



Cancer and Tyrosine Kinase Inhibition

Tyrosine Kinase Inhibition

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Motivation (I)

- In first world countries cancer is the second most common cause of death after cardiovascular diseases.
- For most tumors, treatment is limited to surgery, radiation, or chemotherapy.
 - Radiation and chemotherapy have high toxicity and serve as a treatment when no other option can be found.
- More research is needed to identify the causes of cancer at the molecular level so that more effective therapies can be developed.

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Motivation (2)

- Fighting cancer at the molecular level:
 - In this unit we examine a promising approach to **molecular cancer therapeutics**.
 - The target proteins of these therapies will provide running examples for the rest of the course.
 - The study of these proteins is very interesting from a structural bioinformatics perspective.
 - Lots of data! The PDB contains many structures that are protein targets or are enzymes that interact with these targets.
 - Understanding the structure/function dependencies will require that you recall several topics from earlier courses, for example: exon splicing, post-translational modification, domain interactions, cellular localization, etc.

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Introduction (1)

- Neoplasms (new growth) may be benign or malignant.
 - Benign tumors are usually localized and not harmful.
 - Malignancies are characterized by autonomous cell proliferation and possibly metastatic behavior.
 - Autonomous: proliferation without regulation
 - Metastatic: the ability to travel and invade other (different) tissues.
- Lack of regulation of proliferation may be due to aberrant behavior of signaling molecules.
 - Normally, cell proliferation is controlled by a complicated series of events involving interactions between signal proteins and other molecules.

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Introduction (2)

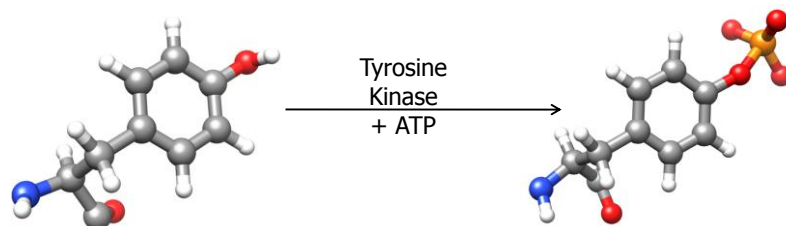
- Signaling molecules include the tyrosine kinases.
 - They are involved in both normal and abnormal cell proliferation.
 - A tyrosine kinase is an enzyme that catalyzes the phosphorylation of some particular tyrosine residue in a target protein.
 - Phosphorylation controls many cellular processes:
 - progression through the cell cycle
 - cell migration
 - metabolism
 - proliferation
 - differentiation

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Introduction (3)

- Tyrosine kinase functionality
 - Phosphorylation: A kinase can transfer a phosphate group from ATP to a tyrosine residue.
 - This is important as one of the mechanisms for signal transmission within a cell.

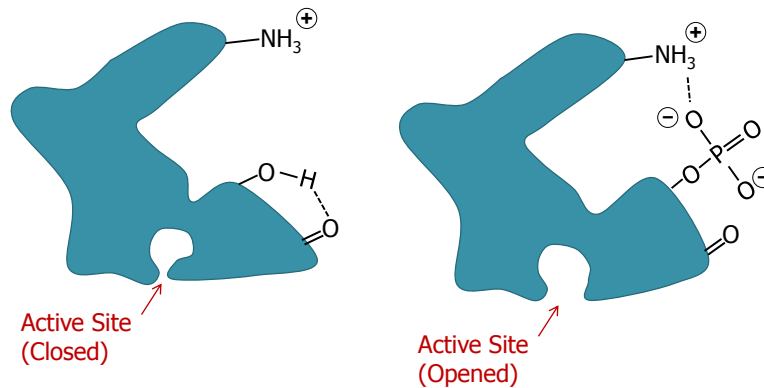


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Introduction (4)

- Phosphorylation can produce a change in conformation:



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Kinase Facts

- There are 518 known kinases in the human genome.
 - About 2% of all genes encode for kinases.
- The average sequence identity for any two kinases is about 30% in the catalytic domain.
- More than 90 tyrosine kinases are known.
 - More than half of these are transmembrane receptors.
 - The rest are cytoplasmic non-receptors.
 - You can think of tyrosine kinases as “relay points” for signal pathways in the cell.
 - The cytoplasmic group includes nuclear kinases.
 - We will focus on the c-Abl (Abelson) nuclear tyrosine kinase.

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The c-Abl Tyrosine Kinase

- The Abelson tyrosine kinase (c-Abl) is localized at several sites within the cell.
 - Sites include: nucleus, cytoplasm, mitochondria, and endoplasmic reticulum.
- c-Abl interacts with several cellular proteins:
 - For example: cell-cycle regulators, cytoskeletal proteins, kinases, phosphatases, signaling adaptors, and transcription factors.
- c-Abl is involved with various cellular processes including: regulation of cell growth, DNA-damage response, and cell migration.

