubularin	MTMR1	Cancer driver, severe X-linked myotubular myopathy	Yes
MTMR2	CC1	Myotubularin	MTMR1	Charcot-Marie-Tooth disease	
MTMR14	CC1	Myotubularin	MTMR14	Myopathy	
SBF1	CC1	Myotubularin	MTMR5	Charcot-Marie-Tooth disease	
SBF2	CC1	Myotubularin	MTMR5	Charcot-Marie-Tooth disease	
DNAJC6	CC1	PTEN	Auxilin	Parkinson's disease	
PTEN	CC1	PTEN	PTEN	Tumor suppressor	Yes
PTPN1	CC1	PTP	PTPN1	Diabetes mellitus type 2	
PTPN22	CC1	PTP	PTPN12	Diabetes mellitus type 1, rheumatoid arthritis, lupus	
PTPN13	CC1	PTP	PTPN13	Cancer driver	Yes
PTPN14	CC1	PTP	PTPN14	Choanal atresia and lymphedema	
PTPN11	CC1	PTP	PTPN6	Oncogene, LEOPARD syndrome 1, metachondromatosis, Noonan syndrome 1, Juvenile myelomonocytic leukemia	Yes
PTPRB	CC1	PTP	PTPRB	Tumor suppressor	Yes
PTPRO	CC1	PTP	PTPRB	Nephrotic syndrome	
PTPRQ	CC1	PTP	PTPRB	Deafness	
PTPRC	CC1	PTP	PTPRC	Tumor suppressor, severe combined immunodeficiency	Yes
PTPRF	CC1	PTP	PTPRD	Breasts and/or nipples, aplasia or hypoplasia	
PTPRZ1	CC1	PTP	PTPRG	Susceptibility to *Helicobacter pylori* infection	
PTPRK	CC1	PTP	PTPRK	Cancer gene	Yes
FIG4	CC1	Sac	FIG4	Yunis-Varon syndrome, Charcot-Marie-Tooth disease, amyotrophic lateral sclerosis, polymicrogyria	
SYNJ1	CC1	Sac	Synaptojanin	Parkinson disease	
EYA1	HAD	EYA	EYA	Melnick-Fraser syndrome, otofaciocervical syndrome, branchiootic syndrome	
EYA4	HAD	EYA	EYA	Deafness, dilated cardiomyopathy	
Dullard	HAD	FCP	DULLARD	Cancer gene	Yes
FCP1	HAD	FCP	FCP1	Congenital cataracts, facial dysmorphism, and neuropathy	
CECR5	HAD	NagD	CUT	Cancer gene	Yes
BPGM	HP	HP1	PGAM	Bisphosphoglycerate mutase deficiency	
PGAM2	HP	HP1	PGAM	Glycogen storage disease	
ACP2	HP	HP2	ACP2	Acid phosphatase deficiency	
MINPP1	HP	HP2	MINPP1	Thyroid cancer	Yes
PDP1	PPM	PPM	PDPc	Pyruvate dehydrogenase phosphatase deficiency	
PPM1D	PPM	PPM	PPM1D	Cancer gene, familial breast cancer	Yes
PPM1K	PPM	PPM	PPM1K	Maple syrup urine disease	
ACP5	PPPL	PAP	ACP5	Spondyloenchondrodysplasia	
PPP6C	PPPL	PPP	PPP6C	Oncogene	Yes
ALPL	AP	AP	AP	Hypophosphatasia	

