5.36 Biochemistry Laboratory
Spring 2009

5.36 Lecture Summary #4 Thursday, February 26, 2009

CI-M assignment: The first draft of your minireview is due on Thursday, March 5th at noon on the MIT class website.

Next Laboratory Session: #7 and 8

Topics: **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 conformation

I. CONSERVED AND VARIABLE FEATURES OF KINASE DOMAINS

A) STRUCTURAL SIMILARITIES

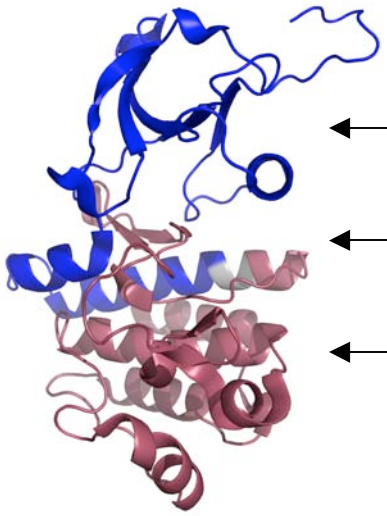
The catalytic domain (or _____ domain) of eukaryotic protein kinases is highly _____ both in sequence and structure.

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

Kinase domains have a _____ structure composed of an

N-lobe (amino lobe) that
- contains a 5-stranded beta sheet and an alpha helix (_____).
- comprises residues _____ to _____ of Abl (shown here).
- contributes to ATP binding.

and a **C-lobe** (carboxy lobe) that
- is made up of multiple alpha helices.
- comprises residues _____ to _____ of Abl (the larger lobe).
- is the location of peptide _____ binding.



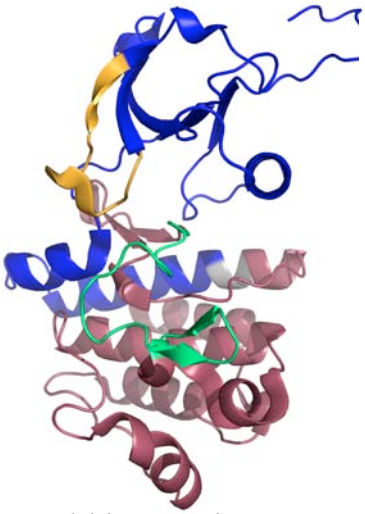
The **hinge** region (between the two lobes) contains several conserved residues that provide the catalytic machinery and make up an essential part of the _____ binding pocket. Among all kinases, Mg-ATP binding is primarily in the _____-lobe and hinge region.

ATP Binding (___) Loop (shown in orange)

- 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.

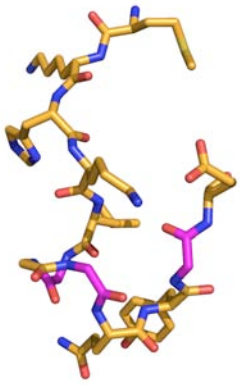
Color scheme for individual atoms:
oxygen (red), nitrogen (blue), carbon (background color), sulfur (yellow), P (orange)

- 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 MKHKL___G___QY___E.



Abl kinase domain

P-loop (orange)



(conserved residues in magenta)

A-loop (green)

Activation (A) Loop (shown in green)

- a principal _____ structure for modulating kinase activity. In the closed form (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 _____-_____-_____ (DFG) motif implicated in ATP binding is located at the N-terminus of the A-loop.