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c-Abl Regulation

- Because of these important functional roles in the cell it is necessary that c-Abl is precisely controlled with respect to both its localization and timing of kinase activity.
 - We start by reviewing the normal structure of c-Abl and then consider aberrant structure leading to cancer.

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Normal Generation of c-Abl

- The generation of a normal c-Abl protein requires three final steps:
 1. N-terminal methionine removal by means of methionine aminopeptidase (MetAP or MAP).
 2. Myristoylation by means of N-myristoyltransferase (Nmt).
 3. The myristoyl group interacts with the kinase domain to help provide regulation by **auto-inhibition**.

More details on the next three slides:

I) c-Abl Methionine Removal

- MAP operates on the N-terminal of the nascent protein, removing the methionine residue before the protein is fully formed (Selmer & Liljas, 2008).

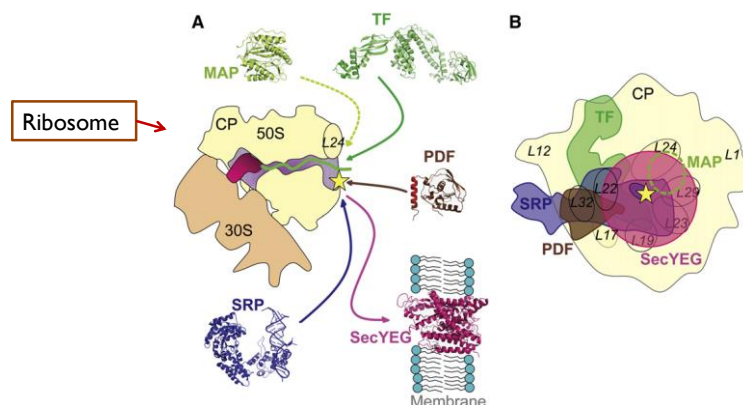
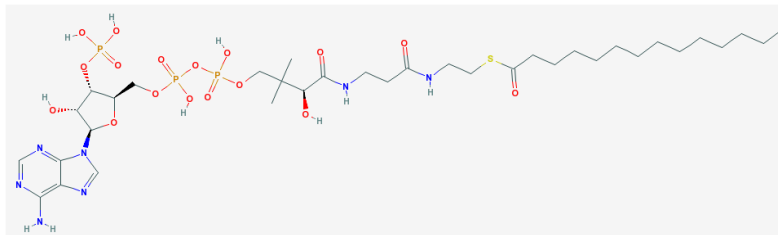


Figure 1. The Battle Field at the Ribosome Polypeptide Exit Site Where Numerous Proteins Compete for the Nascent Chain

2) Myristoylation (1)

- N-myristoylation is the attachment of a 14-carbon fatty acid (called myristate) onto the N-terminal glycine residue of a target protein.
- Myristoyl-CoA (Myristoyl Coenzyme A)

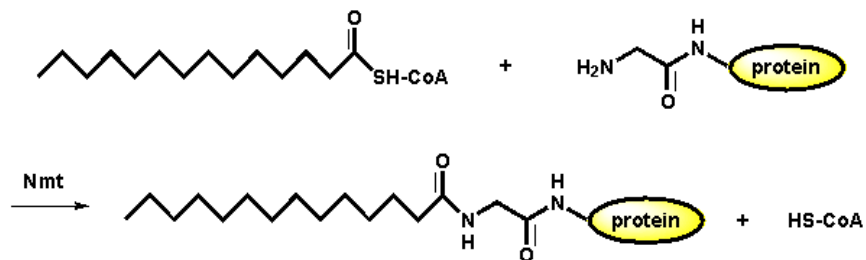


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2) Myristoylation (2)

- Myristoylation is done by the enzyme N-myristoyltransferase (Nmt):

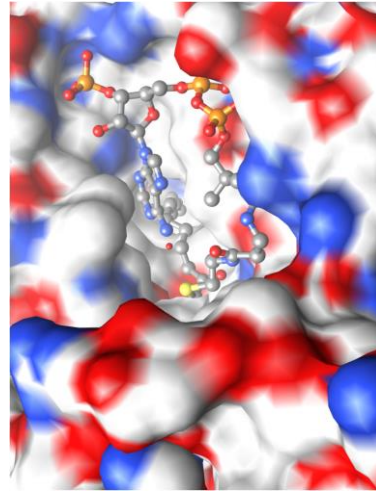
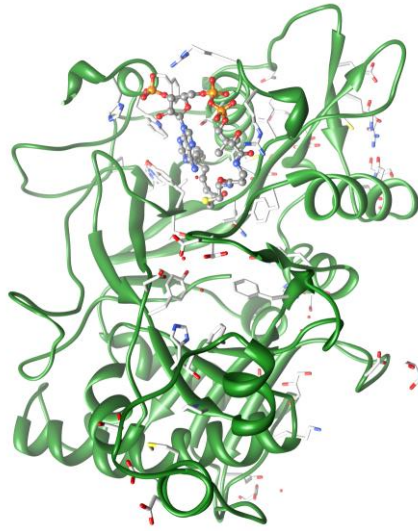