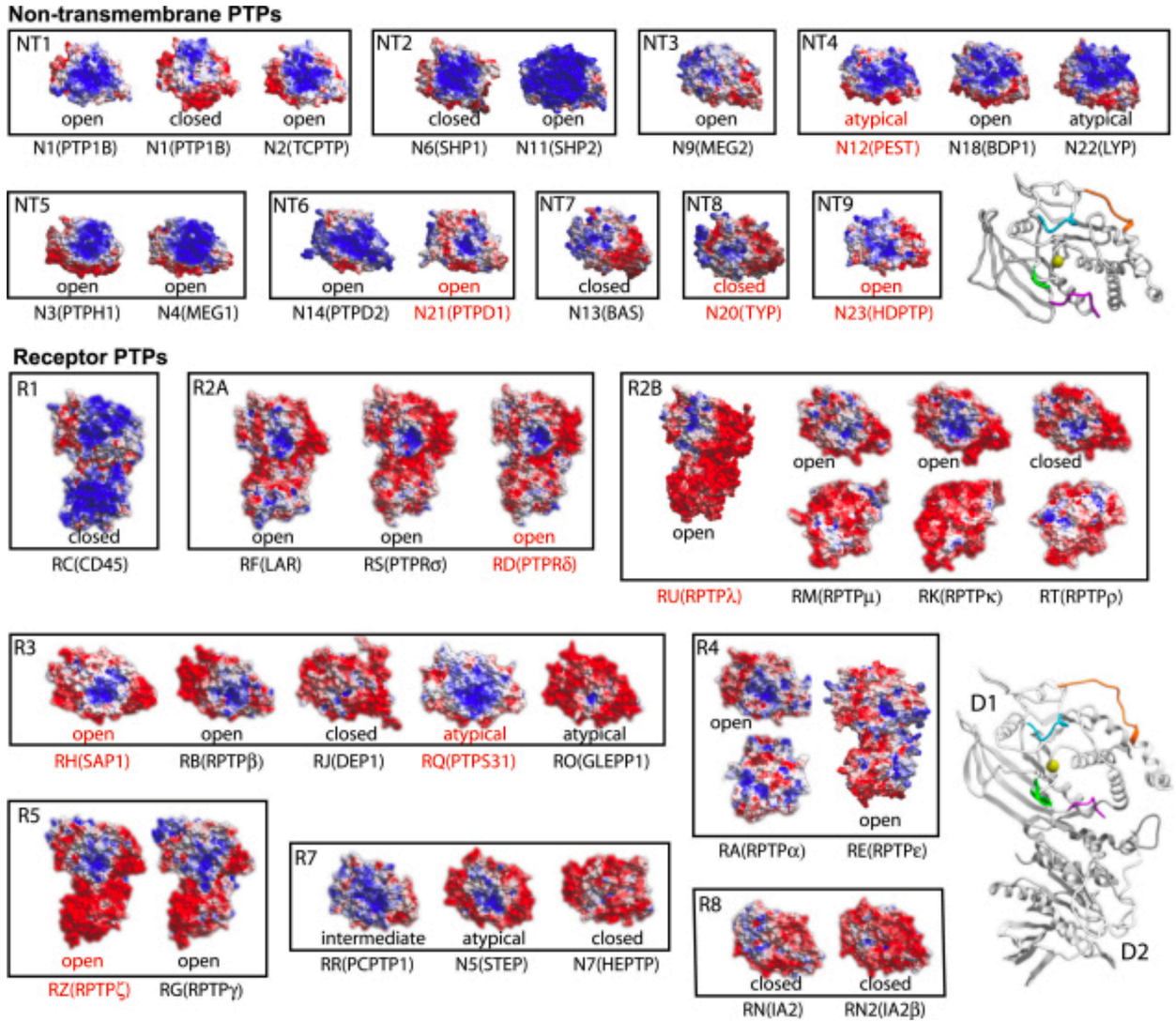
Chen, Dixon, Manning
Science Signalling, 2017

