Propagation of Point Mutation Effect Throughout Tyrosine Kinase c-Kit Structure

probed by Molecular Dynamics Simulations

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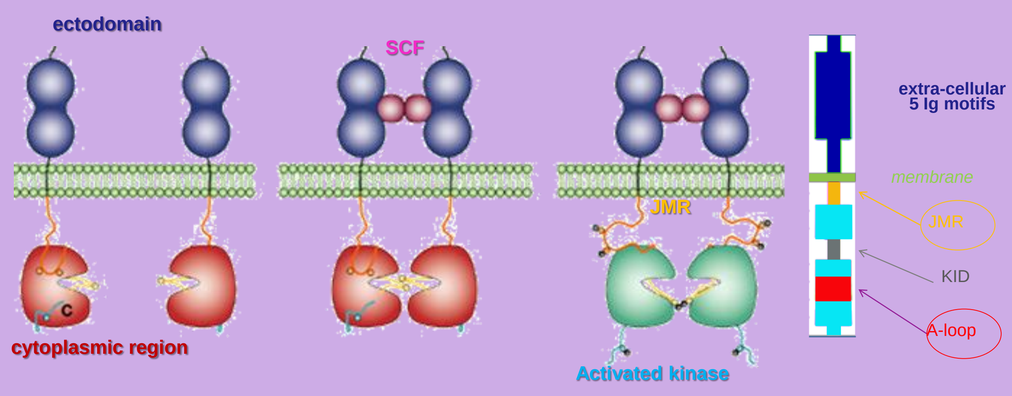
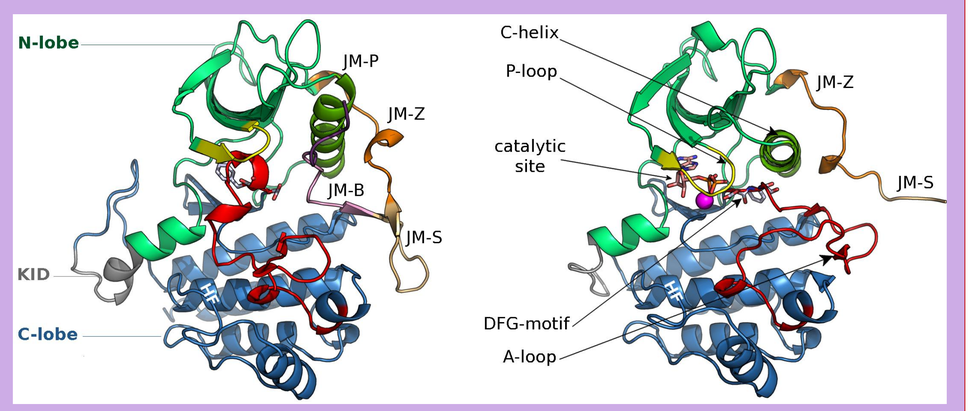
Protein kinases (PKs) play major roles in all aspects of cellular physiology. In particular, **Type III Receptor tyrosine kinase (RTK) c-Kit** transmits external signals crucial for cell survival, growth and proliferation. PKs are among the most studied pharmaceutical targets.



Two structural segments are essential for the stability of either inactive or active state:
- **activation (A-)loop**
- **auto-inhibitory JMR**



The D816V mutation in A-loop is associated with the formation of cancer and mastocytosis and is resistant to imatinib treatment (approved in 2001).