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2) Myristoylation (3)

- N-myristoyltransferase (Nmt) with myristoyl-CoA:

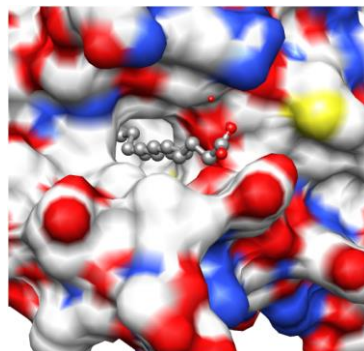


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3) Autoinhibition by the myristoyl group (1)

- Myristoylation is often used for protein localization, the myristoyl group providing an anchor within a cell membrane.
- In the case of Abl, the group is inserted into a pocket of Abl and this insertion acts as an inhibitor.

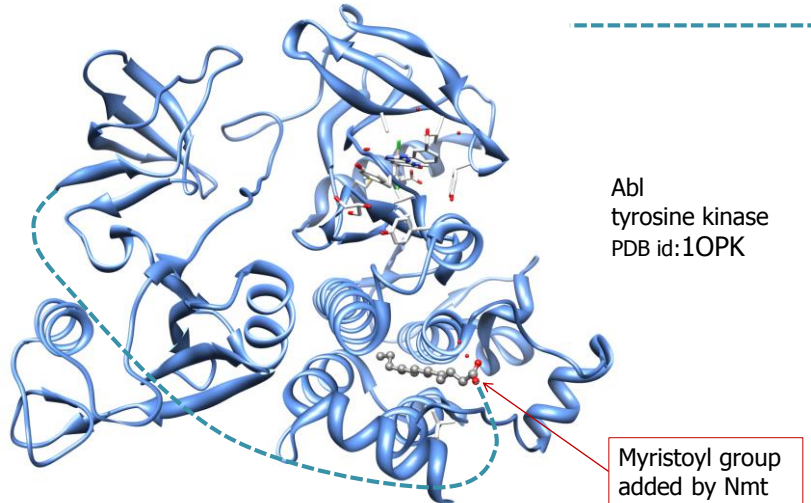


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3) Autoinhibition by the myristoyl group (2)

- The residues upstream from the myristoyl group cannot be resolved by the x-ray analysis.



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Chronic Myelogenous Leukemia

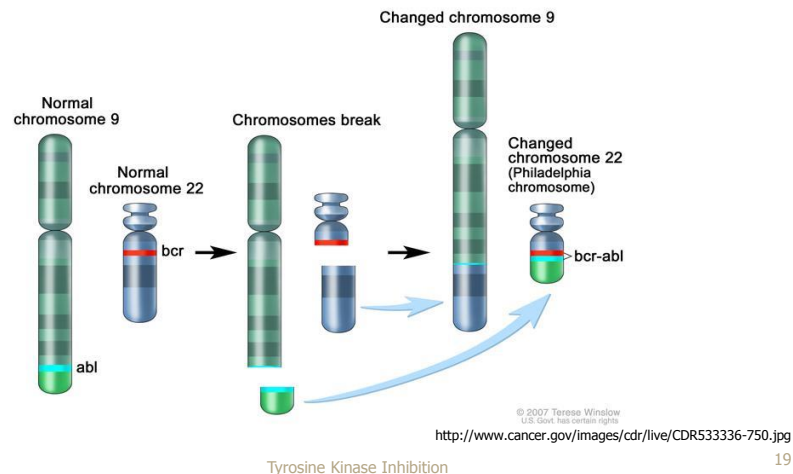
- CML is a cancer of the white blood cells.
 - Incidence is about 3 per 200,000 people (5000 new cases each year in the U.S.A.).
 - It represents about 17% of all cases of adult leukemia.
 - It can be induced by exposure to ionizing radiation.
- At the molecular level, the most common cause of CML relates to the formation of an Abelson tyrosine kinase that does not have the auto-inhibitory myristoyl group.
 - How does this happen?