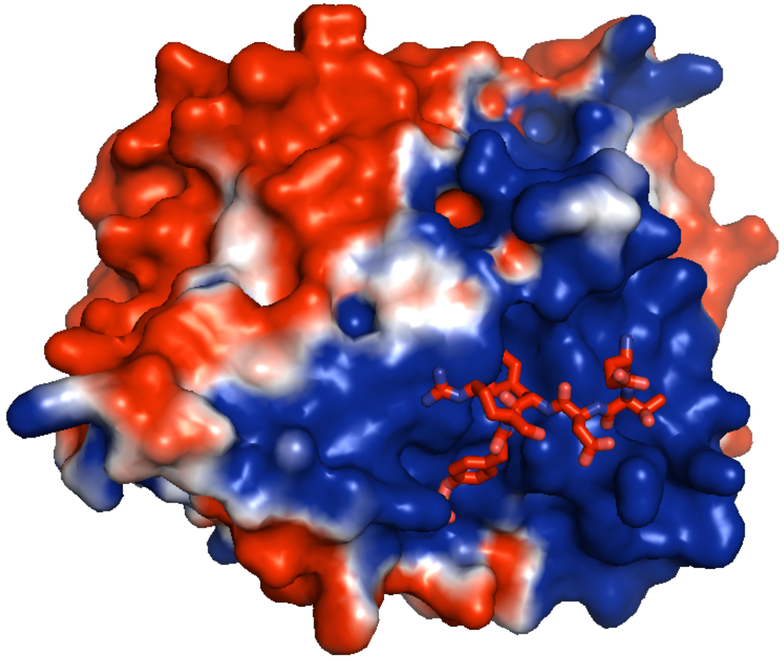
nature
cell biology

PTP1B controls non-mitochondrial oxygen consumption by regulating RNF213 to promote tumour survival during hypoxia

Robert S. Banh[1,2,3], Caterina Iorio[2,11], Richard Marcotte[2,11], Yang Xu[1,2,3,11], Dan Cojocari[1,2], Anas Abdel Rahman[4,5], Judy Pawling[4], Wei Zhang[6], Ankit Sinha[1,2], Christopher M. Rose[7], Marta Isasa[7], Shuang Zhang[3], Ronald Wu[1,2], Carl Virtanen[2], Toshiaki Hitomi[8], Toshiyuki Habu[9], Sachdev S. Sidhu[6], Akio Koizumi[8], Sarah E. Wilkins[10], Thomas Kislinger[1,2], Steven P. Gygi[7], Christopher J. Schofield[10], James W. Dennis[4], Bradly G. Wouters[1,2] and Benjamin G. Neel[2,3,12]

The highly charged active sites of protein tyrosine **phosphatases** exemplify the difficulties of active site drug discovery

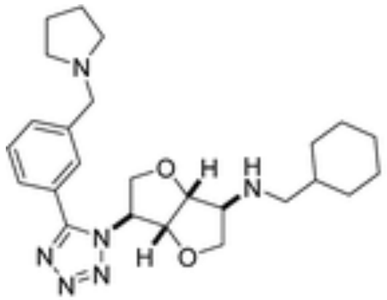




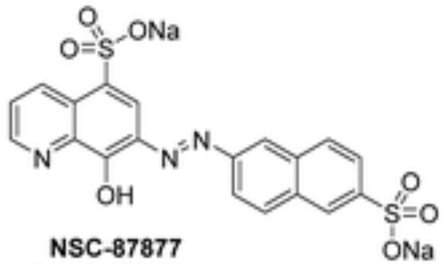
peptide with
negatively charged
pTyr substrate

Barr…Knapp, *Cell*, 2009

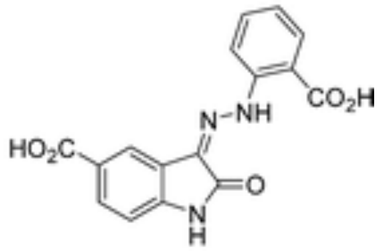
Phosphatase inhibitors with good potency had been developed, but none were bioavailable



