5.36 Biochemistry Laboratory
Spring 2009

Kinase Domains: Structure and Inhibition

I. Conserved and variable features of kinase domains

   A. Structural similarities

   B. Active and inactive forms

II. Abl and Bcr-Abl inhibition by Gleevec

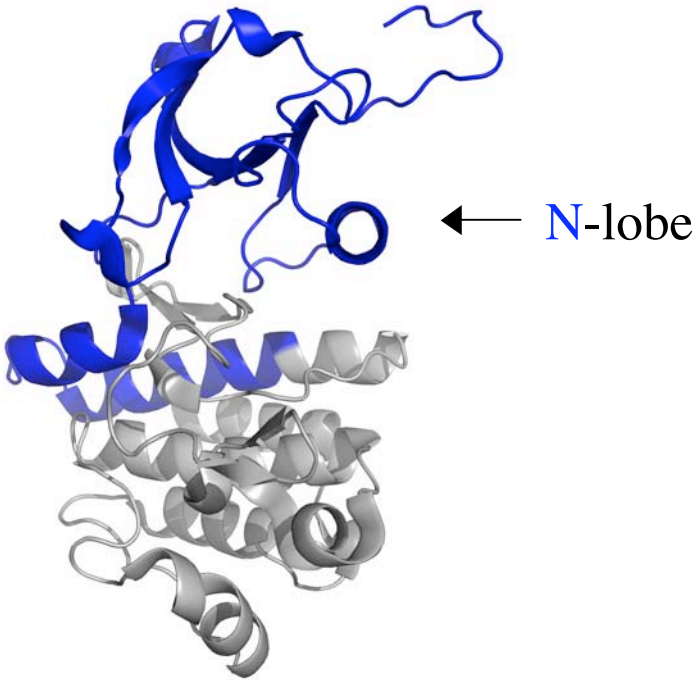
III. Gleevec resistance in Bcr-Abl mutants

   A. Direct interference with Gleevec binding

   B. Destabilization of the inactive form

The catalytic domain (or kinase domain) of eukaryotic protein kinases is highly conserved both in sequence and structure

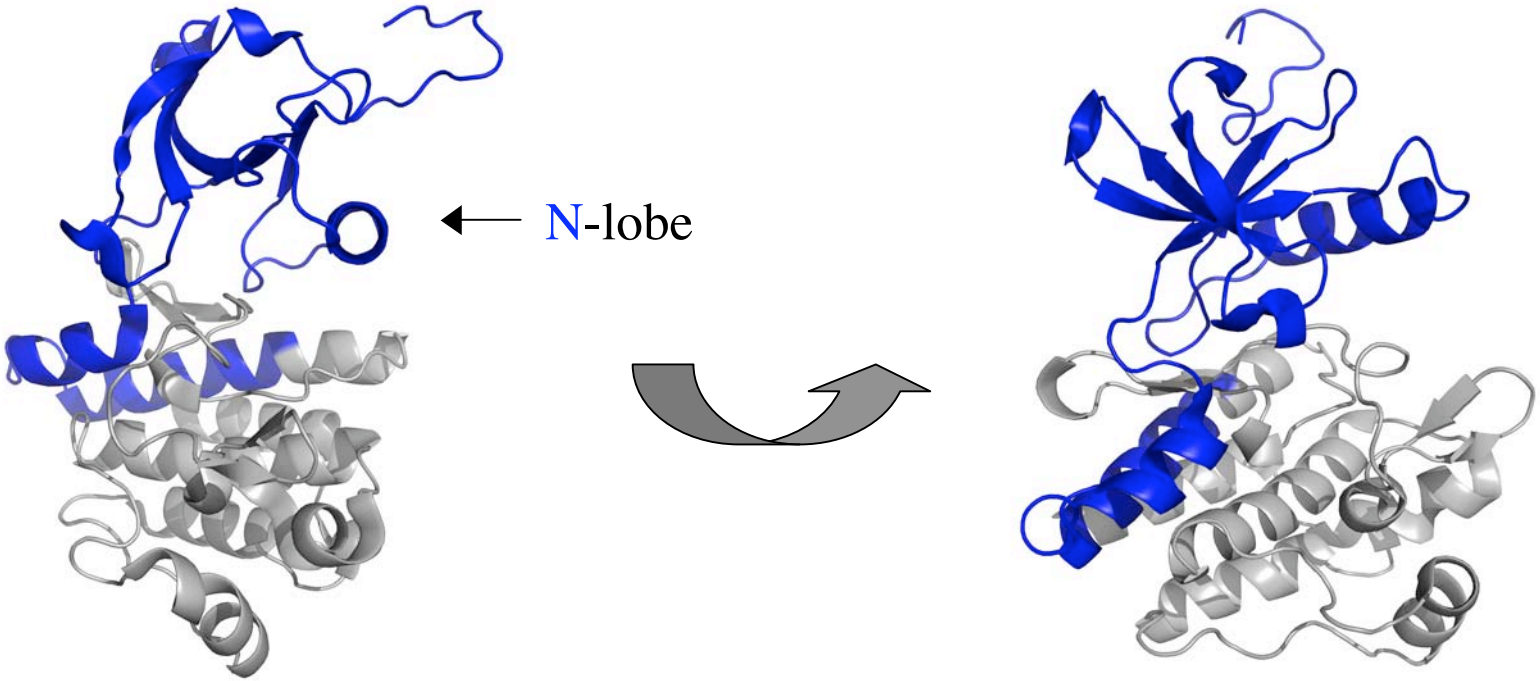
Kinase activity requires binding of the peptide substrate (to be phosphorylated) and Mg-ATP to the catalytic domain.



Kinase domains have a bilobal structure composed of an

N-lobe (amino lobe) that

- contains a 5-stranded beta sheet and an alpha helix (αC).



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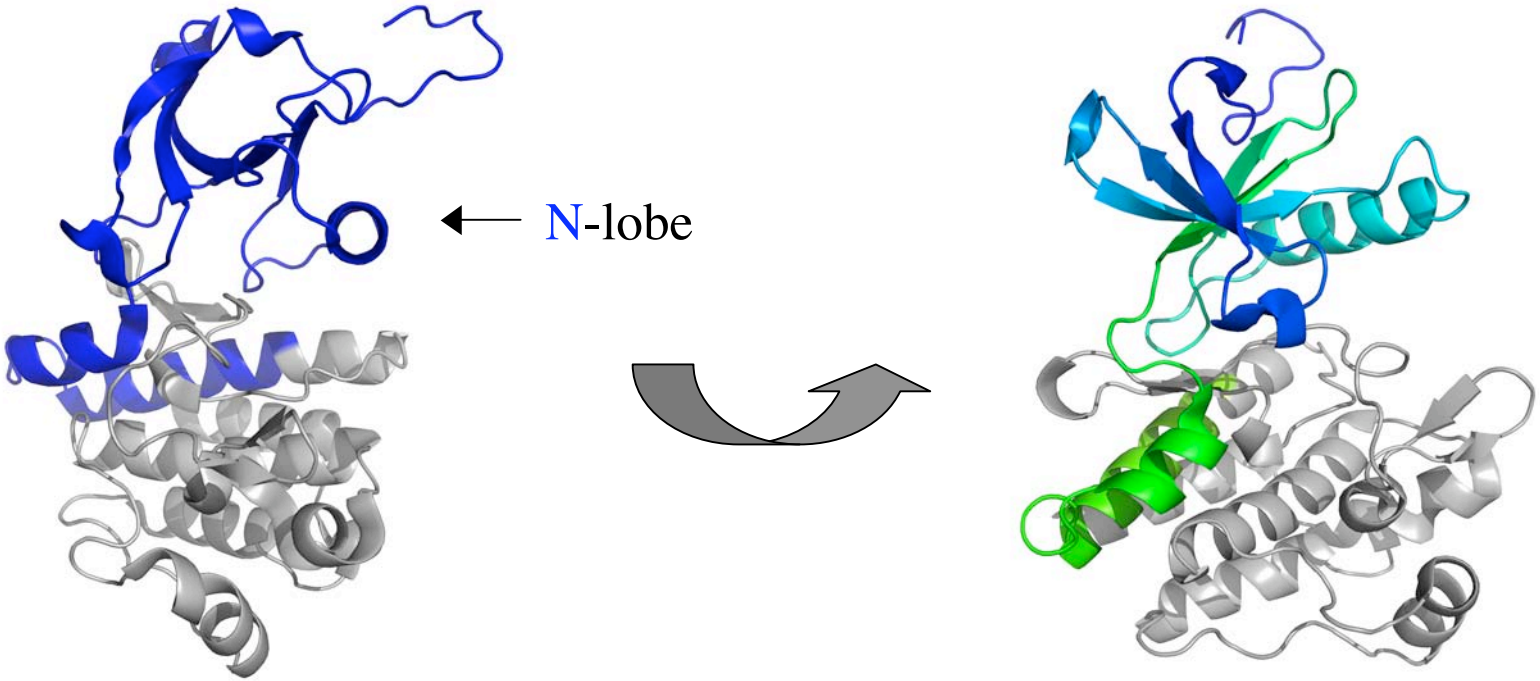
- comprises residues 225-350 of Abl (shown here).
- contributes to ATP binding.