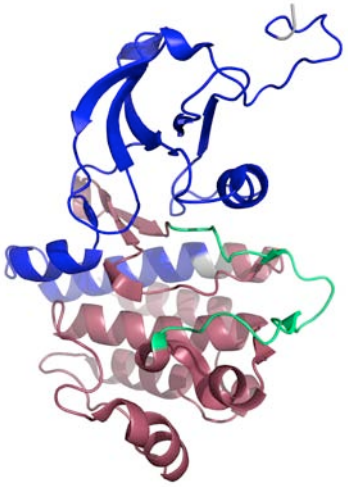
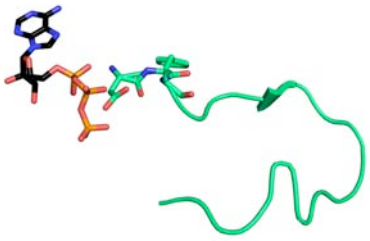
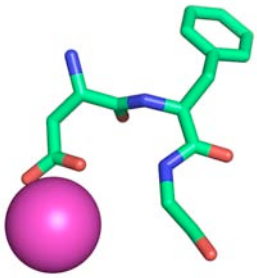
Numbering in the Abl and Bcr-Abl kinase domain:

- **N-lobe**: Abl residues 225-350
  - ➡ **P-loop**: residues _____-_____
- **hinge region**: interface of N and C lobes
- **C-lobe**: 354-498
  - ➡ **A-loop:** residues _____-_____
  - ➡ **DFG motif:** residues _____-_____

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

A) ACTIVE AND INACTIVE FORMS OF PROTEIN KINASES

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

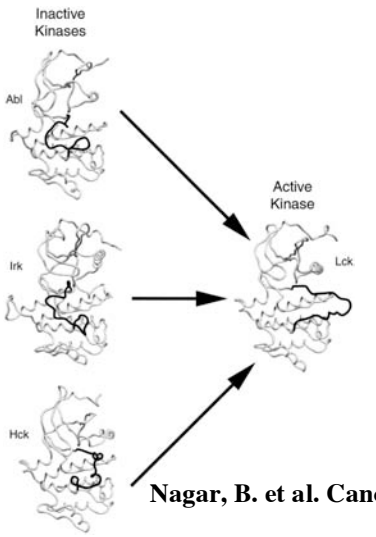
Abl kinase domain ACTIVE conformation	ATP binding orientation with the A loop *(shown with cAPK)*	Asp-Phe-Gly motif
		

Features of an open or _____ 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 _____ side chain interacts with the _____ coordinated to the phophate groups of ATP.

"Happy families are all alike; every unhappy family is unhappy in its own way."
-Tolstoy

 = activation loop

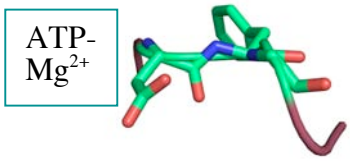
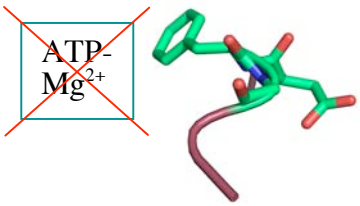
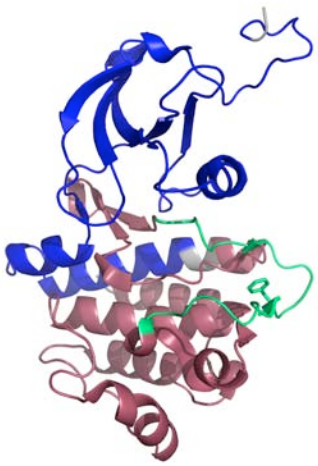
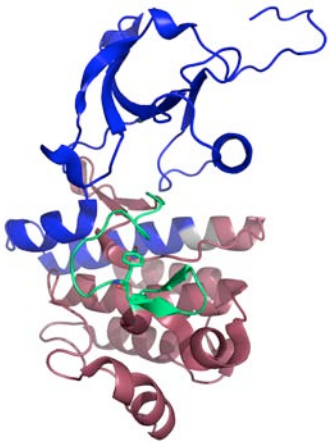


Nagar, B. et al. Cancer Res 2002;62:4236-4243

_____ kinase domains are all alike; every _____ kinase domain is _____ 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 ~_____°.