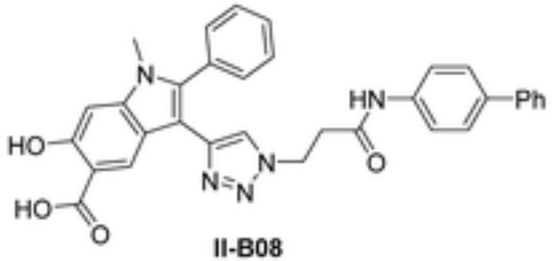
NAT6-297775
IC_{50} (SHP2): 2.5 μM



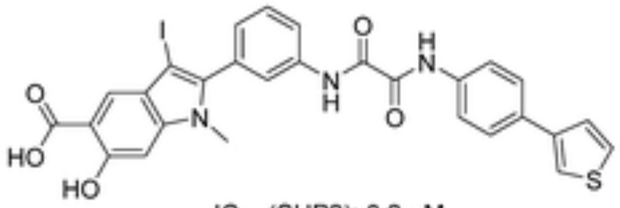
NSC-87877
IC_{50} (SHP2): 0.3 μM



IC_{50} (SHP2): 0.8 μM

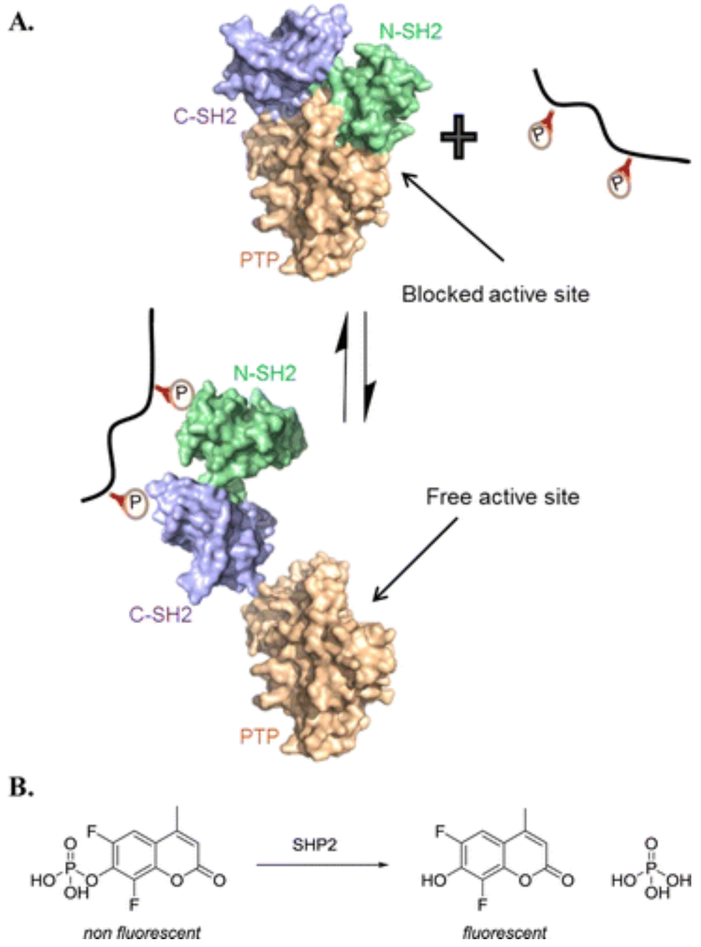