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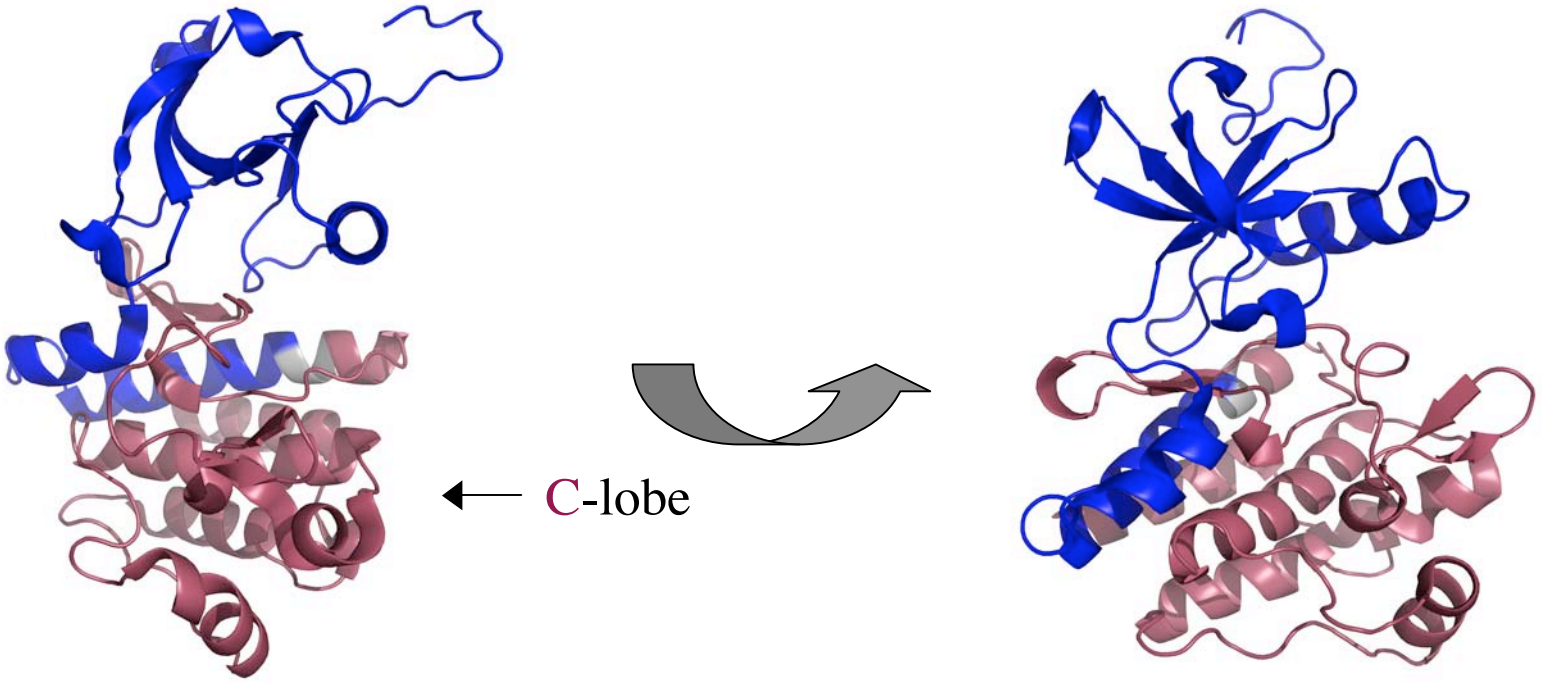
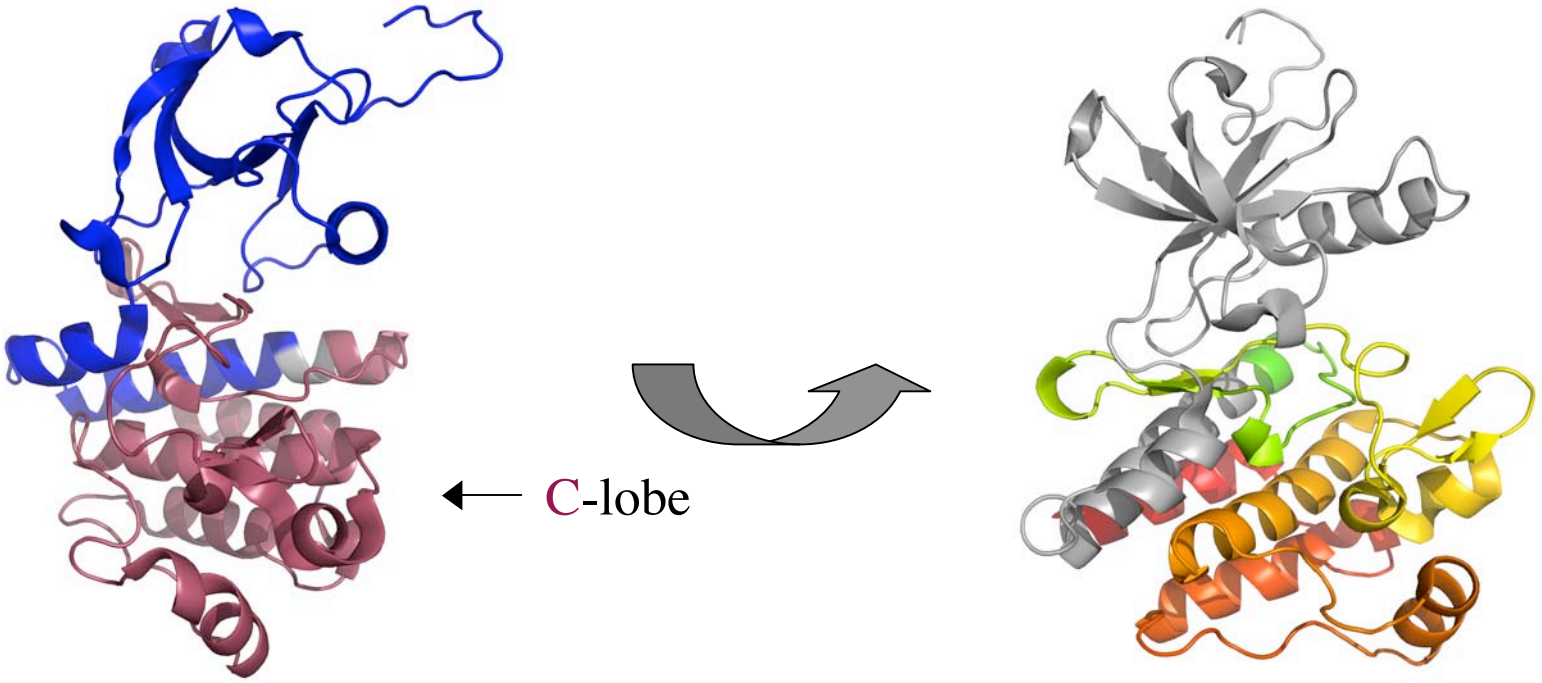


Kinase domains have a bilobal structure composed of an N-lobe and a

C-lobe (carboxy lobe) that

- is made up of multiple alpha helices.
- comprises residues 354-498 of Abl (the larger lobe).
- is the location of peptide substrate binding.



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C-lobe (carboxy lobe) that

- is made up of multiple alpha helices.
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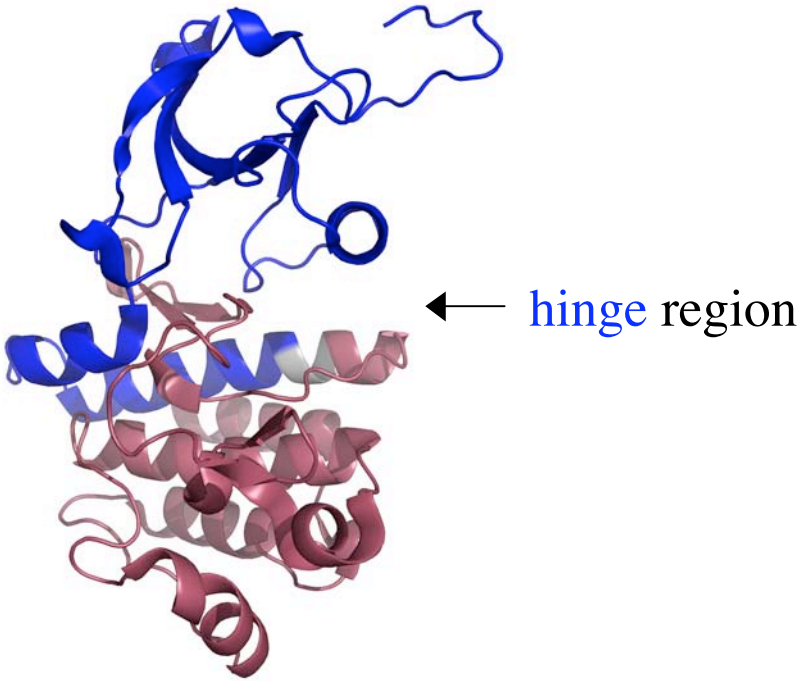
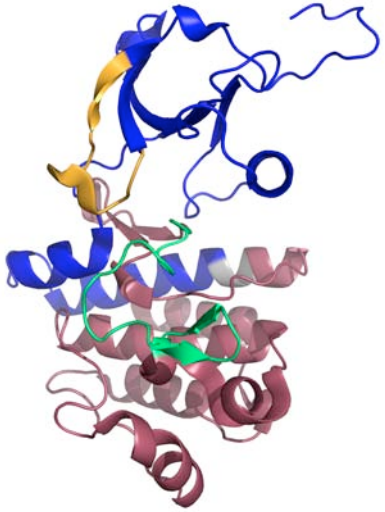
- is the location of peptide substrate binding.

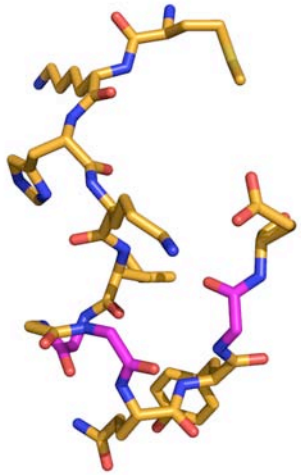
The **hinge** region (between the two lobes) contains several conserved residues that provide the catalytic machinery and make up an essential part of the ATP binding pocket.



Among all kinases, Mg-ATP binding is primarily in the N-lobe and hinge region.

ATP Binding (P) loop





(conserved residues in magenta)

- A ____-rich region in the N-lobe (typically a flexible loop between strands of the beta sheet or between the beta sheet and an alpha helix) that is highly conserved among kinases.

Color scheme for atoms

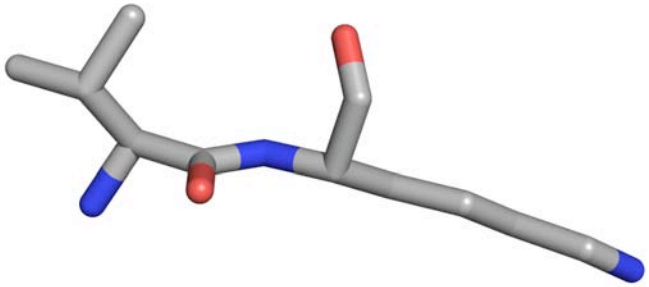
oxygen- red

nitrogen- blue

carbon- black, grey, or background color

sulfur- yellow

phosphorus- orange



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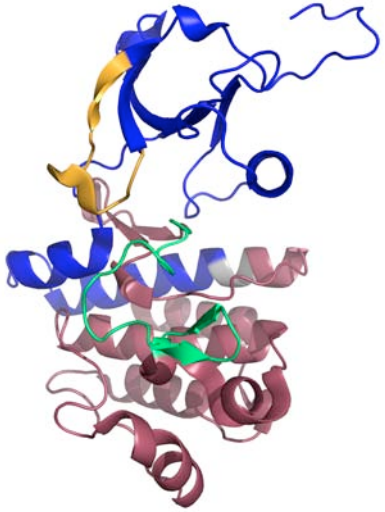
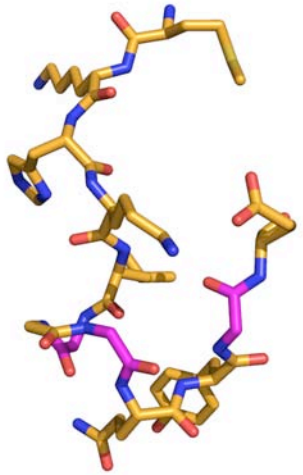
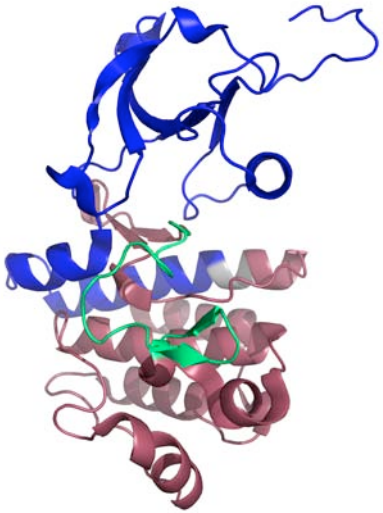


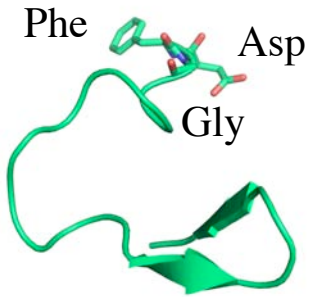


(conserved residues in magenta)

- A Gly -rich region in the N-lobe (typically a flexible loop between strands of the beta sheet or between the beta sheet and an alpha helix) that is highly conserved among kinases.
- The backbone atoms of the conserved P-loop sequence, GXGXXG, interact with the non-transferred phosphate atoms of ATP.
- In Abl, the P-loop sequence is MKHKLGGGQYGE.

Activation (A) loop





- a principal regulatory structure for modulating kinase activity. In the closed form (shown above), the A-loop can block substrate binding to the C-lobe.
- The A-loop can vary significantly in sequence and size between kinase subfamilies.
- A conserved Asp-Phe-Gly (DFG) motif implicated in ATP binding is located at the N-terminus of the A-loop.

In the Abl or Bcr-Abl kinase domains:

- **N-lobe**: Abl residues 225-350
  - ⇒ P-loop: residues 244-255
- **hinge region**: interface of N and C lobes
- **C-lobe**: 354-498
  - ⇒ A-loop: residues 381-402
    - ⇒ DFG motif: residues 381-383

Note that all Abl numbering is provided for isoform 1A of human Abl (swissprot accession number: P00519).