Abl kinase domain ACTIVE conformation	Abl kinase domain INACTIVE conformation	DFG orientation ACTIVE conformation  INACTIVE conformation 
		

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 _____ is unique to Abl and only a few other kinase subfamilies.

Also, in the inactive form, the A-loop blocks the substrate binding region of the C-lobe. Specifically, Tyr393 mimics the _____ Tyr (to be phosphorylated) on the substrate. Tyr393 is typically phosphorylated in the active form, and it is not phosphorylated in the inactive form.

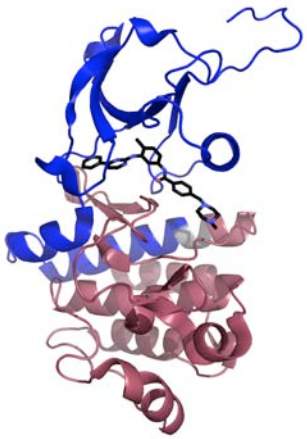
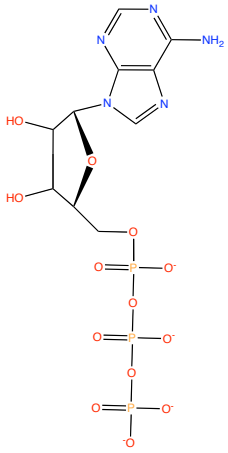
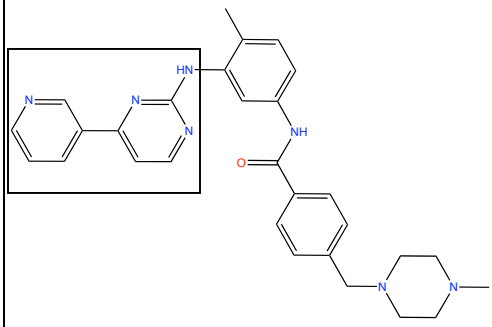
II. ABL AND BCR-ABL INHIBITION BY GLEEVEC

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

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

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

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

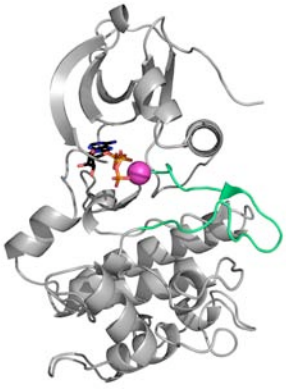
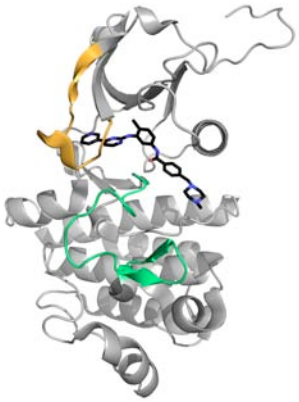
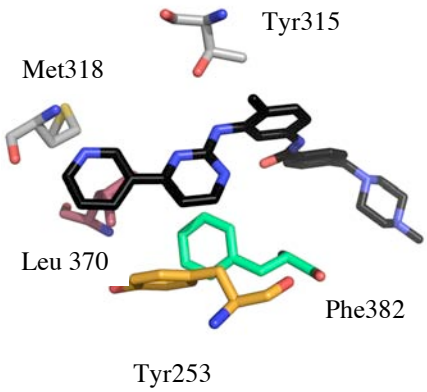
Gleevec-Abl complex	ATP	Gleevec
		

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

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 _____. Gleevec has a unique position in the binding pocket. *(Note: You will identify the additional Abl-Gleevec H-bonds using PyMol in lab session 15.)*

There is _____ overlap in ATP and the Gleevec binding to the Abl kinase domain.

ATP-kinase complex (shown with cAPK)	Gleevec-Abl complex	Hydrogen bonds and hydrophobic "cage"
		

The Gleevec molecule penetrates deeper into the _____ core of the

ATP binding site compared to ATP. 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 (_____) and A-loop (_____).