II-B08
IC_{50} (SHP2): 5.5 μM

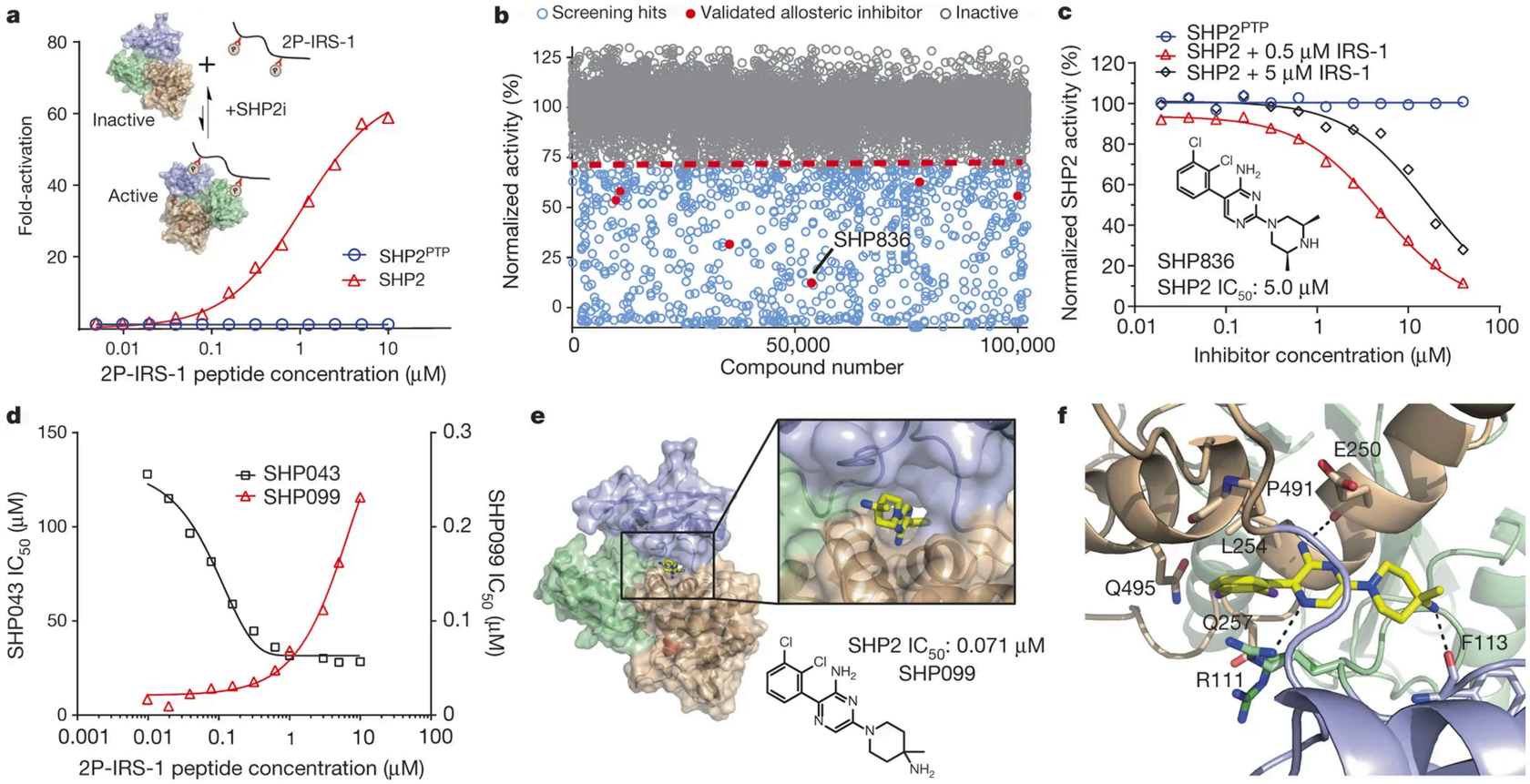


IC_{50} (SHP2): 0.2 μM

A new screening strategy for SHP2



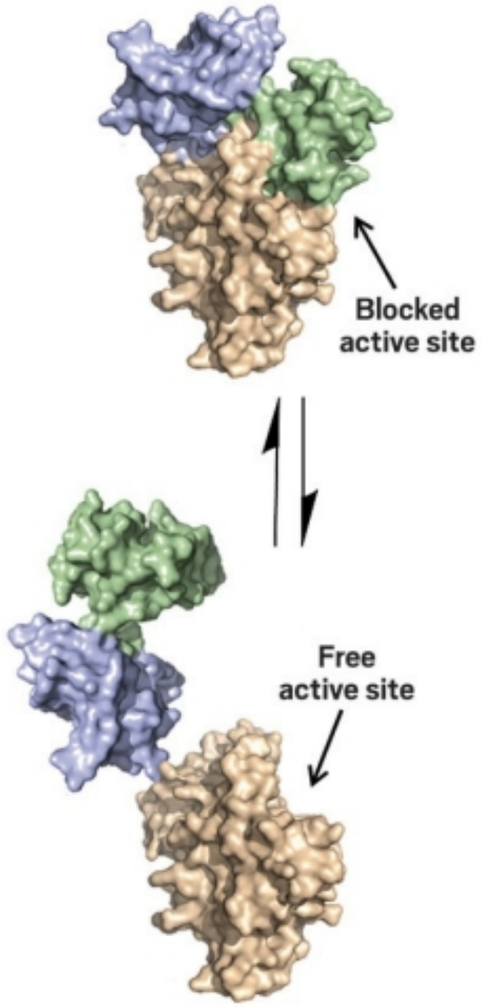
100,000 molecules screened **3 followup assays**