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I. Conserved and variable features of kinase domains

   A. Structural similarities

   B. *Active and inactive forms*

II. Abl and Bcr-Abl inhibition by Gleevec

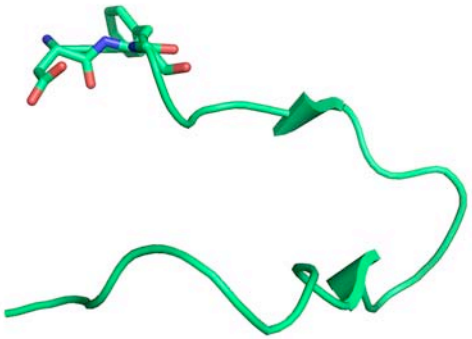
III. Gleevec resistance in Bcr-Abl mutants

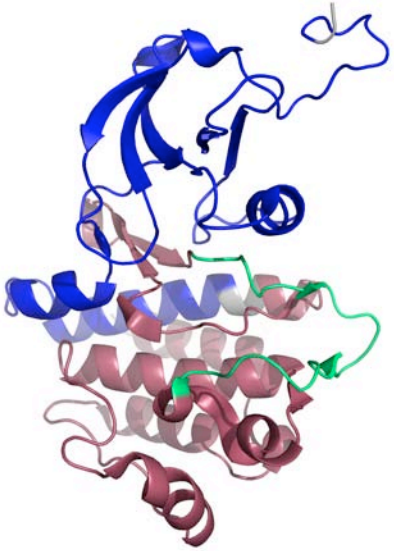
   A. Direct interference with Gleevec binding

   B. Destabilization of the inactive form

In an **active kinase**, the activation (A) loop is in an "open" conformation.

activation loop



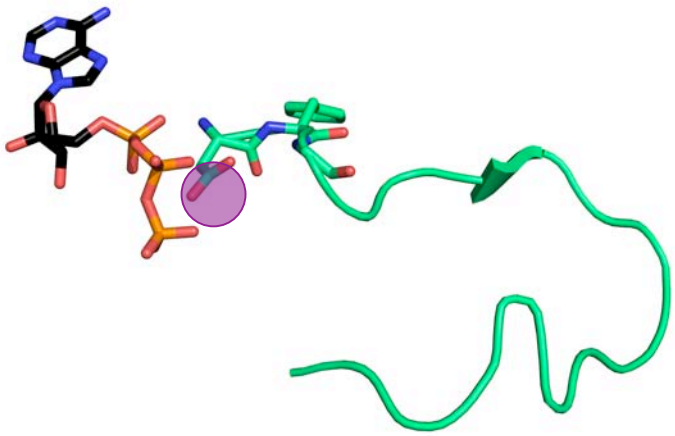


Abl kinase domain: active conformation

Features of an open or extended A loop conformation:

- The body of the A loop does not block the C-lobe, enabling the C-lobe to be available for binding the substrate.
- The Asp within the DFG conserved motif (381 in Abl) is oriented toward the ATP binding pocket.

The Asp side chain interacts with the Mg coordinated to the phophate groups of ATP.



ATP binding the cAPK kinase domain
(PDB: 1atp)

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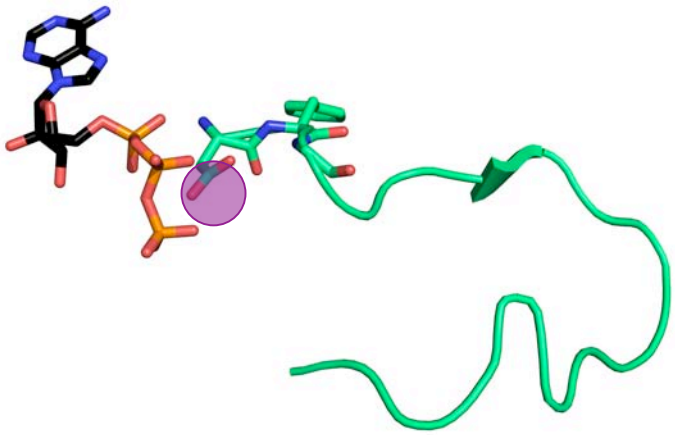
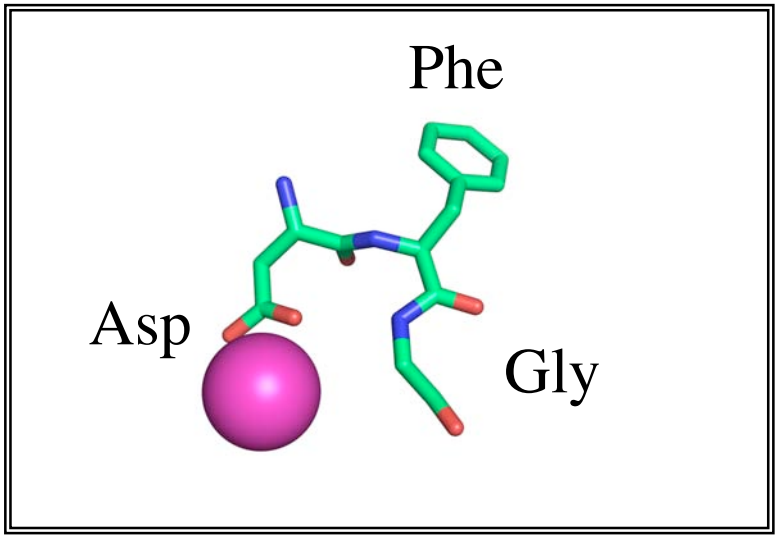




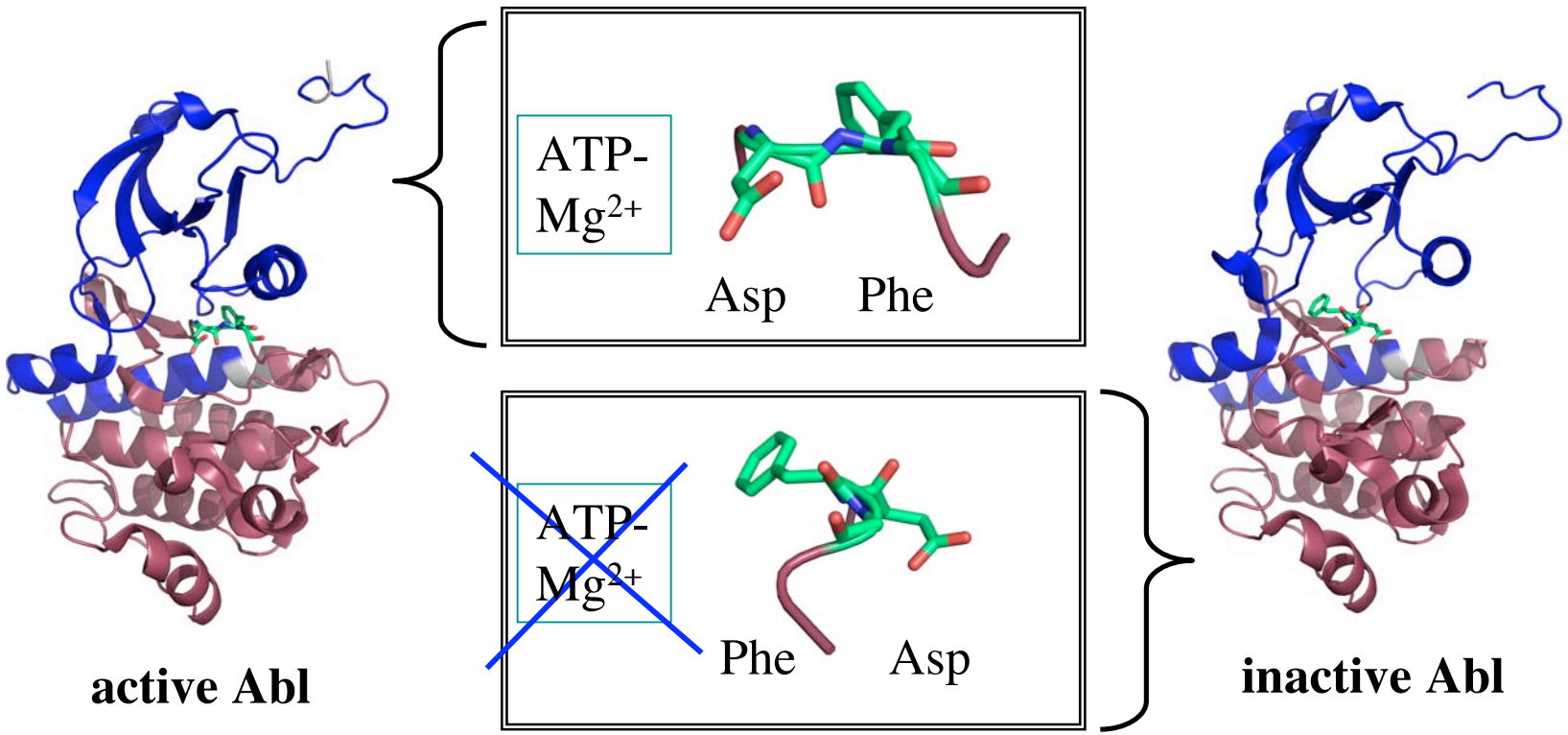
ATP binding the cAPK kinase domain
(PDB: 1atp)

“Happy families are all alike; every unhappy family is unhappy in its own way.”

“ Active kinase domains are all alike; every inactive kinase domain is inactive in its own way.”

The inactive conformation of the Abl kinase domain

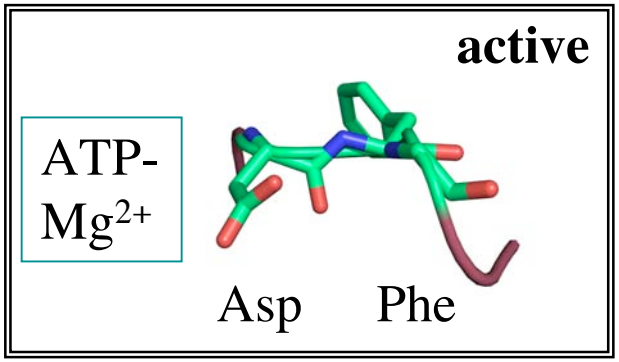
The Abl kinase domain switch from an active to an inactive form results in a conformation change at the start of the A loop. This flips the orientation of the DFG motif by ~180°.

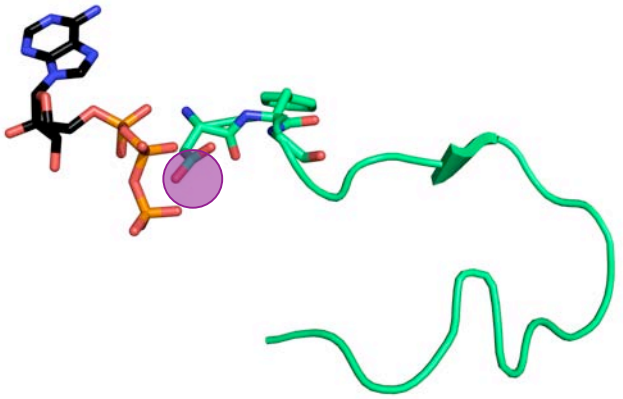


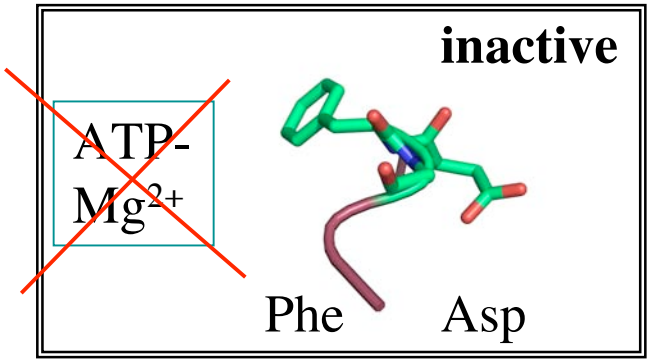
With the Asp side chain is flipped away from the ATP binding site, Mg coordination (with the Mg-ATP complex) is prevented.

The inactive conformation of the Abl kinase domain

Recall that the Asp carboxylic acid functional group binds the Mg^{2+} coordinated to ATP in active kinases.