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CML is Caused by an Oncogene

- About 95% of CML cases are due to a chromosomal abnormality caused by a reciprocal translocation between chromosome 9 and 22:



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The Bcr-Abl Fusion Protein

- Expression of the oncogene leads to the formation of Bcr-Abl, a fusion protein that does not have the myristoylated N-terminus.
 - No myristoyl group → no auto-inhibition and so the protein becomes **constitutively active** (i.e. it works without activation signals from other proteins).

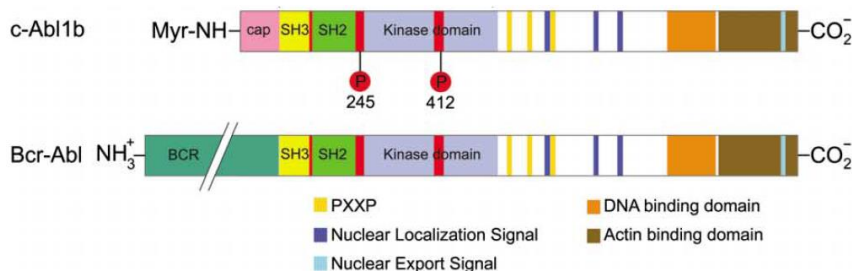


Diagram reference: Nagar et al., Structural basis for the autoinhibition of c-Abl tyrosine kinase, Cell, (112) 2003, 859-871.

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Therapy for CML

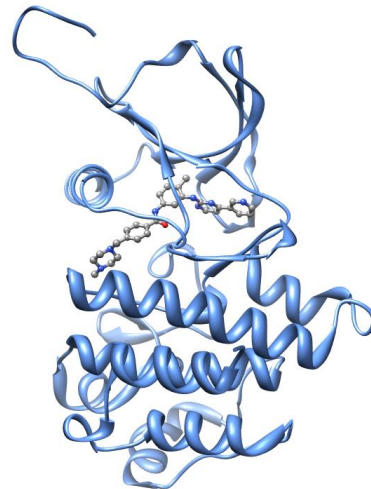
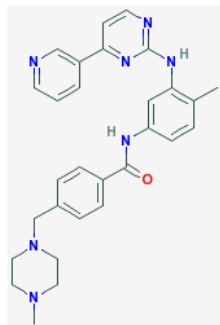
- About 15 years ago, Novartis developed a drug called imatinib mesylate also known as Gleevec (in the USA) or Glivec (in the UK).
- Imatinib is designed to interact with Bcr-Abl and inhibit its activity (of course, it will also inhibit normal Abl).
- This is an exciting advance in cancer therapy because it acts as “the magic bullet” for a particular cancer.
 - Prior to this, chemotherapies involved heavy handed “molecular sledgehammers” that cut back on *all* rapidly proliferating cells.

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Imatinib Mesylate

- Even though Abl is structurally similar to other kinases, imatinib shows a strong preference for Abl with very few side-effects.



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The New Paradigm

- Imatinib and similar drugs have saved thousands of lives.
 - They do not cure the cancer but make it more manageable.
 - Patients stay on the drug for the remainder of their lives.
 - There is the possibility that a mutation in the Philadelphia oncogene produces a fusion protein that is resistant to imatinib.
- Research is now underway to find more kinase targets that will yield to inhibitory drugs.

More Molecular Therapies for Cancer? (1)

- Can this success story be repeated for other cancers? Hopefully: Yes, but CML has attributes that make it an easy candidate for therapeutic intervention.
 - The correlation between CML and a particular malformed protein is very high.
 - Some cancers do not have such an obvious cause.
 - For example, AML (Acute Myeloid Leukemia) is a heterogeneous disease: mutations can occur in any of several tyrosine kinases.

More Molecular Therapies for Cancer? (2)

- Mutations giving gain of function are easier to correct than mutations giving loss of function.
 - Bcr-Abl is a fusion protein that results in overactive functionality. The corrective therapy requires an inhibitor.
 - Mutations in a tumor suppressor protein (such as p53) cause loss of function. The corrective therapy requires restoration of functionality. This is a much harder problem.
- Specificity of inhibition is crucial.
 - Researchers were surprised to find that imatinib binds almost exclusively with Abl and Bcr-Abl even though these kinases have high sequence similarity to other kinases.
 - So, there are relatively few side effects (for adults).
 - Exposure to imatinib during pregnancy carries an increased risk of serious fetal abnormalities or spontaneous abortion.

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References

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- Wright, M.H., Heal, W.P., Mann, D.J., Tate, E.W., Protein myristoylation in health and disease. *J. Chem. Biol.* (3) 2010, 19-35.
- Website for Nmt: <http://mendel.imp.ac.at/myristate/SUPLalignment.htm>

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