The Nature and Location of Oncogenic Mutations modulate Receptor KIT Activation Mechanisms and Drug Sensitivity

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INTRODUCTION

Receptor tyrosine kinases regulate cell growth and proliferation.

In absence of Stem Cell Factor (SCF), the cytoplasmic domain of receptor tyrosine-kinase **KIT** is mainly in its inactive state. Upon extracellular binding of SCF, the intracellular Juxta-Membrane Region (JMR) and the Activation loop (A-loop) undergoe large conformational rearrangements leading to activation. [1]

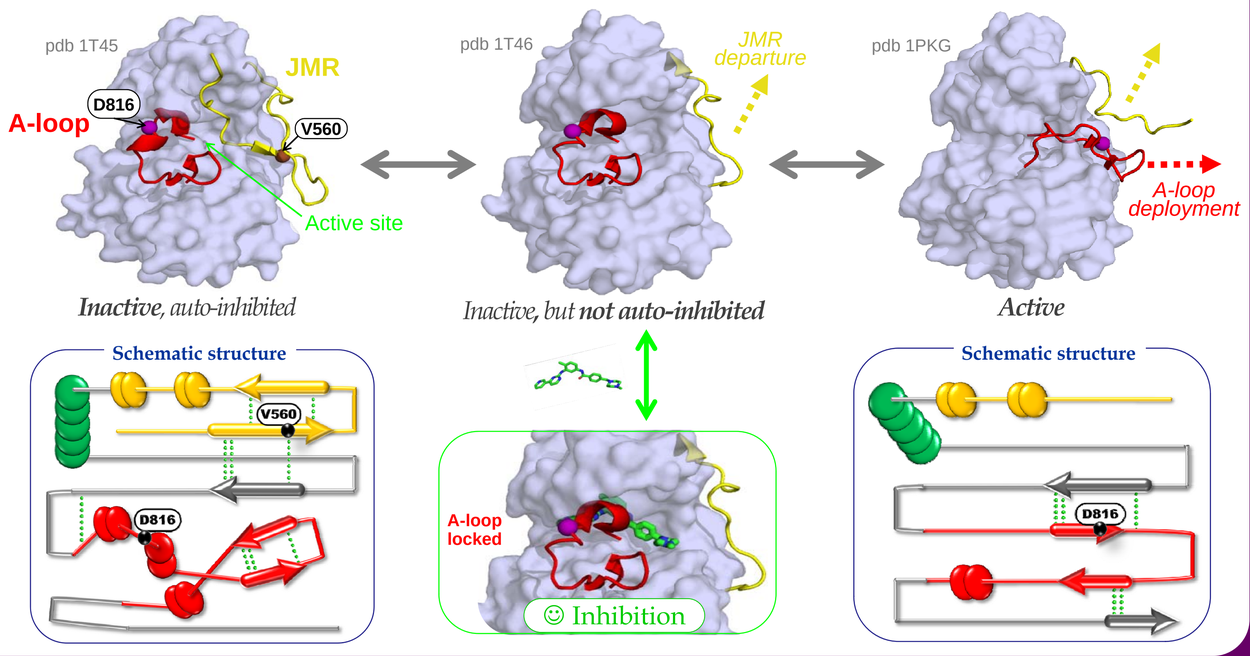
Mutations D816H/V (in the A-loop) or **V560G/D (in the JMR)** can induce activation, provoking cancers and mastocytoses [2]. Biological constants of some mutants have been assessed *in vitro* [2] :

Position	Mutation	Activation rate compared to WT	Sensitivity to inhibitors
A-loop	D 816 **V**	++	- -
	D 816 **H**	+	-
JMR	V 560 **G**	++++	+ +
	V 560 **D**	++++	+ +

How does the **mutation point** decide of the **activating** or **resistant** nature of the mutant?

How does the types of substituted **amino acid** impact on the activation or resistance **rates** ?

X-ray structures of KIT WT Cytoplasmic domain (JMR + Kinase domain):



PREVIOUS RESULTS (E. Laine) [3]

Impact of D816V mutation
revealed by MD simulations, NMA and pocket detection on KIT WT and mutant

- Increases global conformational stability and MD convergence
- Increases conformational exploration
- Promotes concerted global motions
- Promotes JMR independent motions
- Short-range structural effect : destabilization of the A-loop
- Long range structural effect : strengthening of JMR β-sheets

JMR structuration – long-range mutation effect – facilitates its concerted departure.
A-loop destructuration facilitates its opening out.