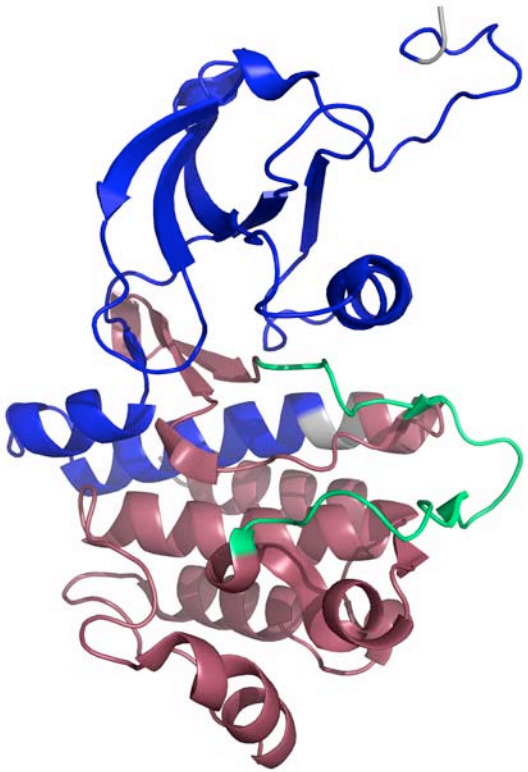




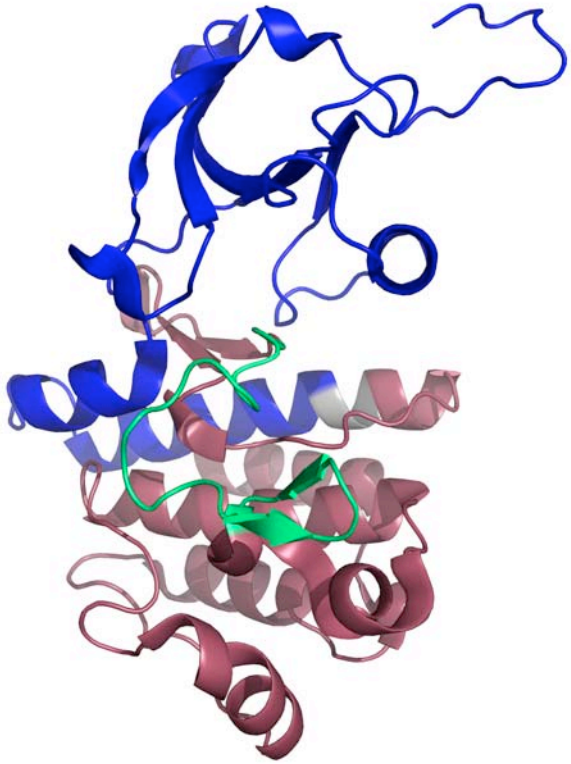
While the DFG motif is conserved among all protein kinases, the DFG flip is unique to Abl and only a few other kinase subfamilies.

The inactive conformation of the Abl kinase domain

Also, in the inactive form, the A-loop blocks the substrate binding region of the C-lobe.



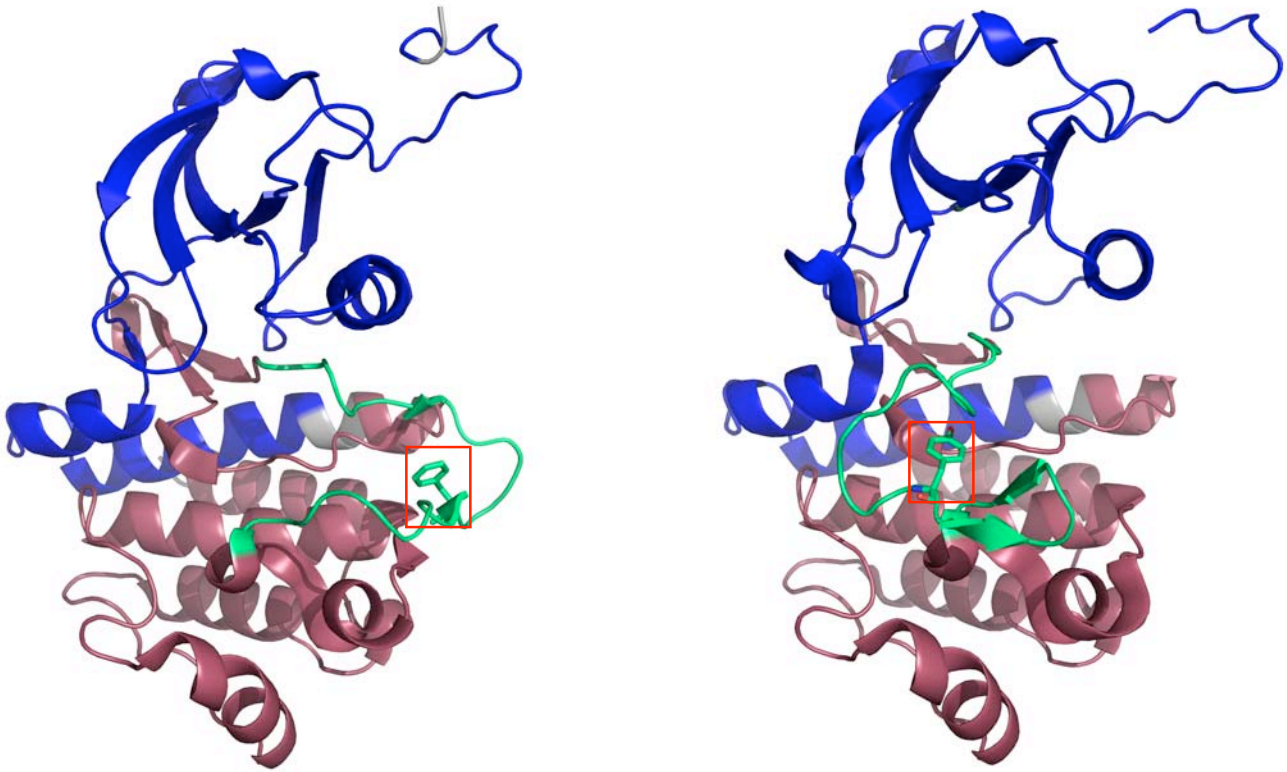
active



inactive

The inactive conformation of the Abl kinase domain

Specifically, Tyr393 mimics the target Tyr (to be phosphorylated) on the substrate.



active **inactive**

Tyr393 is typically phosphorylated in the active form, and is not phosphorylated in the inactive form.

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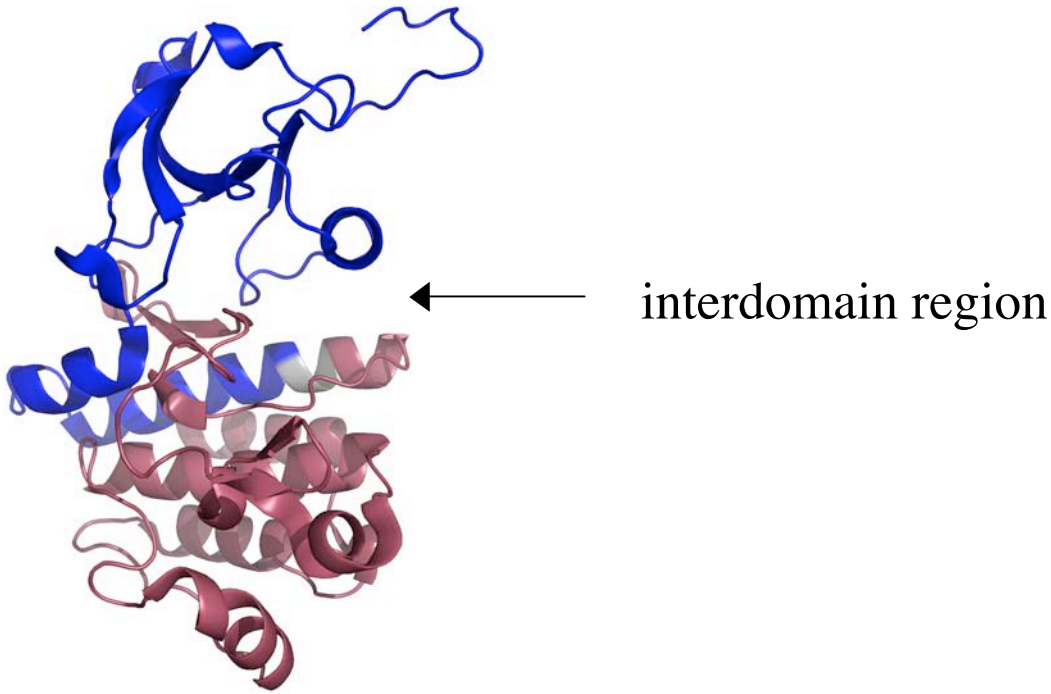
Gleevec inhibition of the Abl (and Bcr-Abl) kinase domains:

The vast majority of kinase inhibitors are ATP competitive inhibitors that bind in the kinase domain hinge region.



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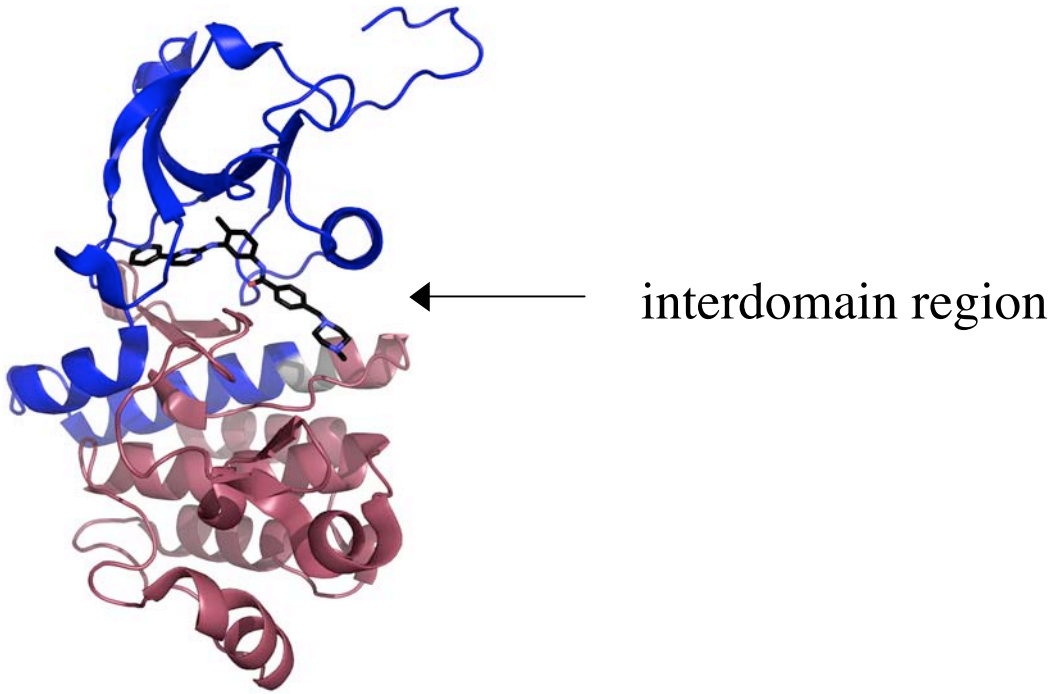
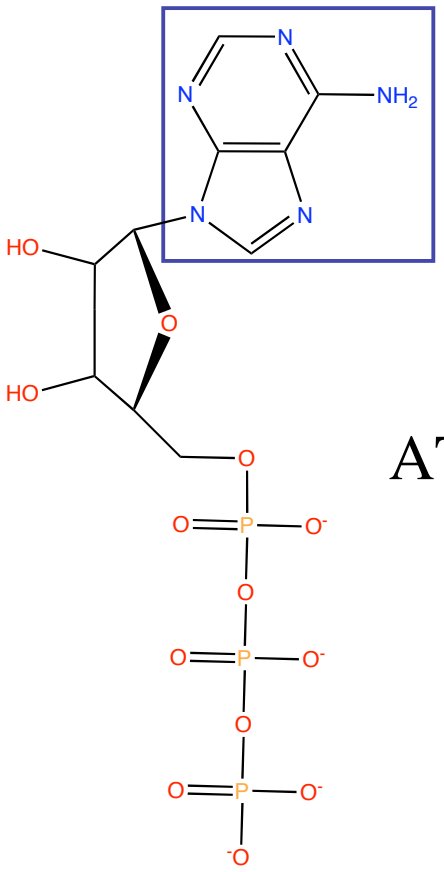


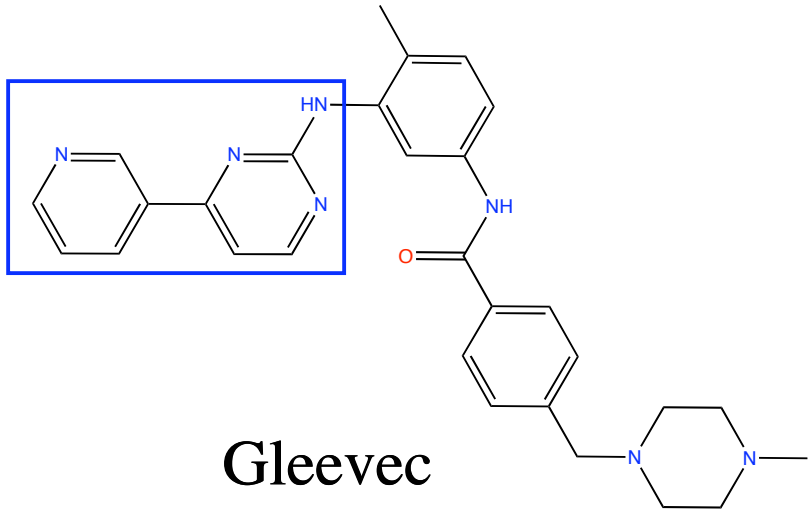
As with most kinase inhibitors, Gleevec competes with ATP to bind in the hinge region of the kinase domain.