Methods

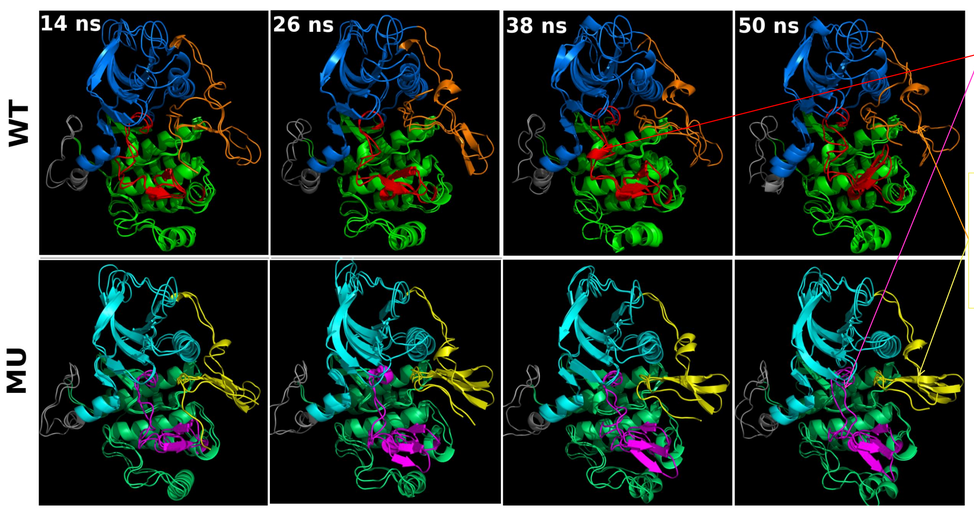
❖ Multi-approach procedure combining:
- **Molecular Dynamics (MD) Simulations** of the inactive auto-inhibited state, wild-type (WT) and D816V mutant (MU)
- **Normal Mode Analysis (NMA)** of representative MD conformations (similar to consensus mode philosophy [1])
- **Potential Binding Site Detection** [2] at the surface of 4 Å-displaced conformations along selected modes

❖ **Local Feature Analysis (LFA)** [3], based on **Principal Component Analysis (PCA)** of the MD trajectories, was used to model the effect of the mutation through the residue network of the protein

[1] Batista & Perahia, 2010 – [2] Le Guilloux et al., 2009 – [3] Zhang & Wriggers, 2006

Results [A]

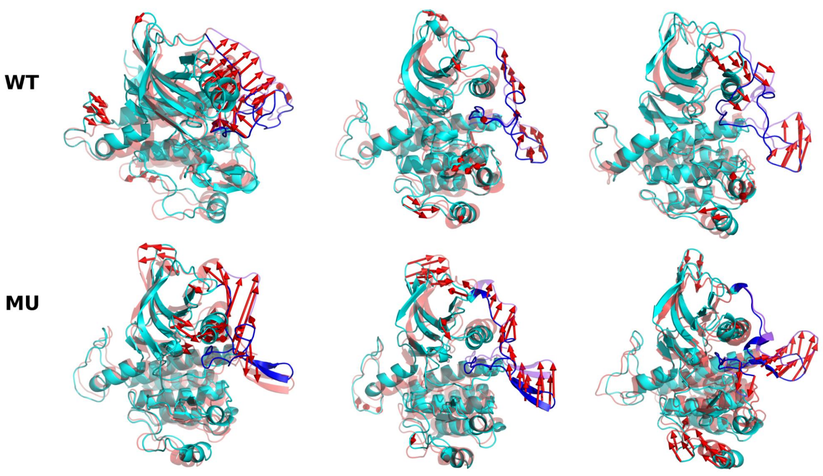
1) MD simulations: local and long-range effects of D816V



local unfolding of A-loop 3_{10} helix

long-range stabilization of JMR anti-parallel β-sheet

2) NMA: concerted and independent motions of JMR

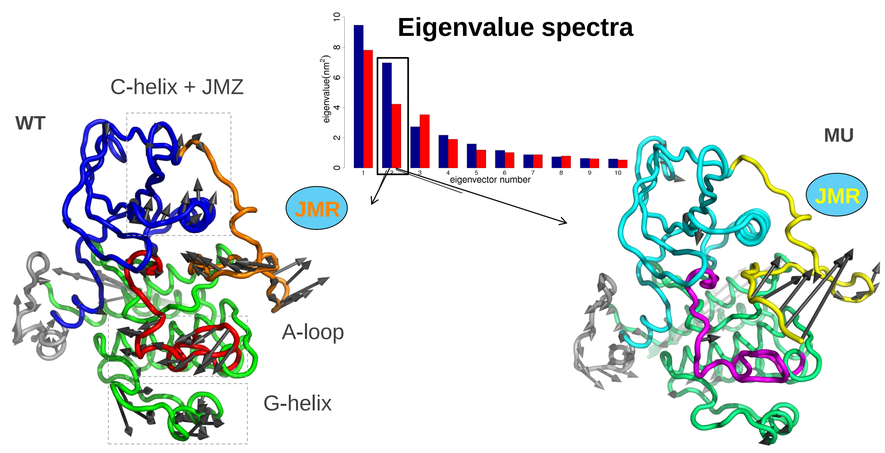


selected normal modes illustrating concerted motions of JMR away from its auto-inhibitory position.