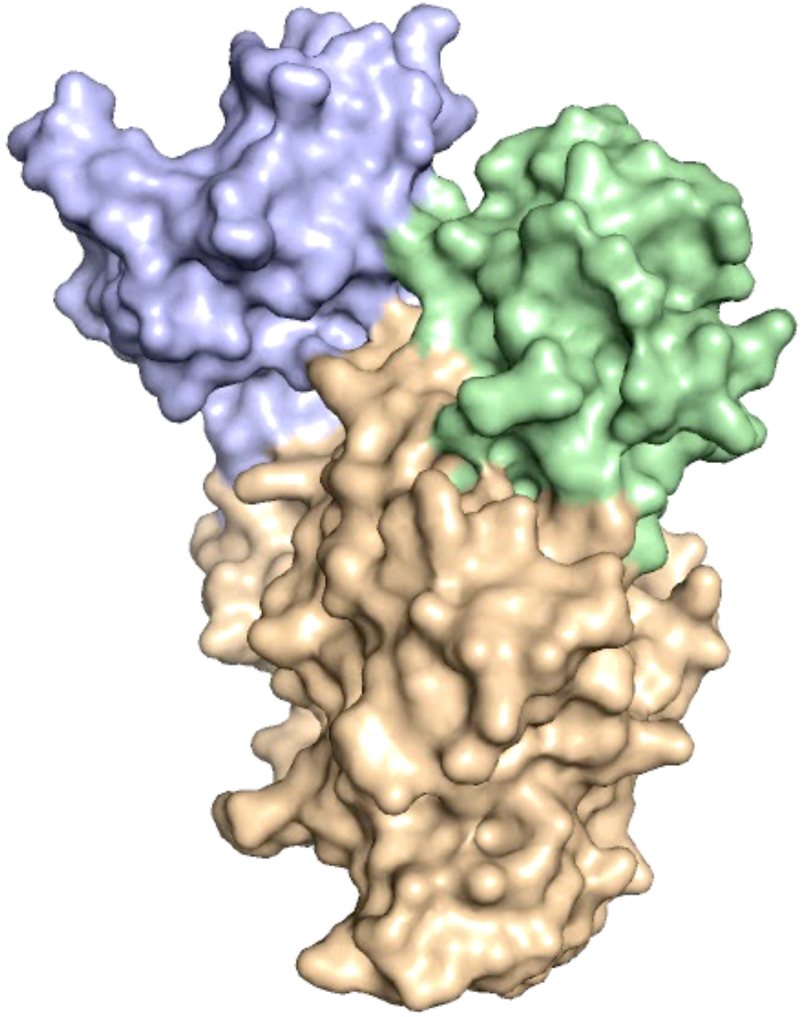


Contrast with active site inhibitor

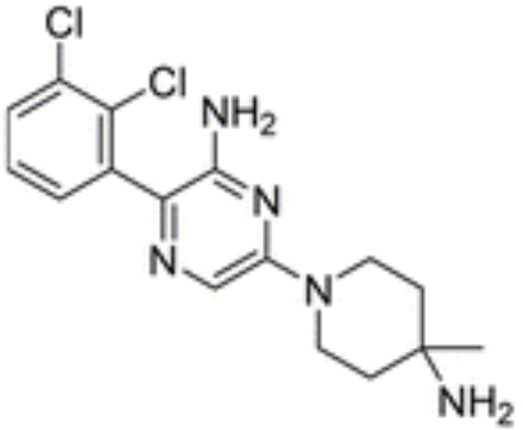
SHP836 - is a published ion channel inhibitor!

