Bio-Molecular Model Building

Exam Exercise

Spring 2015

Author:
Cedric Lood
Yi Ming Gan

Supervisors:
Prof. M. De Maeyer
dr. J. De Raeymaecker
dr. X. Qing

May 16, 2015

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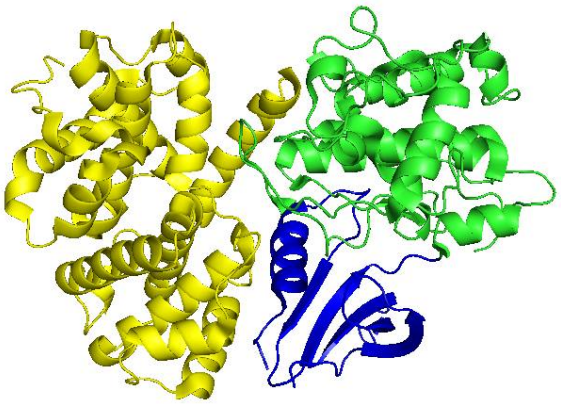
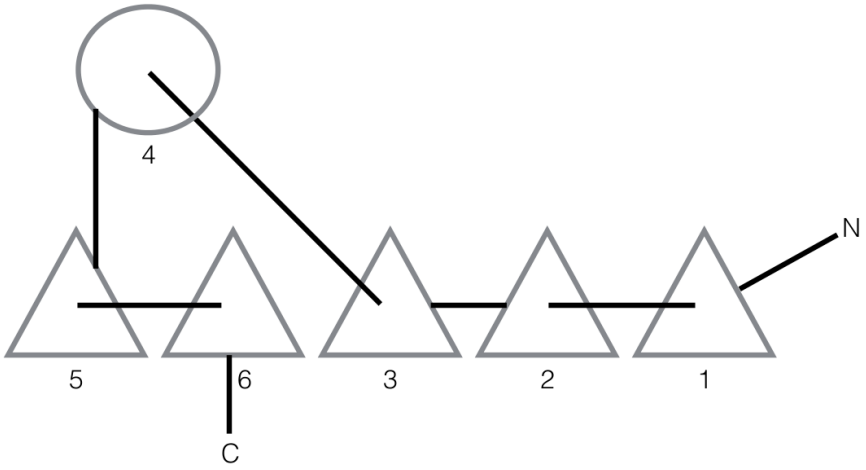
1 Question 1 - Kinases