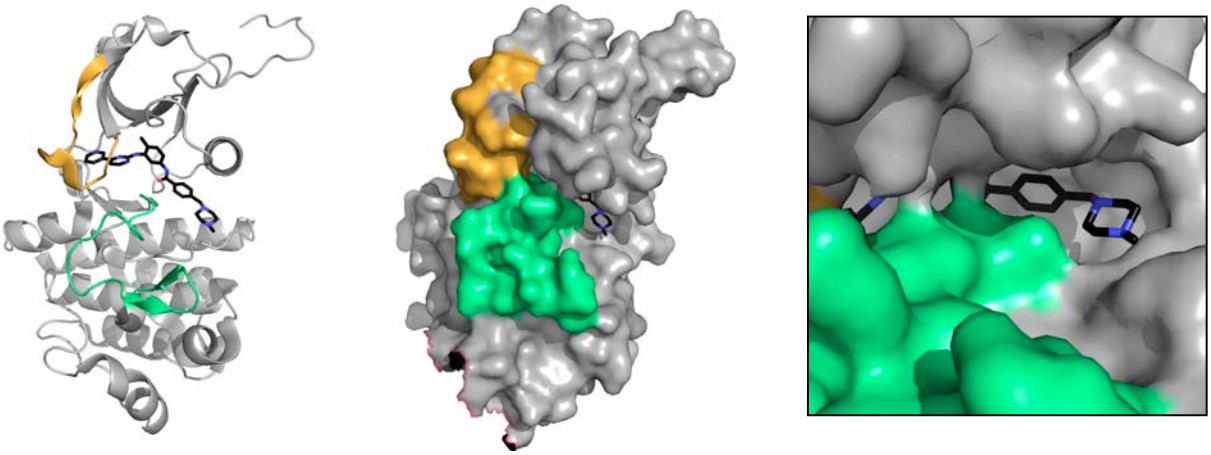
Phe382 is part of the conserved _____ motif. The Phe382 orientation toward the pyrimidine ring is critical for Gleevec binding.

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 (see figures on page 4).

Gleevec binds Abl in the _____ conformation!

The _____ 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 form of the Abl kinase domain:



Side note: 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.

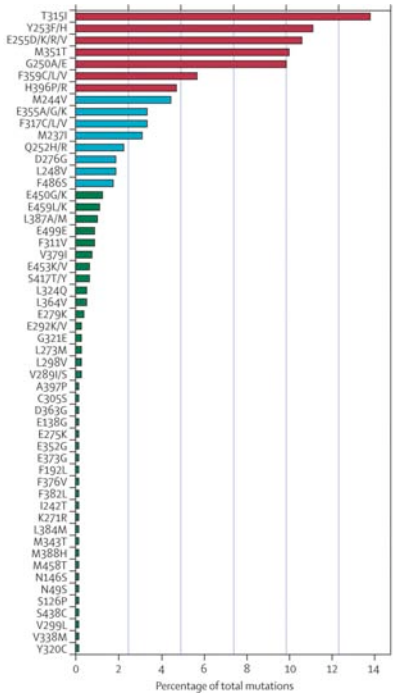
If Bcr-Abl is constitutively active, how can Gleevec bind to the Bcr-Abl kinase domain in CML cells?

Possibilities include:

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

2. The Gleevec "_____" the Bcr-Abl protein as it is _____, prior to taking on the active conformation

III. GLEEVEC RESISTANCE IN BCR-ABL MUTANTS



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

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

M244, L248, F317, ____396, ____417

Common mutations in patients with _____ phase CML:

Q252, Y253, _____255, T315, E459, F486

A 2006 study comparing the kinase activity of 5 common mutations with wild type (wt) Bcr-Abl found:

T315I, ____351____, and H396P < wt
E255K comparable to wt
Y253F > wt

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

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

A) DIRECT INTERFERENCE WITH GLEEVEC BINDING

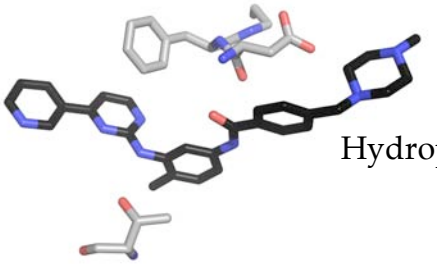
Kinase domains contain a _____ residue that partially or fully blocks a hydrophobic region deep in the ATP binding pocket.