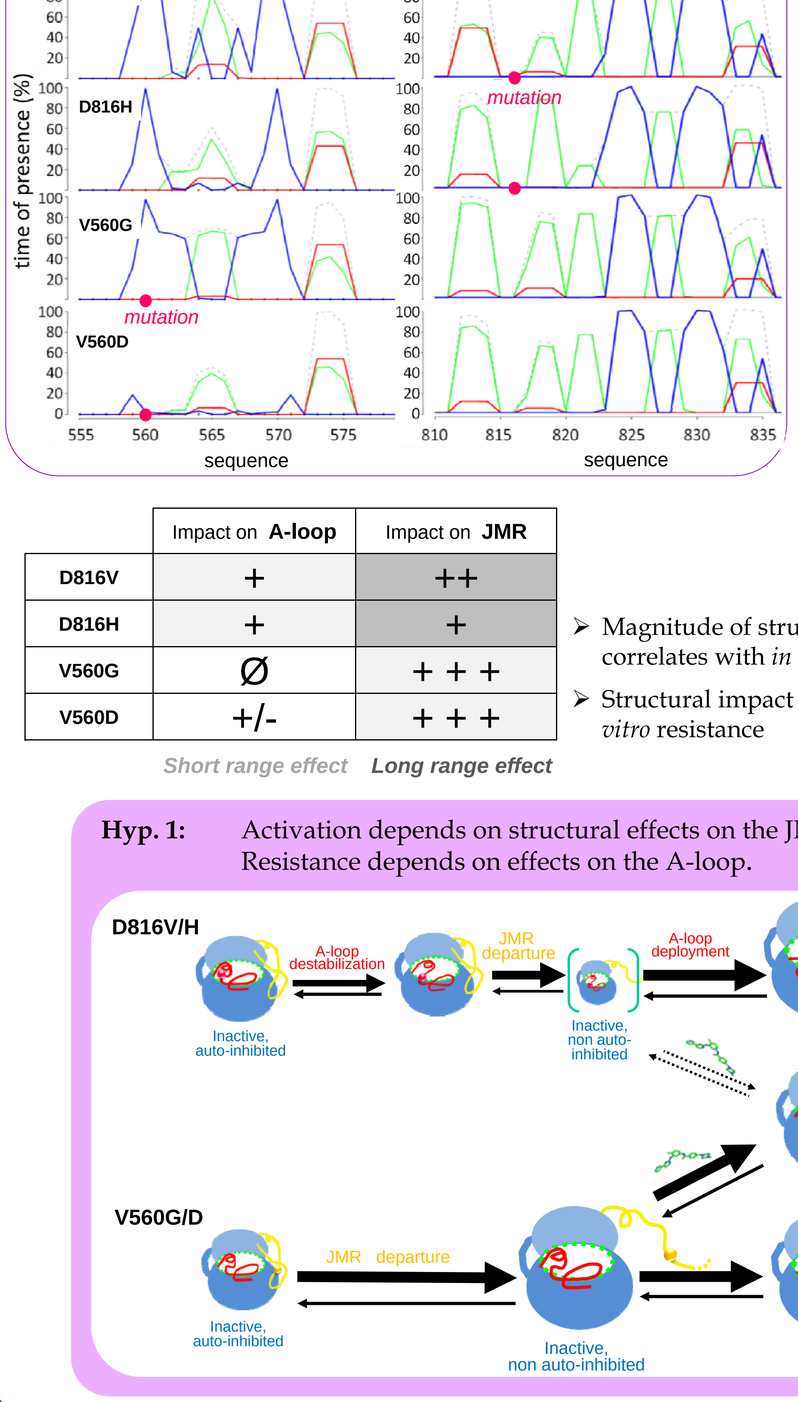
METHODS

MD simulations of the inactive state of KIT^{D816H} and $KIT^{V560G/D}$ cytoplasmic domain . (AMBER, ff99SB, 2 50 ns or 70 ns, all atoms in explicit water)