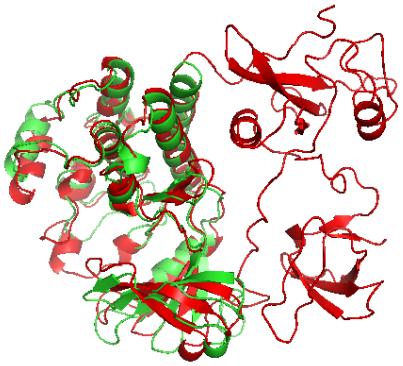
1.1 Part a



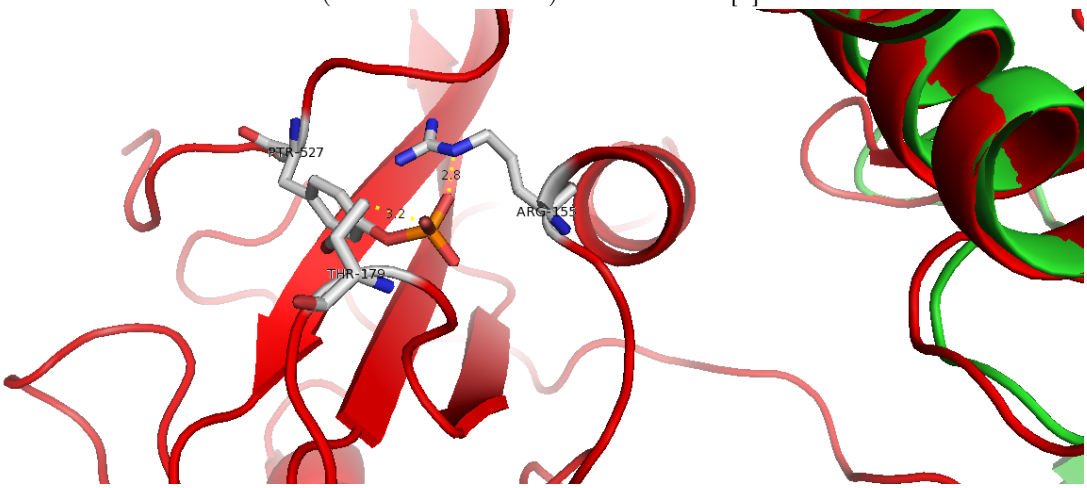
1.2 Part b



2 Question 2 - Kinase active/inactive forms

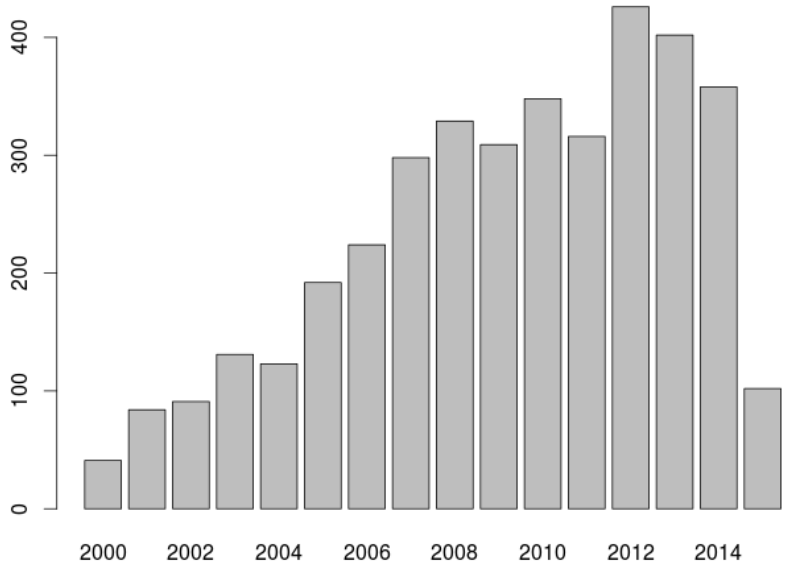


The role of regulatory domains in the kinases are to induce conformational changes that switch the kinase from one form (inactive or active) to the other [1].



3 Question 3

```
> table(years)
years
2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015
  41   84   91  131  123  192  224  298  329  309  348  316  426  402  358  102
> barplot(table(years))
```



4 Question 4

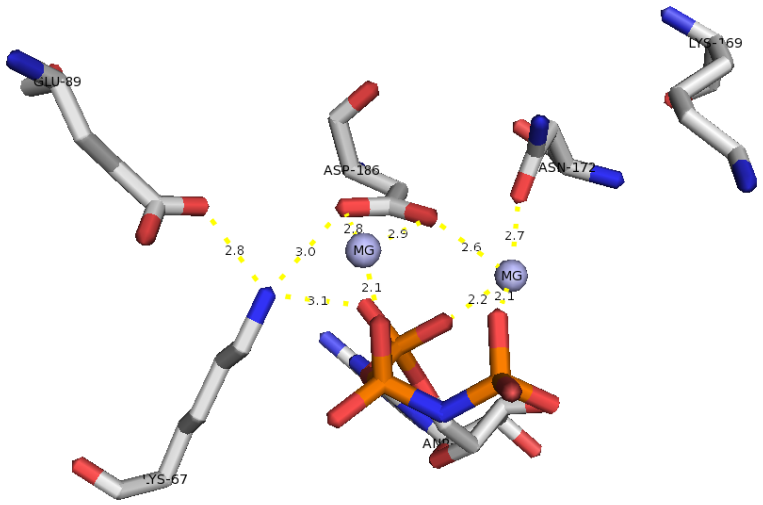
4.1 Part a

The molecule bound is Phosphoaminophosphonic Acid-Adenylate Ester, or ANP. Along with 2 Magnesium Ions.

4.2 Part b

ANP is an analog of ATP that cannot be hydrolized by the kinase. Therefore it stays bound to the active site of the kinase and allows for the crystal structure of the molecule to be established.