In contrast to many kinase inhibitors, only part of the Gleevec molecule blocks ATP binding.

Specifically, only the pyridine and pyrimidine rings of Gleevec interfere directly with ATP binding, blocking the adenine base.

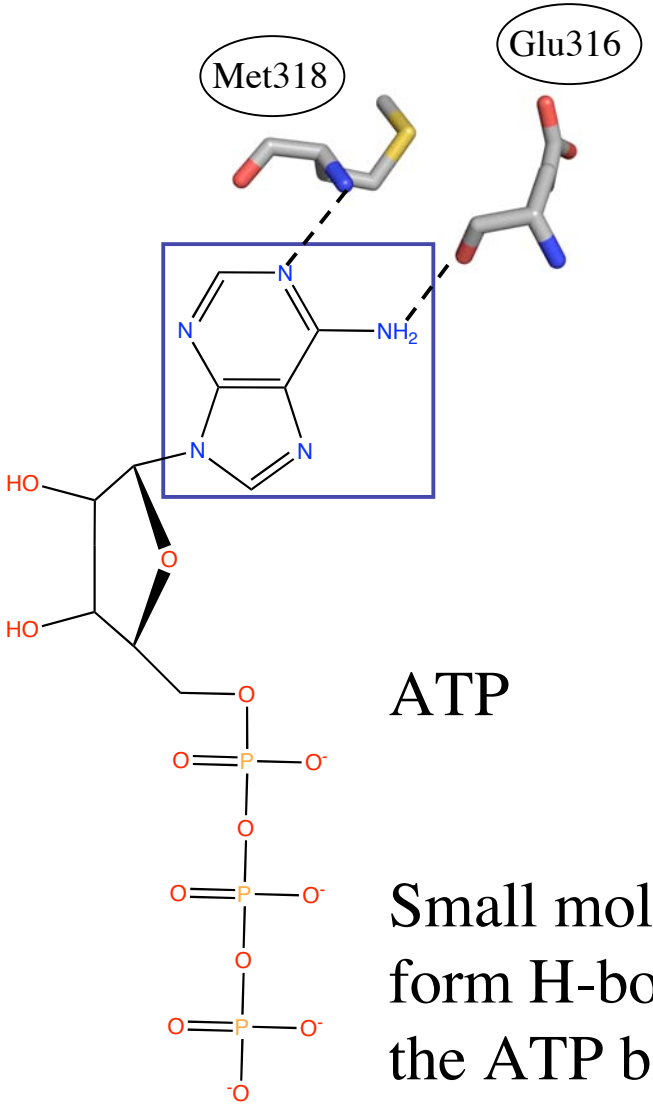


ATP

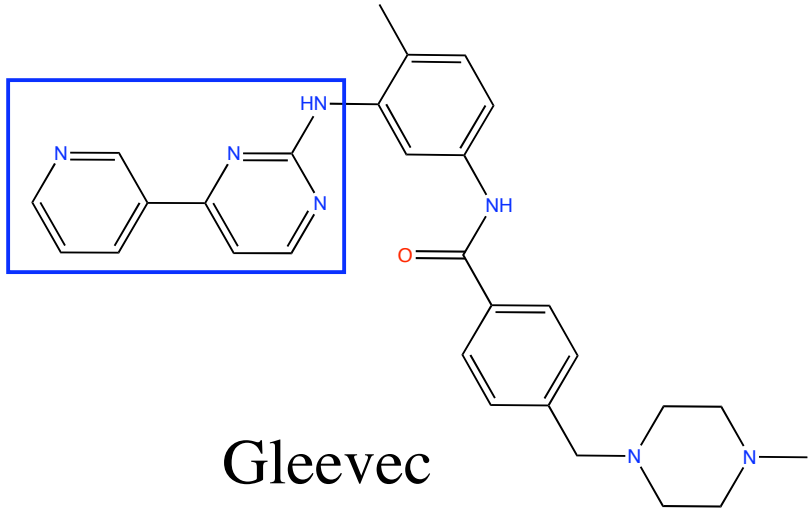


Gleevec

In active Abl, the adenine base of ATP forms two hydrogen bond with the protein backbone in the hinge region.



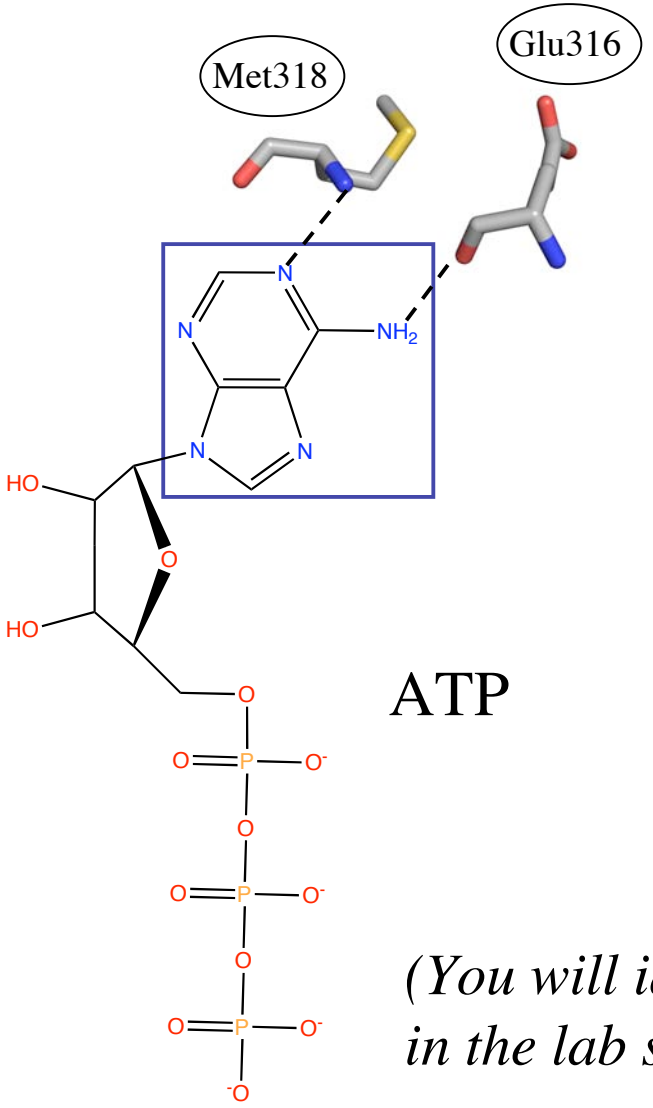
ATP



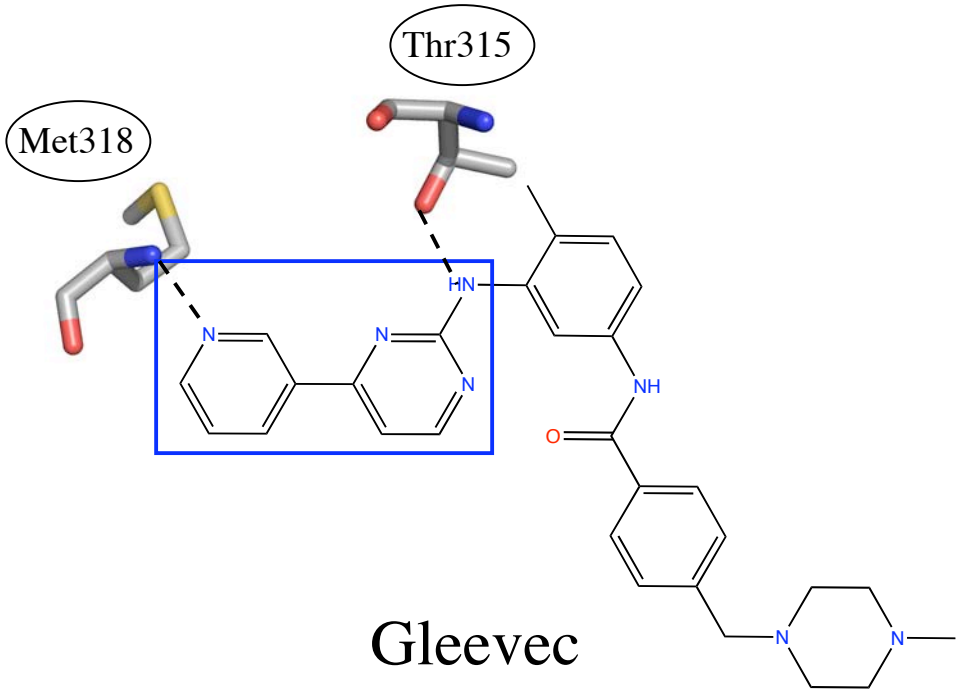
Gleevec

Small molecule inhibitors of numerous kinases form H-bonds with the corresponding residues in the ATP binding pocket of the target kinase.

Although Gleevec forms similar hydrogen bonds, there is no H-bond formed with Glu 316. Gleevec has a unique position in the binding pocket.



ATP

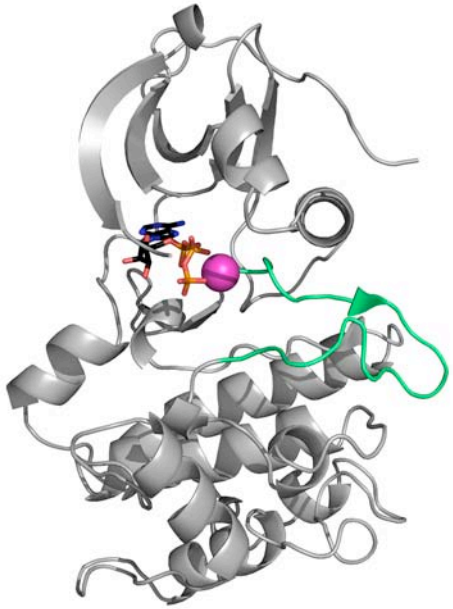


Gleevec

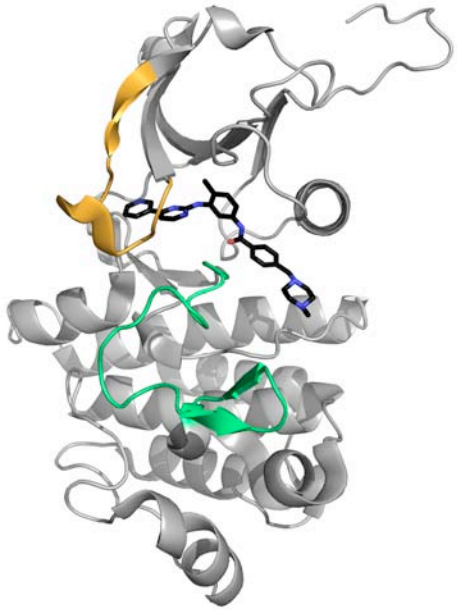
(You will identify the additional Abl-Gleevec H-bonds in the lab session 15 structure viewing excerise.)

There is minimal overlap in the ATP and the Gleevec small molecule binding orientations.

The Gleevec molecule penetrates deeper into the hydrophobic core of the ATP binding site compared to ATP.