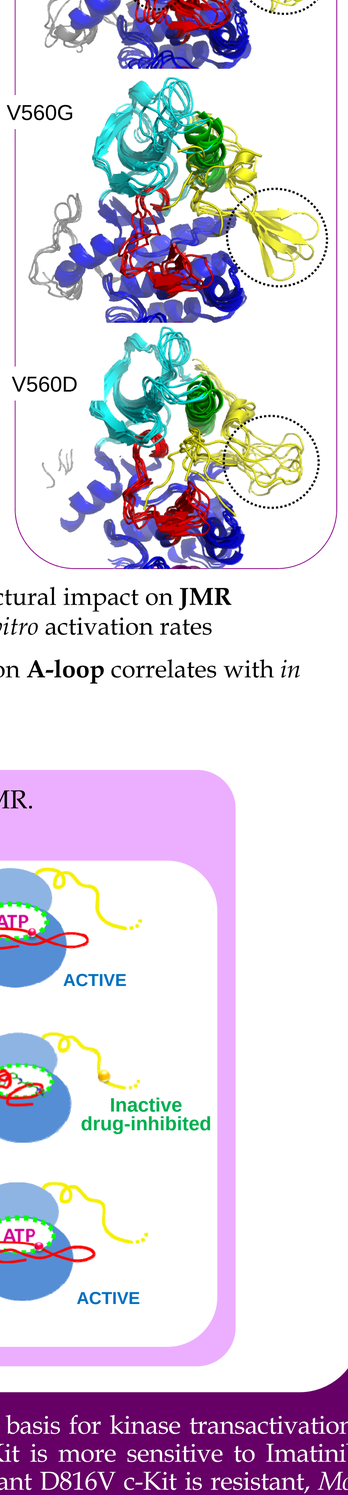
- **Convergence analysis**
- **Secondary structure analysis**
- **MD Conformations clustering**
- **Principal Component Analysis**

RESULTS I : Impact on Secondary Structure

Secondary structure summation



MD snapshots superimposition



	Impact on **A-loop**	Impact on **JMR**
D816V	+	++
D816H	+	+
V560G	Ø	+ + +
V560D	+/-	+ + +

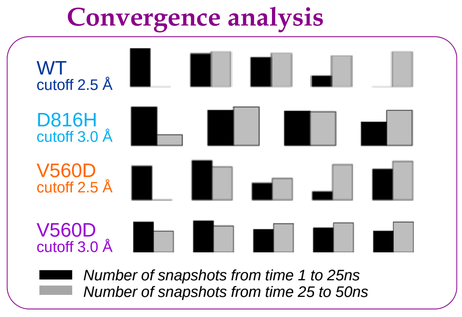
Short range effect *Long range effect*

- Magnitude of structural impact on **JMR** correlates with *in vitro* activation rates
- Structural impact on **A-loop** correlates with *in vitro* resistance

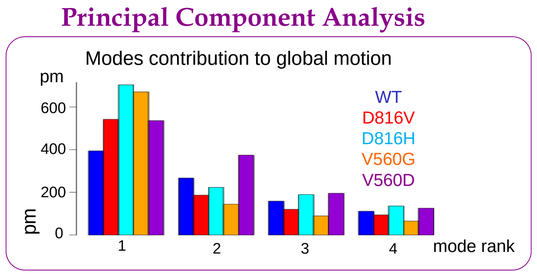
Hyp. 1: Activation depends on structural effects on the JMR.
Resistance depends on effects on the A-loop.

RESULTS II : Impact on Convergence & Internal Dynamics

Convergence analysis



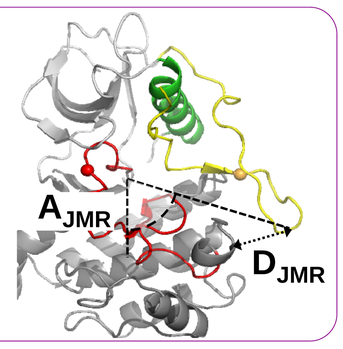
Principal Component Analysis



- Conformational stabilization and higher exploration
- Emergence of predominant motions

Geometrical features

D_{JMR}	WT	D816H	D816V	V560G	V560D
mean (Å)	12.1	13.3	13.1	12.9	17.2
min (Å)	8.4	9.8	9.3	8.4	11.3
max (Å)	16.1	17.7	18.9	17.9	24.3
‰ D_{JMR} > 16 Å	0.2	14	24	4.8	738
A_{JMR}	WT	D816H	D816V	V560G	V560D
mean (°)	75.8	79.7	77.1	76.5	87.1
min (°)	66.3	71.2	68.5	65.3	74.8
max (°)	85.1	90.3	91.6	87.1	105.0
‰ A_{JMR} > 85°	0	18	15	0.3	668
‰ A_{JMR} < 70°	19	0	0.6	55	0