4.3 Part c



As shown in the figure, a salt bridge is formed between Glu89 and Lys67. Lys67 forms a salt bridge directly with the α-phosphate oxygen of the ANP molecule. Asp186 forms H-Bond with the Lys67 and also coordinates the Magnesium Ion, that in turns coordinates the β-phosphate oxygen of the ANP molecule. Asn172, in collaboration with Asp186 coordinates the second Magnesium Ion which interacts with the α and γ-phosphate oxygens of the ANP molecule [2].

4.4 Part d

The AUTHOR section from the PDB file reveals the same list of names as the list of the article's authors:

```
AUTHOR    K.C.QIAN,L.WANG,E.R.HICKEY,J.STUDTS,K.BARRINGER,C.PENG,
AUTHOR   2 A.KRONKAITIS,J.LI,A.WHITE,S.MISCHE,B.FARMER
```

5 Question 5

We found 2 proteins of interest: 1JKK and 3F5U. Both have reasonable resolutions and R-Free values are similar. 1JKK boasts a 'up to 1.5 A' resolution of the catalytic domain. However, after visualizing the B-Factors using pymol, we decided to go with 3F5U.

5.1 Part a

The AUTHOR section from the PDB file reveals the same list of names as the list of the article's authors:

```
AUTHOR    L.K.MCNAMARA,D.M.WATTERSON,J.S.BRUNZELLE
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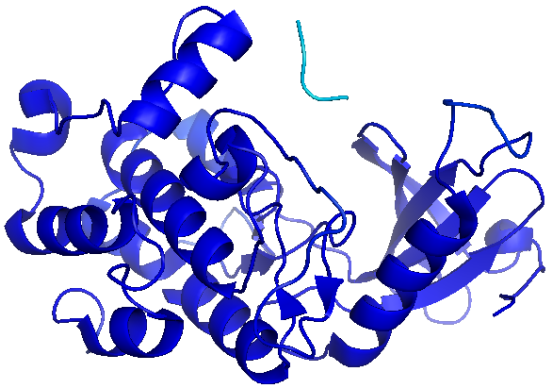
5.2 Part b

5.3 Part c

Overall, this structure has low B-factors. There is however a loop region, located around amino acids 291-294, which displays higher B-Factors (around 100). The reason for this seems to be lying in the fact that this loop is part of a flexible regions at the C-Terminus, which is not captured by the X-Ray crystalography.

5.4 Part b



5.5 Part d

The domains present in DPAK1-Human http://www.uniprot.org/uniprot/P53355 are:

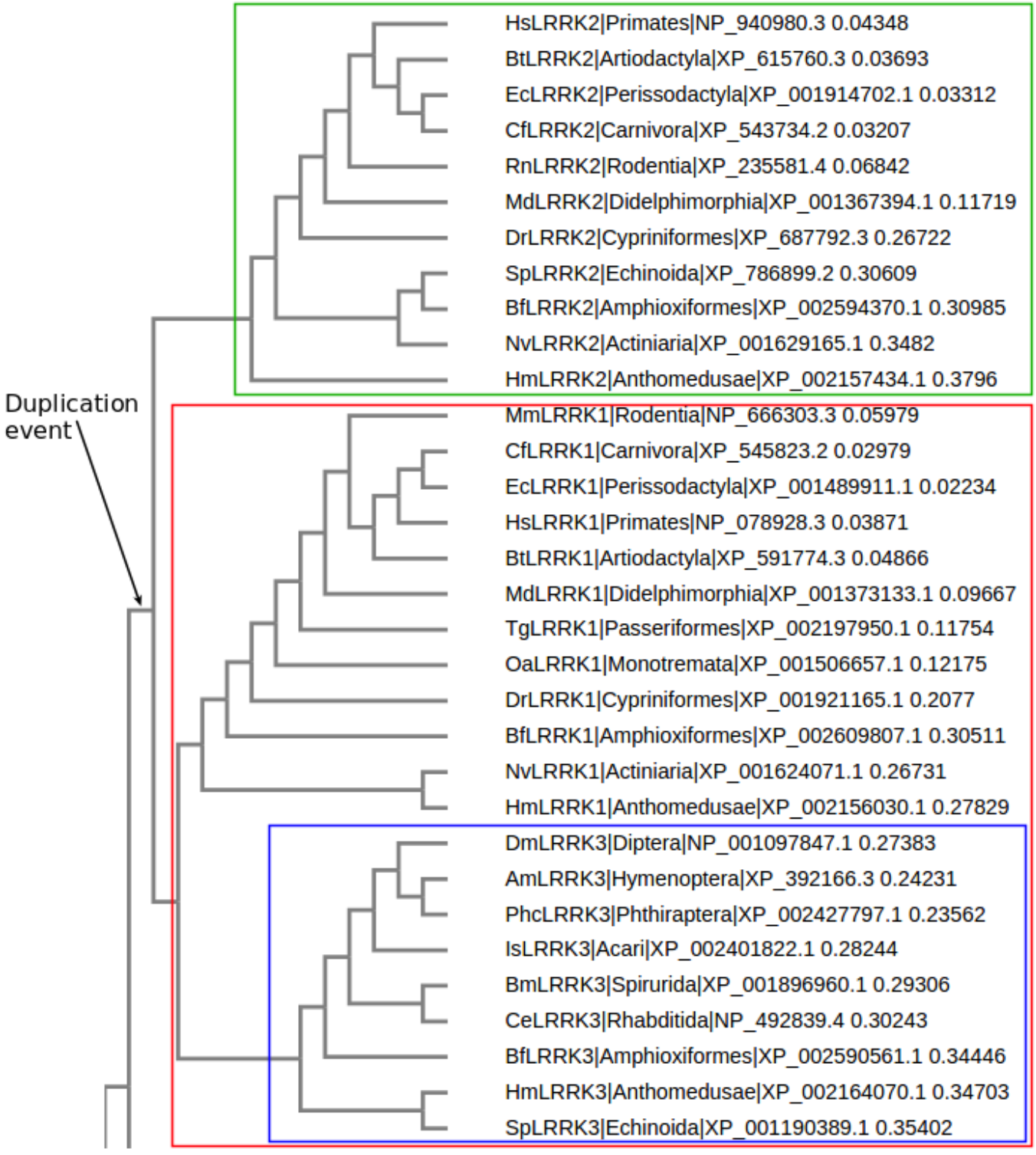
- Kinase whose function is to catalize the transfer of phosphate groups to specific substrates.
- Ankyrin domains (multiple found) mediate protein-protein interactions. https://en.wikipedia.org/wiki/Ankyrin_repeat