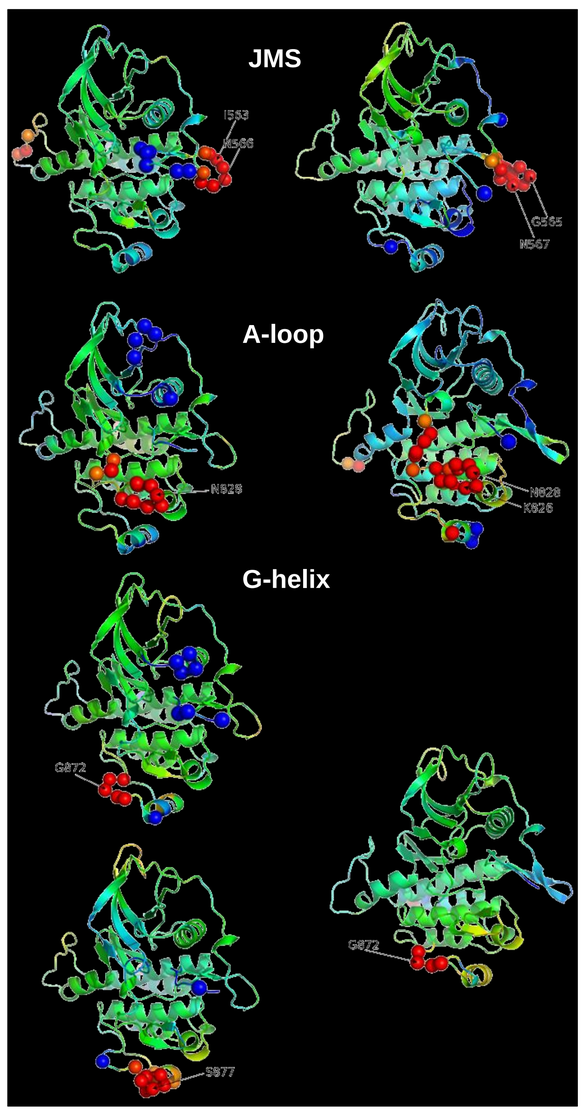
3) Path-of-pockets: inactive-to-active transition facilitated by D816V

Results [B]

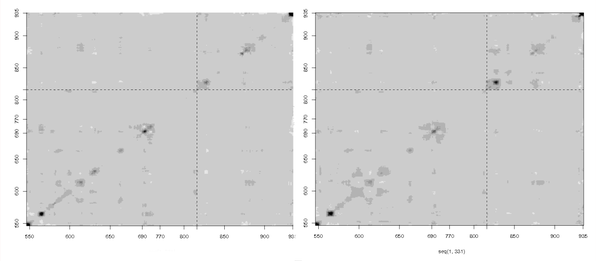
1) PCA: coupling/decoupling between JMR and kinase domain



2) LFA: propagation of D816V mutation signal



Protocol based on residual correlations and interaction patterns



- Clustering in **local dynamic domains**
- Recording of interactions
- ➢ **Through where does the signal pass ?**

Conclusion and Perspectives

❖ Proposed **D816V activating molecular mechanism**. The D816V mutation not only induces a local destabilization of A-loop, but also a decoupling between the kinase domain and JMR, resulting in: (i) folding of JMR anti-parallel ß-sheet, (ii) weakening of the interaction network between JMR and kinase domain, (iii) independent and concerted motions of JMR that could trigger the inactive-to-active state transition. The freedom of movement allowed to JMR in MU could promote **an alternative molecular recognition mechanism leading to receptor dimerization**.

❖ The identification of **preferred routes/hubs** through which the mechanistic and thermodynamic effects of the D816V mutation are propagated will help design **allosteric inhibitors** able to trap the protein in an inactive or non-functional conformation.