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

A small gatekeeper residue allow an inhibitor to access the "gated" hydrophobic regions of the binding pocket. A larger residue _____ blocks inhibitor binding.

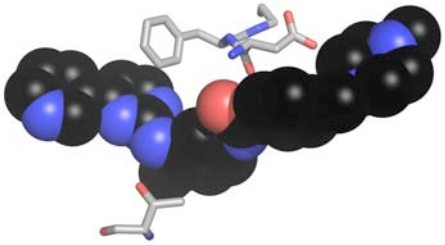
ATP binding is not affected because ATP does not access the "gated" part of the binding pocket.

The gatekeeper residue is a conserved _____ in _____% of all human kinases. This is residue _____ in Abl



Hydrophobic pocket

gatekeeper (Thr315)



gatekeeper (Thr315)

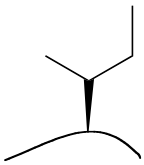
In some kinases, the gatekeeper residue has a bulkier side chain compared to Thr, and this precludes the binding of small molecule inhibitors in the hydrophobic pocket.

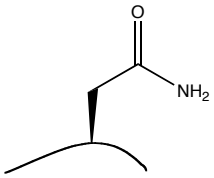
Abl mutations at the gatekeeper position (315) from a Thr to a bulkier residue block inhibitor penetration past the gatekeeper and and confer Gleevec resistance.

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	Pro/P	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	Val/V	GUU, GUC, GUA, GUG
START	AUG	STOP	UAG, UGA, UAA





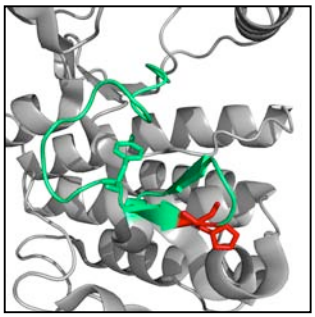
The Thr315_____ Abl mutant demonstrates high kinase activity even in the presence of 10 μM Gleevec (STI571).

The T315I mutation makes up ~13% of reported Bcr-Abl mutations.

Other mutants that interact directly with Gleevec (but not ATP) include F317 and F359. Those two mutants make up a combined total of 14% of all reported Bcr-Abl mutations.

B) DESTABILIZATION OF THE INACTIVE CONFORMATION

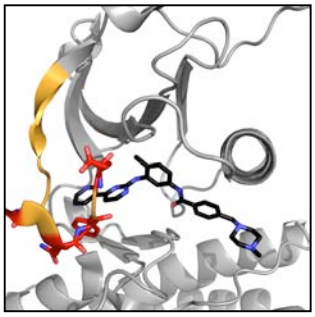
The majority of mutations result in a _____ of the _____ (Gleevec-binding) form of the Abl kinase domain.



A-loop mutations

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 _____ mutant that you are working with in lab.



P-loop mutations

P-loop mutants may destabilize the inactive conformation of the P-loop (residues 244-255). Mutants have been identified for every X residue in the P loop consensus sequence, GXGXXGX: _____250, Gln(Q)252, Tyr253, _____(E)255.

Ie. Tyr253 mutations result in the loss of a loop-stabilizing H-bond with the carboxy group of Asn322. In addition, the Tyr253 forms part of the hydrophobic cage for Gleevec (see additional figure on p. 5).