SHP2 brings new optimism for allosterically targeting **phosphatases**



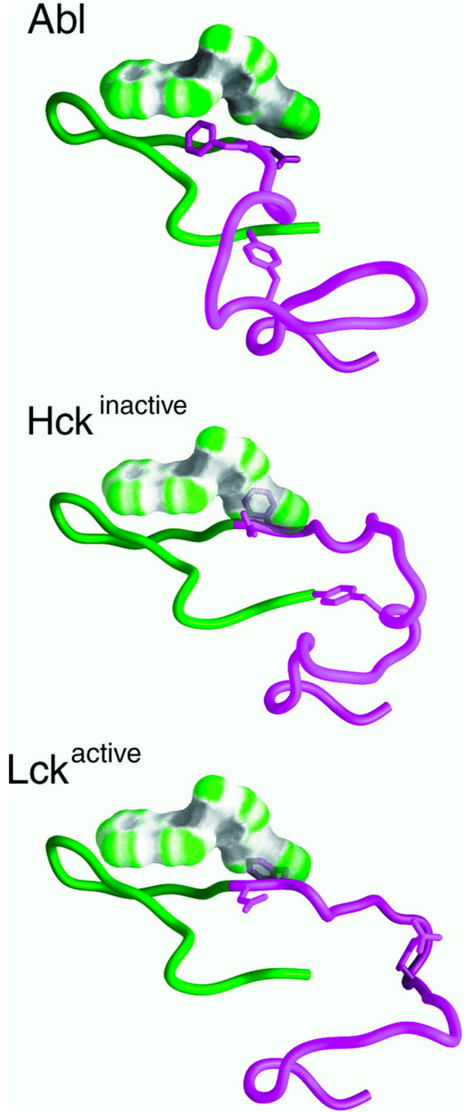


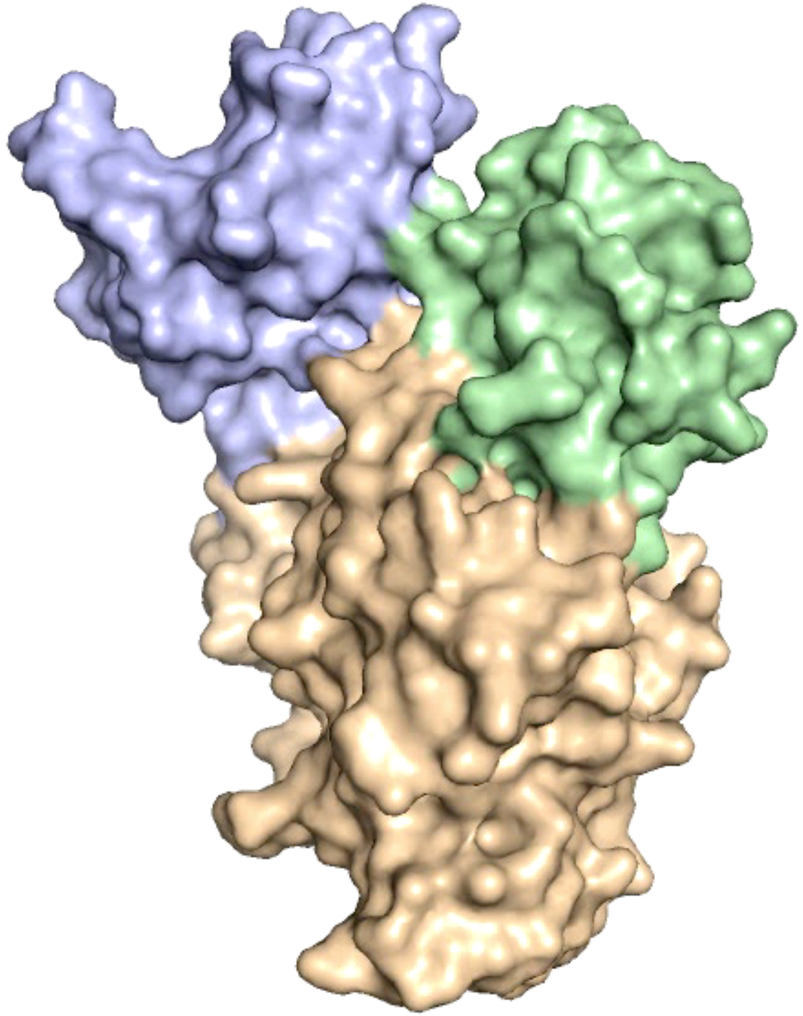
Novartis: SHP099



Chen…Fortin
Nature, 2016

Both kinases and phosphates can be inhibited by targeting specific inactive conformations



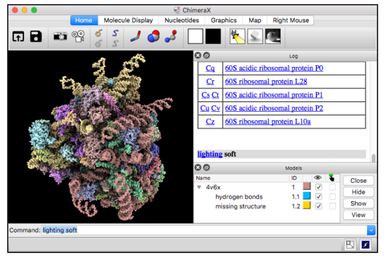


Tomorrow

Chemical space (100,000 molecules is nothing)
Starting with a scaffold
(development of PLX4032/Vemurafenib)
and how crystallography is useful for getting all these structures in the first place

Install ChimeraX:

https://www.rbvi.ucsf.edu/chimerax/download.html



UCSF ChimeraX Early Access

UCSF ChimeraX is the next-generation visualization program from the Resource for Biocomputing, Visualization, and Informatics at UC San Francisco, following UCSF Chimera.

Features

Compared to Chimera, ChimeraX has a more modern user interface, better graphics, and much faster handling of large structures. For more information, see:

- ChimeraX Advantages, User Guide, Documentation Index, Change Log
- UCSF ChimeraX: Meeting Modern Challenges in Visualization and Analysis. (Goddard *et al.*, *Prot Sci*. 2018)
- Integrative Modeling Demo (2016)
- Structures at the Experimental Forefront (2016)
- Next-Generation Graphics (2015)

Although similar in many aspects, **ChimeraX is not backward compatible with Chimera and does not read Chimera session files.**

Missing Features