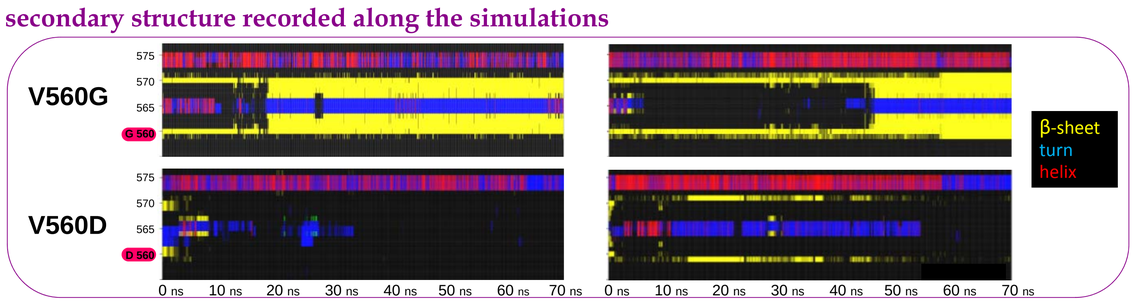


- Increased values of JMR-PKD distance and angle in all mutants.
- Wider panel of accessible angles in V560G mutant.

Hyp. 2 : A-loop mutants and JMR mutants show similar activation mechanism *via* JMR collective motions and facilitated departure from auto-inhibitory position

RESULTS III : Similar *in vitro* Impact *vs.* opposite Structural Effects

JMR secondary structure recorded along the simulations

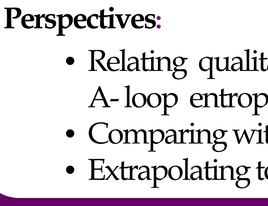


- JMR β-sheets show an abrupt and strong elongation in KIT^{V560G} *vs.* an almost total disappearance in KIT^{V560D}

Hyp 3: KIT activation mechanism requires a particular structuration of the JMR. Disturbance of this structuration leads to constitutive activation

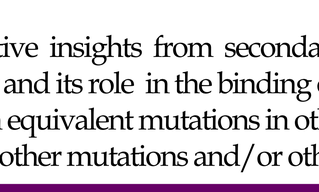
Internal and external JMR H-bond network impacted by V560G/D mutations

WT



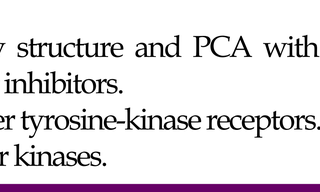
Val has high ability to stabilize β-sheets [4]

V560D



ASP, as charged residues, trends to destabilize β-sheets [4]

V560G



Gly stabilizes β-sheets when paired to aromatic residue [4]

A JMR mutation induces a loss of 15% (V560D) or 30%(V560G) of H-bond time occupancy between residues 787 (C-lobe) and 560 (JMR)

Hyp 4: β-sheets disturbance in JMR induces a loss of H-bonds with the rest of the protein. This facilitates JMR departure from its inhibitory position

CONCLUSIONS

JMR structuration and motions play predominant roles in KIT activation mechanisms. Short-/long-range mutation effects would impair its auto-inhibitory function and facilitate KIT **activation** (D816 and V560 positions) and/or **inhibitors binding** (V560 position).

The **A-loop** structural integrity seems required for the binding of inhibitors. Short-range effects of a mutation at position D816 may contribute in the mechanism of **drug resistance**.

The magnitude of impacts of these mutations on KIT structural and dynamical behavior depends on the **type of amino acid** substitution. It correlates with *in vitro* **auto-activation rates** and **drug sensitivities.**

Perspectives:

- Relating qualitative insights from secondary structure and PCA with quantitative analysis of A- loop entropy and its role in the binding of inhibitors.
- Comparing with equivalent mutations in other tyrosine-kinase receptors.
- Extrapolating to other mutations and/or other kinases.

[1] C.D. Mol *et al* (2003) Structure of a c-kit product complex reveals the basis for kinase transactivation, *J.Biol.Chem*. [2] MJ. Frost *et al* (2002) Juxtamembrane mutant V560G c-Kit is more sensitive to Imatinib (STI571) compared with wild-type c-Kit whereas the kinase domain mutant D816V c-Kit is resistant, *Mol Cancer Ther*. [3] E. Laine, I. C. de Beauchêne, C. Auclair, J.-F. Mouscadet, D. Perahia, L. Tchertanov (2011). Mutation D816V Alters the Internal structure and dynamics of c-KIT Cytoplasmic Region: Implications for Dimerization an Activation Mechanism, *PloS*. [4] E. Hutchinson (1998) Determinants of strand register in antiparallel β-sheets of proteins, *Protein Science*.