- Roc domain, which is a GTPase domain. GTP binding to the ROC domain activates kinase activity. http://www.copewithcytokines.de/cope.cgi?key=ROC%20domain
- The death domain (DD) is a protein interaction module composed of a bundle of six alpha-helices. https://en.wikipedia.org/wiki/Death_domain

5.6 Part e

The sequences provided are all related and display homology. Some of the organism in which the protein is found belong to varied kingdom such as Animal, Bacteria, Plants, which indicates that it must originates from an ancient gene that existed even before the split between Eukaryotes and Prokaryotes on the tree of life.

To further our analysis, we used the guide tree created when we performed the multiple sequence alignment, along with the results of the alignment. We found that the gene had had multiple duplication event during evolution. For example, we analyzed the duplication event (indicated on the figure) that gave rise to the proteins indicated by LRRK1, LRRK2, and LRRK3. All of which can be fount in organism belonging to the Eukaryotic domain. On the picture below, you can see that we emphasized 3 sub-clusters. The green and the red cluster show the LRRK1 and LRRK2+LRRK3 groups (the blue cluster can be hypothesized to have come from a duplication event early in the Animals/Invertebrates branch).

Using the results from the sequence alignment, you can observe that the alignment scores between the protein within the same cluster (for example LRRK1) are better than the ones from another cluster (LRRK2 in our example). This makes sense when you think about the evolutionary distance between the 2 genes coding for these paralog genes.



References

[1] Huse M. and Kuriyan J. The conformational plasticity of protein kinases. *Cell*, 2002.

[2] Kevin C. Qian, Lian Wang, Eugene R. Hickey, Joey Studts, Kevin Barringer, Charline Peng, Anthony Kronkaitis, Jun Li, Andre White, Sheenah Mische and Bennett Farmer. Structural basis of constitutive activity and a unique nucleotide binding mode of human pim-1 kinase. *The Journal of Biological Chemistry*, 2005.