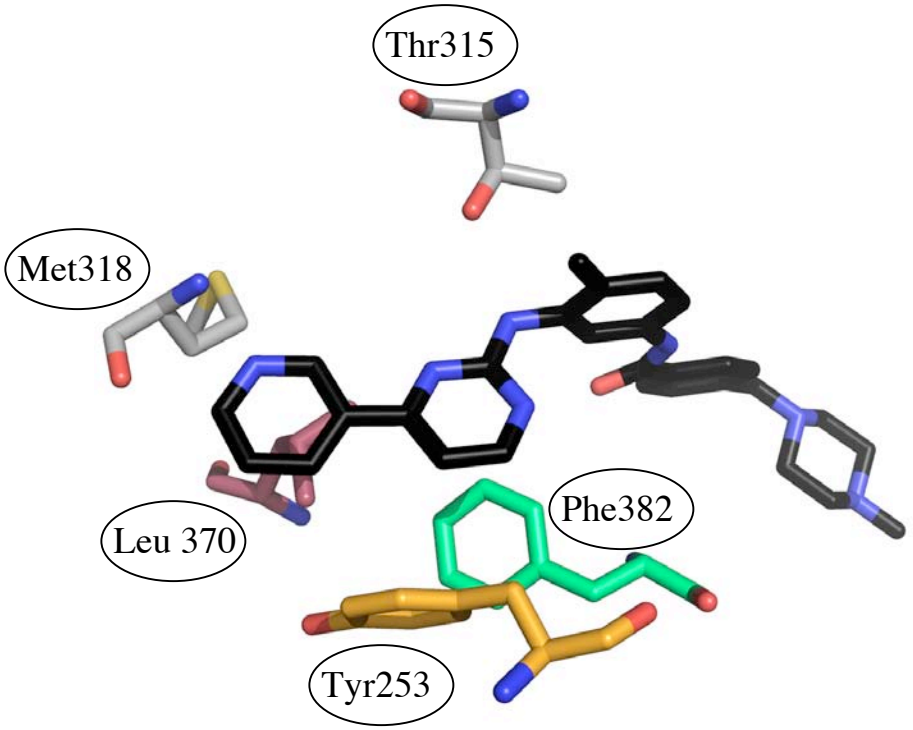
ATP-kinase complex (shown with cAPK)



Gleevec-Abl complex

The majority of the Gleevec binding energy comes from van der Waals and hydrophobic interactions (NOT just H-bonds).

For example, a hydrophobic “cage” around Gleevec’s pyridine and pyrimidine rings is formed by Leu 370 and residues from the P-loop (Tyr 253) and A-loop (Phe 382).



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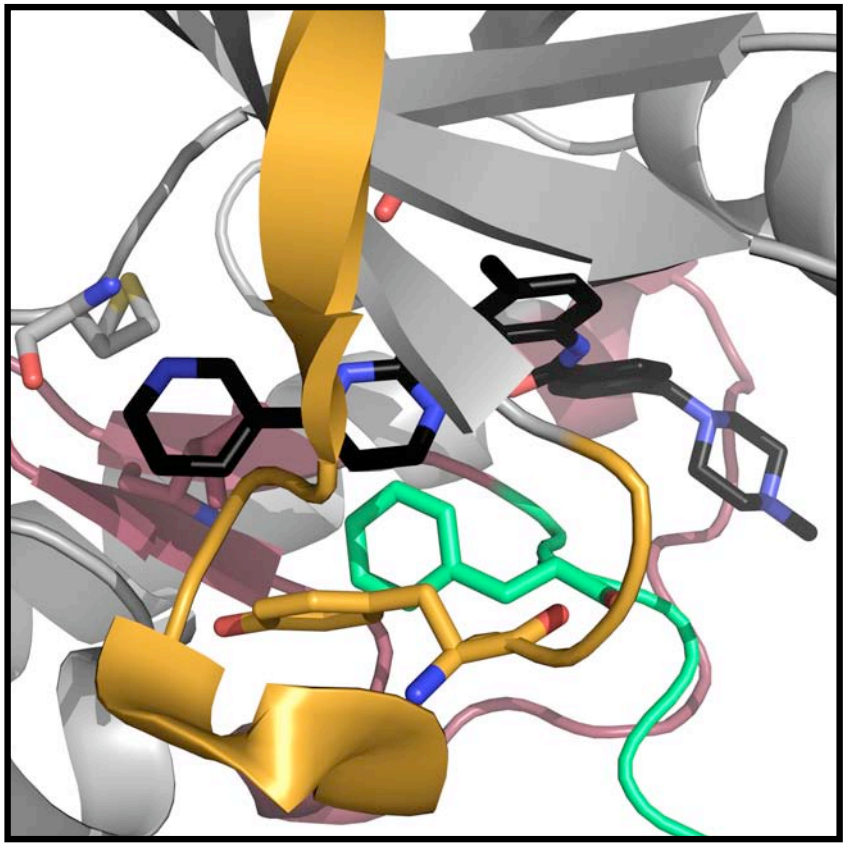
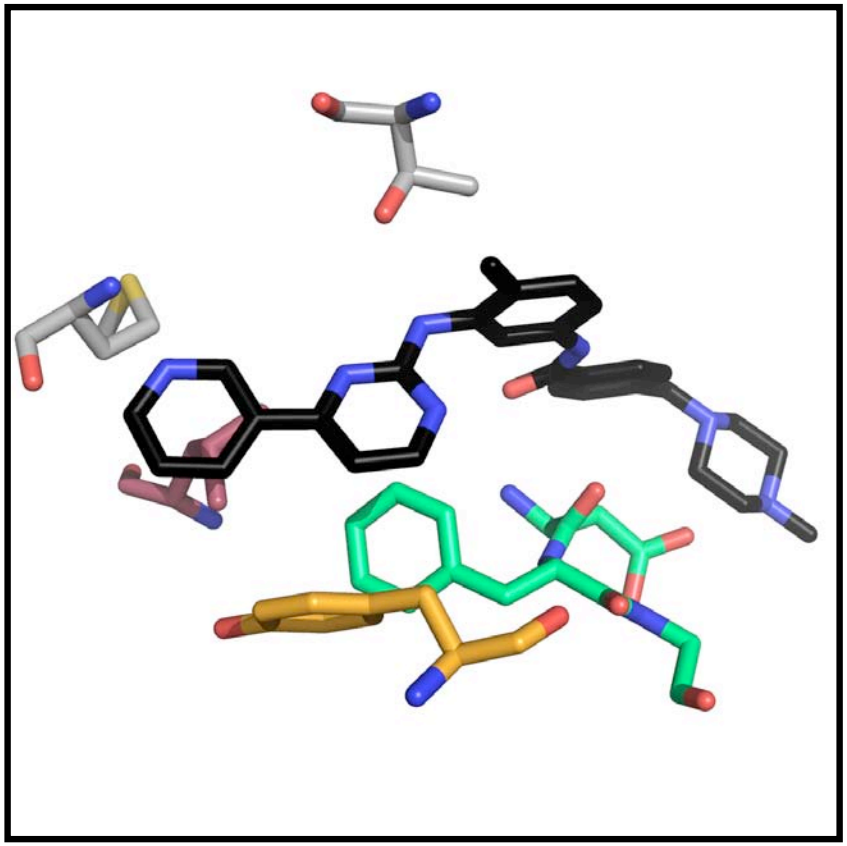
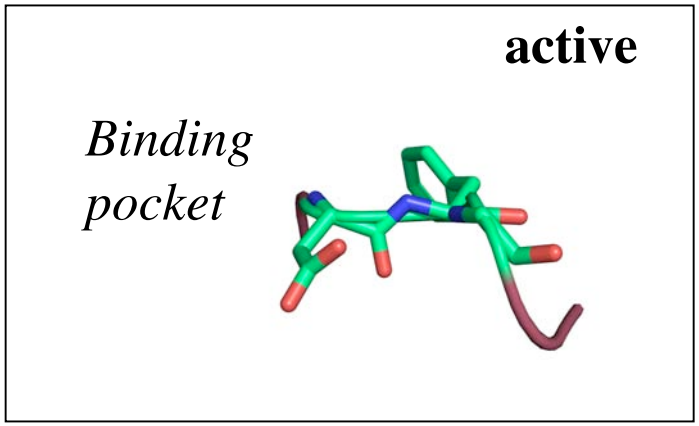
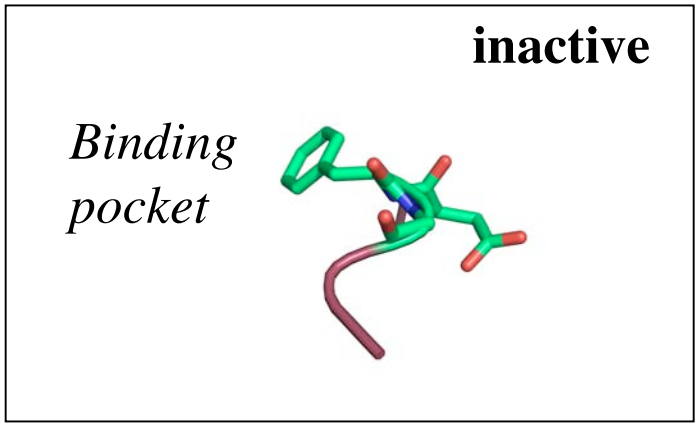




Phe382 is part of the conserved DFG motif. The Phe382 orientation toward the pyrimidine ring is critical for Gleevec binding.

Recall that in the active form, the Asp381 side chain is oriented toward the ATP binding pocket. In the the inactive form the Phe side chain is oriented toward the binding pocket.

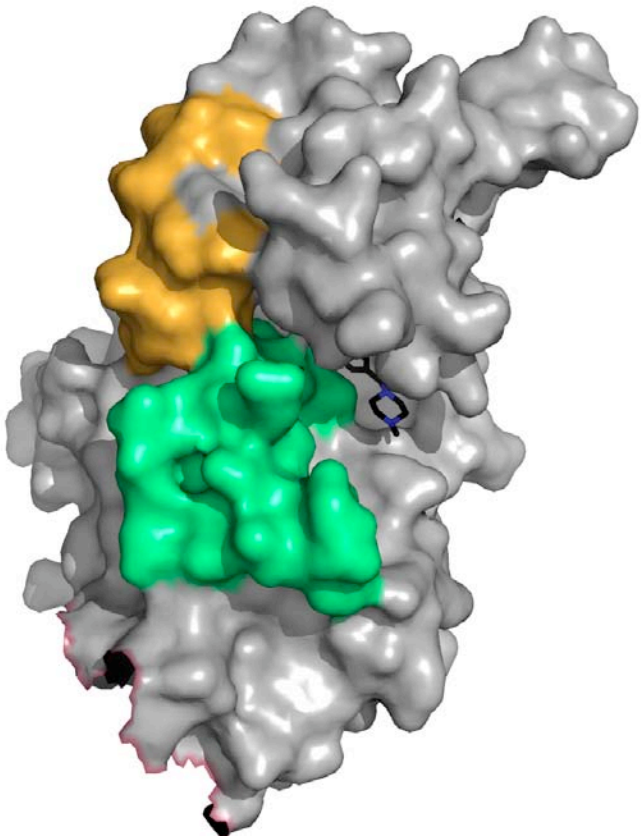


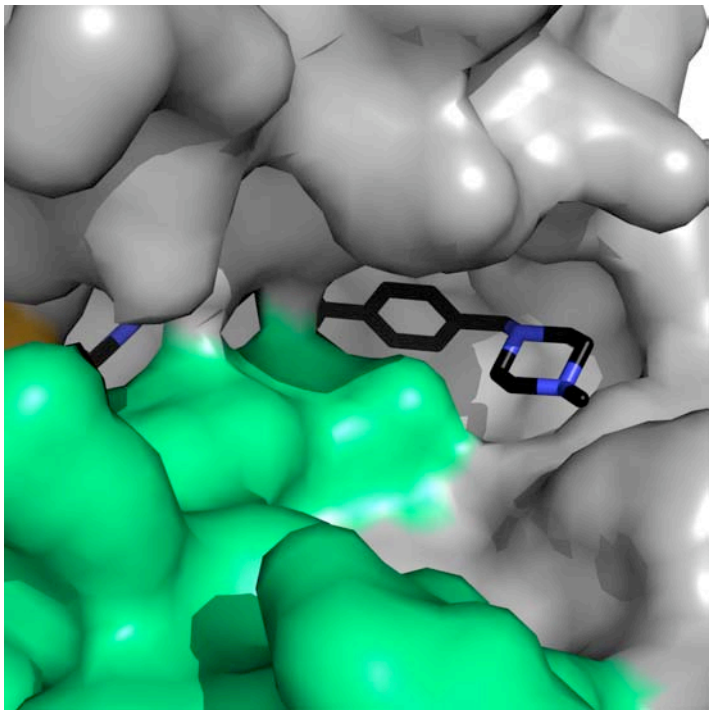


Gleevec binds Abl in the INACTIVE conformation!