While ChimeraX has several completely new features and other advantages, it will not substantially replace Chimera for some time. Current capabilities are somewhat limited and mostly implemented as commands only (not yet as graphical interfaces). Missing features relative to Chimera include calculation of axes/planes/centroids, dock prep, "worms" to show residue attributes, custom attributes, label by attribute, color key, 2D label GUI, per-model clipping, trajectory analysis other than simple playback, structure building, loop modeling, and many others. Chimera capabilities grew significantly over several years, and likewise, ChimeraX will contain more and more of these important features as development proceeds.

Downloads

- Download is **free for academic, government, nonprofit, and personal use**; commercial users, please see licensing.
- Using **a newer computer** (≤ 3 years old) **is recommended** for ChimeraX because it employs graphics features that require or work best on a recent system.
- ChimeraX **commands and their syntax may change**.

Daily Builds
Production Releases
Platform Notes
Virtual Reality Notes

Daily Builds

Daily builds are generated automatically each night from the development source code (see the change log). While a given build may have unforeseen problems, these are often fixed by the next day.

Operating System	Distribution	Notes
Windows 10 64-bit	chimerax-daily.exe built: 2020-01-05 02:26:23 PST committed: 2020-01-03 16:01:37 PST size: 433.8 MiB md5: c83061d877049be05d0a74b8aaff490d	Download is a Windows installer. Tested on Windows 10. See Windows notes below.
	chimerax-daily.dmg built: 2020-01-05 02:48:36 PST	