The **specificity** of Gleevec for Abl relies on the binding of Gleevec to the inactive form and the differences between the inactive forms of Abl and other protein kinases.

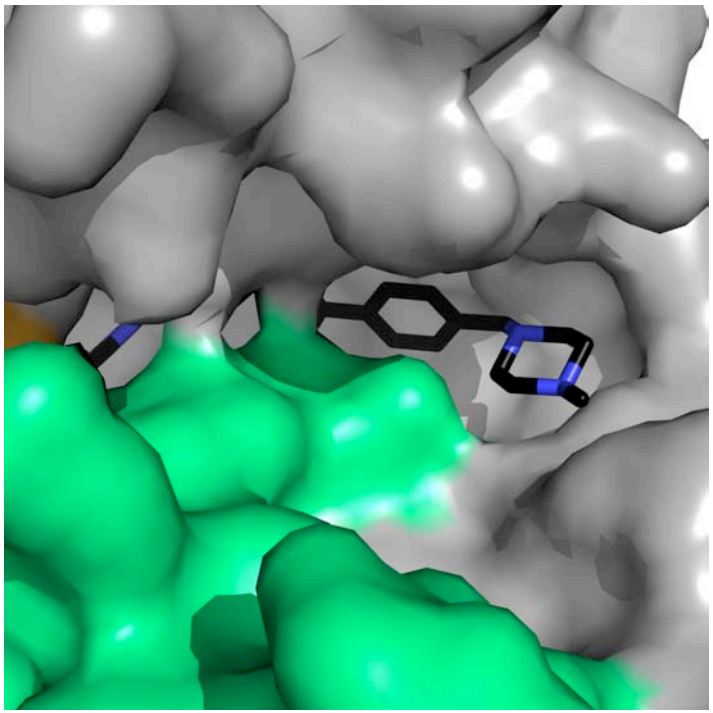
Another look at the binding pocket in the inactive Abl kinase domain:





Side note: piperazine rings in pharmaceuticals

Piperazine rings are often included in drugs to increase solubility.



While the ring may participate in H-bonds with the target protein, it is often solvent exposed, and in many cases does not contribute to the drug binding.

If Bcr-Abl is constitutively active, how can Gleevec bind to the Bcr-Abl kinase domain in CML cells?

Possibilities include:

1) The orientation of the activation loop is dynamic, transiently passing through an inactive conformation that can bind Gleevec.

2) The Gleevec "traps" the Bcr-Abl protein as it is translated, prior to taking on the active conformation

Kinase Domains: Structure and Inhibition

I. Conserved and variable features of kinase domains

   A. Structural similarities

   B. Active and inactive forms

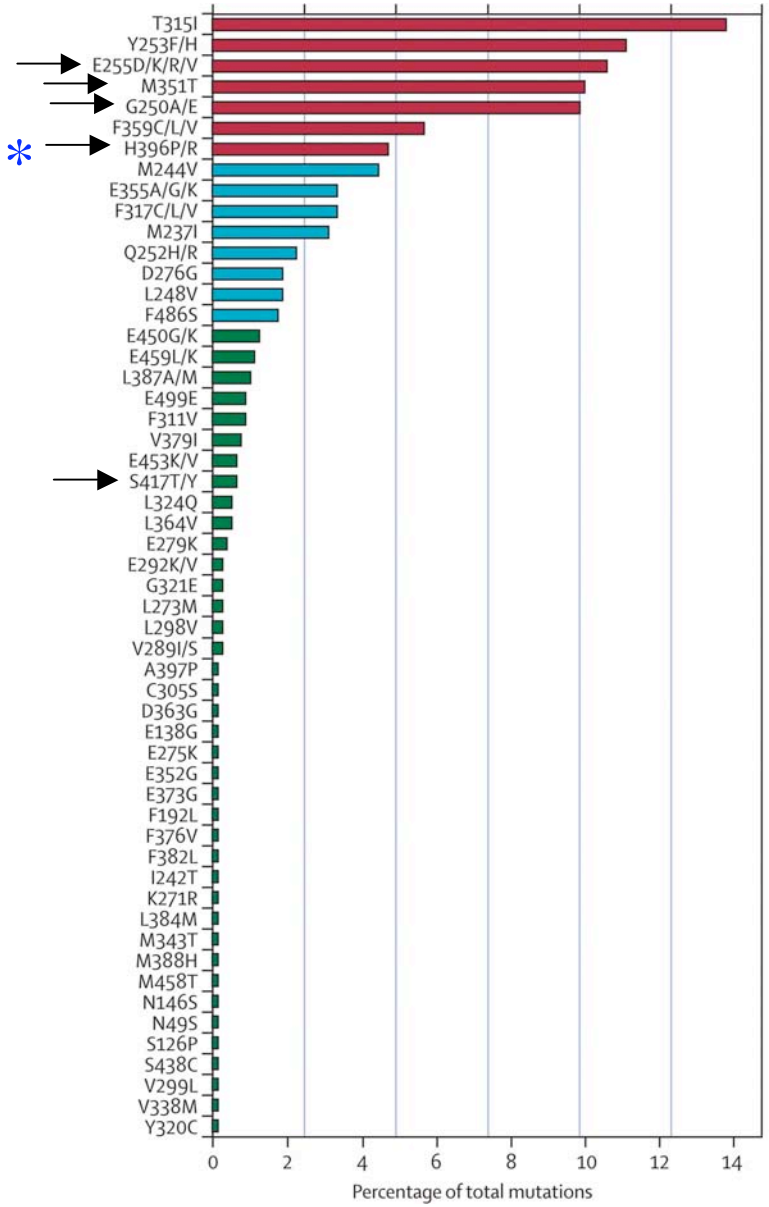
II. Abl and Bcr-Abl inhibition by Gleevec

III. Gleevec resistance in Bcr-Abl mutants

   A. Direct interference with Gleevec binding

   B. Destabilization of the inactive form

Our class selected target mutants that include some of the most prevalent mutations found in CML patients.



Apperley, J. F. *Lancet Oncol* **8**, 1018-1029 (2007)

Common mutations in patients with chronic phase (early) CML:

M244, L248, F317, H396, S417

Common mutations in patients with advanced phase CML:

Q252, Y253, E255, T315, E459, F486

A 2006 study comparing the kinase activity of 5 common mutations found:

T315I, M351T, and H396P < wt

E255K comparable to wt

Y253F > wt

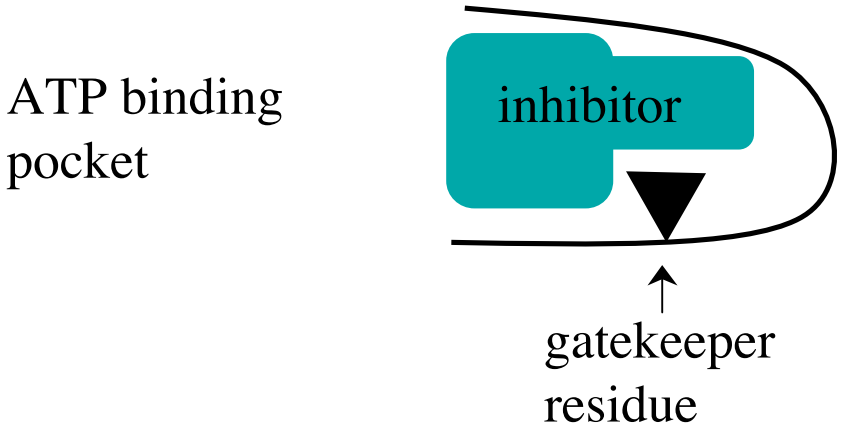
How can single amino acid mutations in Bcr-Abl confer Gleevec resistence?

- Directly interfere with Gleevec binding (ie. sterics)
- Destabilize the inactive (Gleevec binding) conformation of Abl

Gatekeeper residue in the ATP binding pocket

Kinase domains contain a gatekeeper residue that partially or fully blocks a hydrophobic region deep in the ATP binding pocket.

The gatekeeper residue contributes to the selectivity of kinases for small molecule inhibitors.

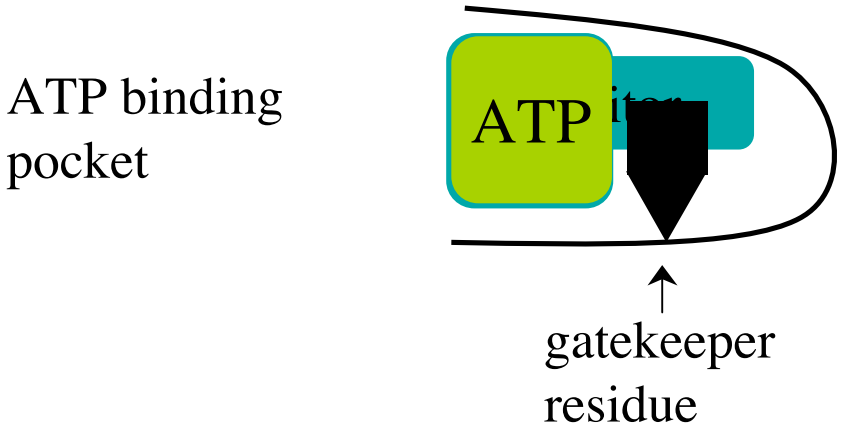


A small gatekeeper residue allow an inhibitor to access the “gated” hydrophobic regions of the binding pocket.

Gatekeeper residue in the ATP binding pocket

A larger residue sterically blocks inhibitor binding.

ATP binding is not affected because ATP does not access that part of the binding pocket.



Gatekeeper residue in the ATP binding pocket

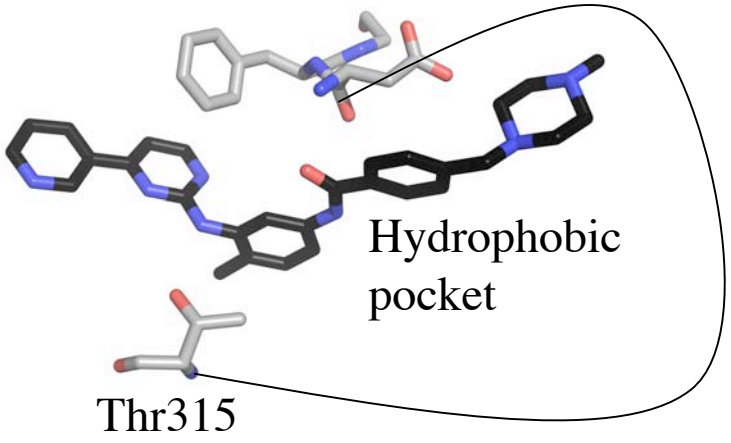
The gatekeeper residue is a conserved Thr in 20% of all human kinases.

Thr315 in Abl

Thr338 in Hck

Thr106 in p38

Thr766 in EGFR



In other kinases, the gatekeeper residue has a bulkier side chain compared to Thr, and this controls the kinase's sensitivity to small molecule inhibitors that bind in the ATP pocket.

Gatekeeper residue in the ATP binding pocket

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Thr315 in Abl

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Thr106 in p38

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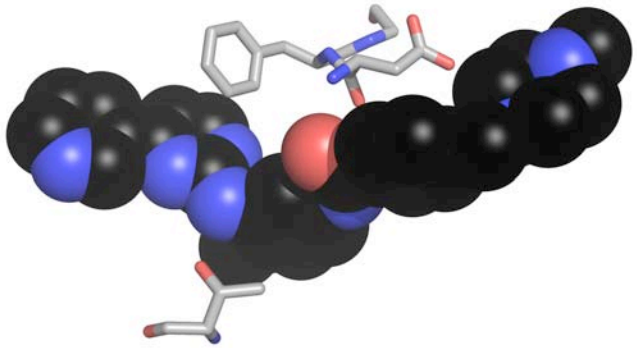


Mutations from Abl Thr 315 to a bulkier residue block penetration past the gatekeeper and and confer Gleevec resistance.

Mutations identified for Thr315

Question: what residues are bulkier than Thr and can be accessed with a single base pair substitution?

Thr 315 is coded by ACT

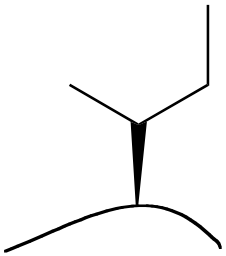
Ala/A	GCU, GCC, GCA, GCG	Leu/L	UUA, UUG, CUU, CUC, CUA, CUG
Arg/R	CGU, CGC, CGA, CGG, AGA, AGG	Lys/K	AAA, AAG
Asn/N	AAU, AAC	Met/M	AUG
Asp/D	GAU, GAC	Phe/F	UUU, UUC
Cys/C	UGU, UGC	PrøP	CCU, CCC, CCA, CCG
Gln/Q	CAA, CAG	Ser/S	UCU, UCC, UCA, UCG, AGU, AGC
Glu/E	GAA, GAG	Thr/T	ACU, ACC, ACA, ACG
Gly/G	GGU, GGC, GGA, GGG	Trp/W	UGG
His/H	CAU, CAC	Tyr/Y	UAU, UAC
Ile/I	AUU, AUC, AUA	VałV	GUU, GUC, GUA, GUG
START	AUG	STOP	UAG, UGA, UAA

Mutations identified for Thr315

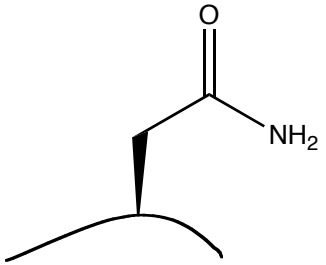
Question: what residues are bulkier than Thr and can be accessed with a single base pair substitution?

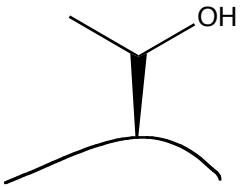
Thr 315 is coded by ACT

Ile (ATT)



Asn (AAT)





The Thr315Ile Abl mutant demonstrates high kinase activity even in the presence of μM conentrations of Gleevec (STI571)

Image removed due to copyright restrictions.

See Fig. 4 in Mercedes, E. B. et al. "Clinical Resistance to STI-571 Cancer Therapy Caused by BCR-ABL Gene Mutation or Amplification." *Science*. 293 (2001): 876-880.

Mercedes, E. G. et al. *Science* **293**, 876-880 (2001)

The Thr315Ile Abl mutant demonstrates high kinase activity even in the presence of μM conentrations of Gleevec (STI571)

The Thr315Ile mutation makes up ~13% of reported Bcr-Abl mutations.

Other mutants that interact directly with Gleevec (but not ATP) are F317 and F359.

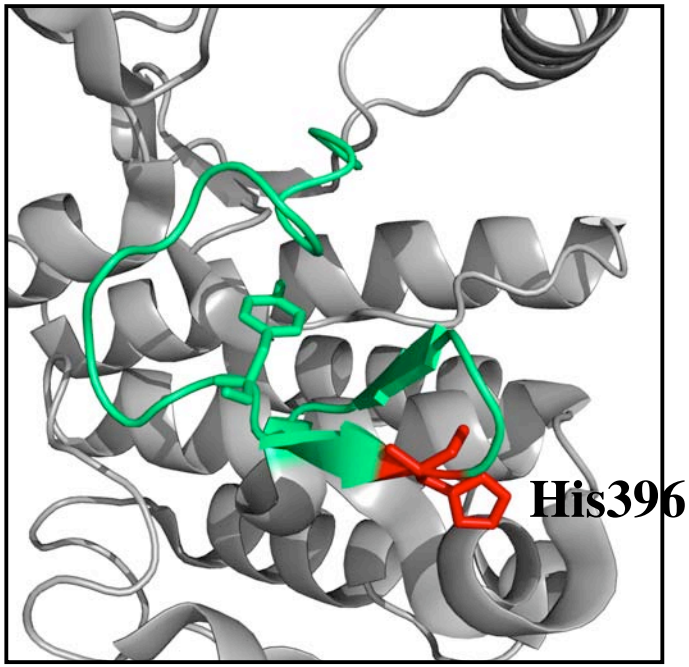
Mutations in these two residues make up a combined total of 14% of all reported Bcr-Abl mutations.

The majority of mutations result in a destabilization of the inactive (Gleevec-binding) form of the Abl kinase domain

- Mutations found within the A-loop (381-402) of the C-lobe can destabilize or prevent rearrangement to the inactive conformation of that loop.

The majority of mutations result in a destabilization of the inactive (Gleevec-binding) form of the Abl kinase domain

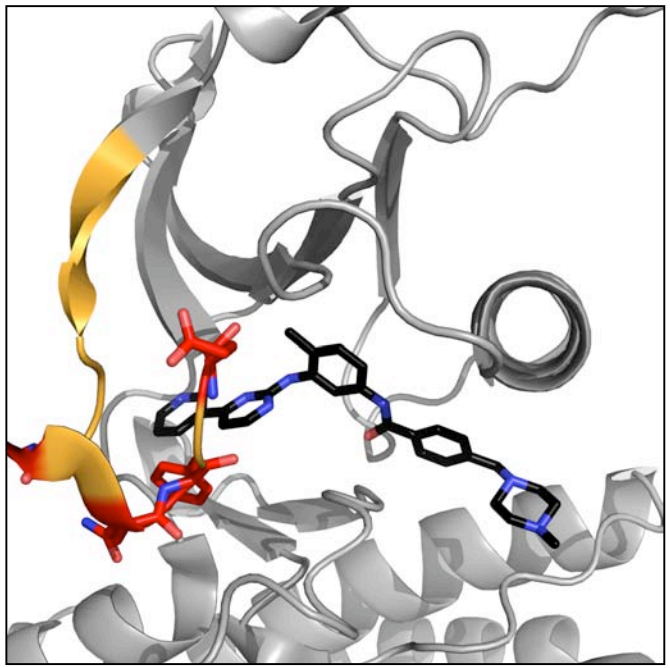
- Mutations found within the A-loop (381-402) of the C-lobe can destabilize or prevent rearrangement to the inactive conformation of that loop.



This includes the H396P mutant that you are working with in lab.

The majority of mutations result in a destabilization of the inactive (Gleevec-binding) form of the Abl kinase domain

- P-loop mutants may destabilize the inactive conformation of the P-loop.



Mutants have been identified for every X residue in the P loop consensus sequence, GXGXXGX:

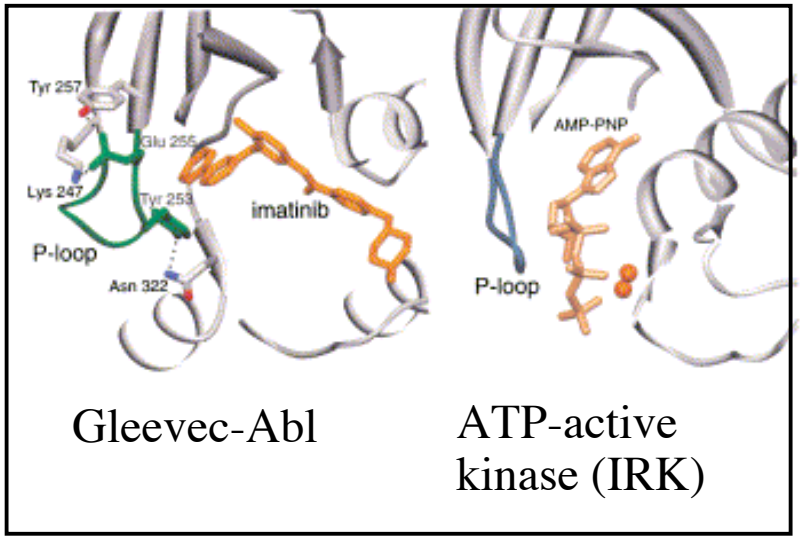
Gly250, Gln(Q)252, Tyr253, Glu(E)255

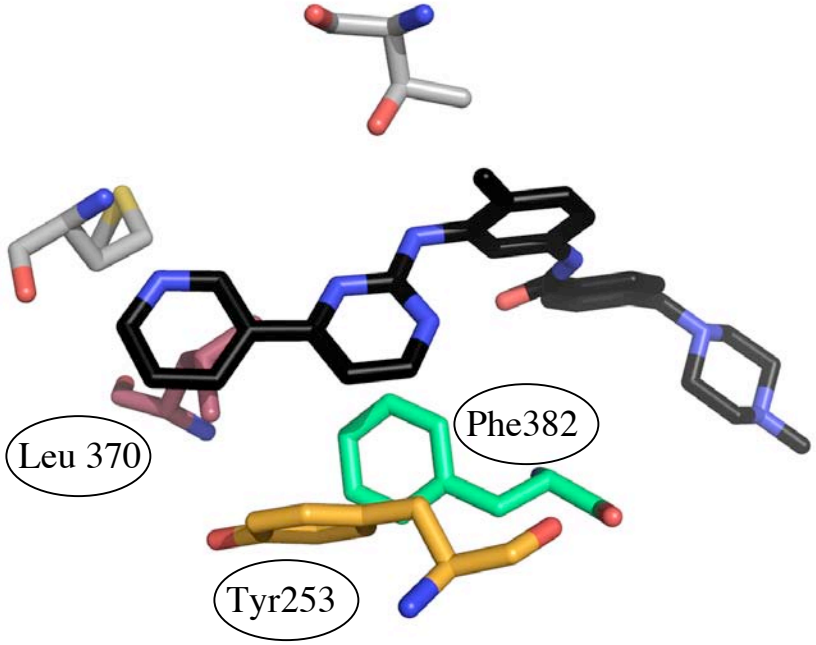
The majority of mutations result in a destabilization of the inactive (Gleevec-binding) form of the Abl kinase domain

Example 1:

Tyr253 mutations result in the loss of a loop-stabilizing H-bond with the carboxy group of Asn322.

Recall that Tyr253 also forms part of the hydrophobic cage for Gleevec.



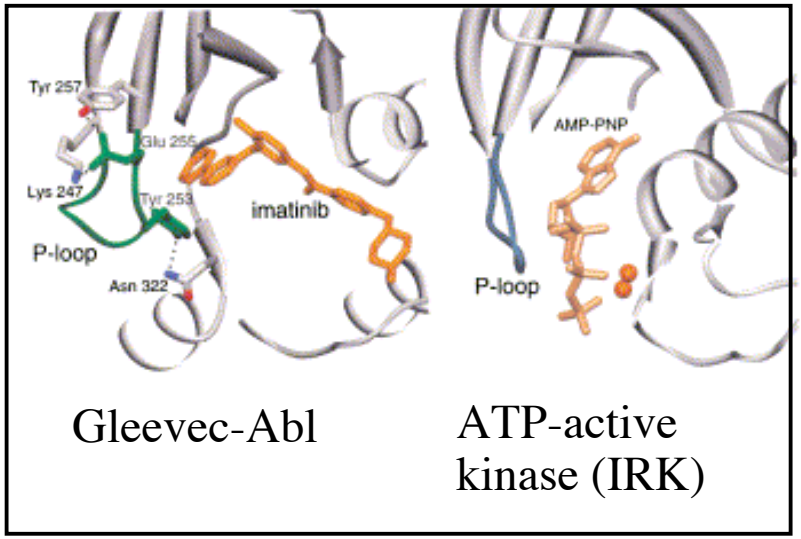


P-loop models from
Shah, N. P. et al. *Cancer Cell* **2**, 117-125 (2002)

The majority of mutations result in a destabilization of the inactive (Gleevec-binding) form of the Abl kinase domain

Example 2:

E255 mutations can similarly disrupt hydrogen bonds that stabilize the distorted (Gleevec binding) P-loop conformation.



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