Receptor Tyrosine Kinase KIT: A New Look for an Old Receptor

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Julie Ledoux[1] and Luba Tchertanov[1]

Université Paris-Saclay, ENS Paris-Saclay, Centre Borelli, 4 avenue des Sciences, 91190 Gif-sur-Yvette, France
julie.ledoux@ens-paris-saclay.fr

Abstract. RTK KIT regulates a variety of crucial cellular processes via its cytoplasmic (CD) domain composed of the tyrosine kinase domain crowned by highly flexible domains - juxtamembrane region, kinase insert domain and C-tail, key recruitment regions for downstream signalling proteins. We generated the 3D model of the full-length CD attached to the transmembrane helix to prepare a structural basis for characterization of the interactions of KIT with its signalling proteins (KIT INTERACTOME). This generic model of KIT in inactive state was studied by molecular dynamics simulation under conditions mimicking the natural environment of KIT. With the accurate atomistic description of the multidomain KIT dynamics, we explained its intrinsic (intra-domain) and extrinsic (inter-domain) disorder, and represented the conformational ensemble of KIT through free energy landscapes. Strongly coupled movements within each domain and between distant domains of KIT prove the functional interdependence of these regions, described as allosteric regulation, a phenomenon widely observed in many proteins. We suggested that KIT in inactive state encodes all properties of the active protein and post-transduction events.

Keywords: Receptor Tyrosine Kinase · RTK · full-length KIT cytoplasmic region · intrinsically disordered regions · phosphotyrosine · modelling · molecular dynamics · conformational transition · allosteric regulation · free energy landscape

Receptor tyrosine kinases (RTKs) control various signalling pathways in cells. Their remarkable conformational plasticity enables the specific recognition of many molecules such as ligands, substrates or proteins. In solution, RTKs are at equilibrium between different conformations ranging from an inactive auto-inhibited state to a fully active state. Ligand-induced activation of RTKs leads to recruitment and activation of multiple downstream signalling proteins which alter the expression of genes governing cell physiology [9]. Explicit elucidation of signalling events is an important and unresolved problem in cell biology. The initiation of these cascade-like processes involves different domains of RTK, each performs specific actions, finely concerted by a regulated allosteric mechanism controlling all functional biological processes [4, 11].

Focusing on the RTK KIT, an important target in oncology [10, 3], we will discuss this RTK as a key regulator of intracellular signalling mediated by regions possessing multiple phosphorylation sites: juxtamembrane region (JMR), kinase insert domain (KID), activation loop (A-loop) and C-terminal tail. Since these regions are very flexible or disordered, their properties are not yet well understood. As KIT is a multi-functional protein, its different regions regulate catalytic processes and/or events that activate and control the signalling cascade. To complete the functions required in more than one region, these regions should be directly or collaterally coupled.

We generated the 3D model of the inactive full-length KIT attached to the transmembrane helix (Fig. 1.), to (i) prepare a structural basis for the characterisation of interconnections between functional regions - JMR, tyrosine kinase (TK) domain, KID and C-tail -, and (ii) establish the interactions of KIT with its signalling proteins. Then, we investigated this model by molecular dynamics (MD) simulation in conditions mimicking its natural environment. We suggested that such atomistic description of KIT will fully elucidate structural and dynamical properties of its different functional regions. To the best of our knowledge, we have presented for the first time a model of a full-length cytoplasmic region of an RTK KIT attached to a transmembrane helix and its molecular dynamics simulations under conditions that mimic its natural environment [8].

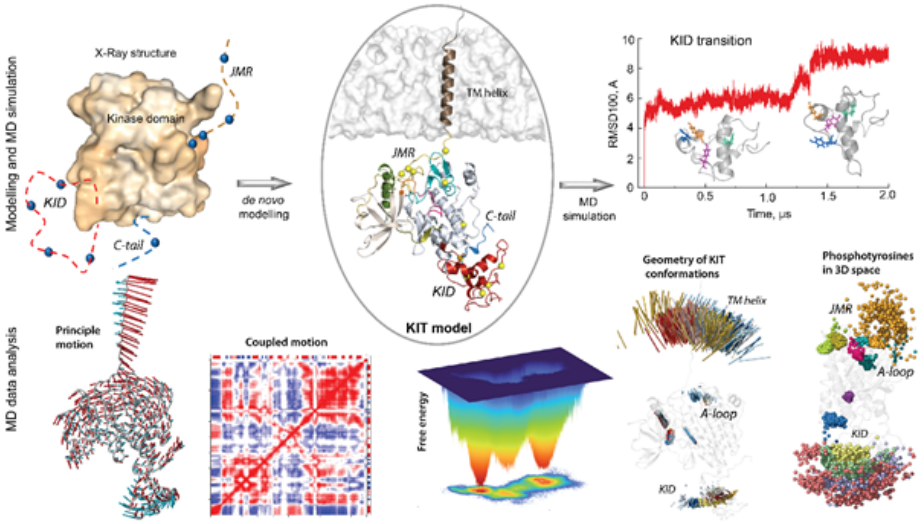

Fig. 1. The 3D model of the inactive full-length KIT attached to the transmembrane helix. Modelling and study by MD simulations (top panel) analysed to (i) characterise the global motions and coupling, (ii) to represent the MD conformations as the free energy landscape, (iii) to describe the relative positions of the KIT functional regions and the position of phosphotyrosine residues.

Analysis of the simulation data (three 2-μs MD trajectories) put in evidence that the multidomain RTK KIT is a modular protein consisting of a quasi-stable TK domain crowned by at least four intrinsically disordered (ID) regions – JMR, KID, A-loop and C-tail. These ID regions belong to two types – the very elongated (extended) and poorly folded regions (JMR, A-loop and C-tail), and the globule-like (collapsed) KID having a high level of the helical structures. KIT ID regions contain transient structures (helices and β-strands) and their local architecture displays various degrees of compaction and elongation. Therefore, the structure of each ID domain of KIT represents a very complex mixture of a broad variety of differently folded conformations which describe a reversible folding-unfolding process, specific for each ID domain. In particular, the KID, composed of transient helices linked by coils, is the most disordered domain in respect to other KIT domains, but shows a globule-like shape stabilised by non-covalent interactions [5, 7]. Also, the inherently disordered KID shows different positions derived from two types of motions - linear (translation) and angular (rotation) displacement - regarding the stable TK domain. The elongated regions (JMR, A-loop and C-tail) show rather local disorder as evidenced by alternating positions of their short segments relative to the stable TK domain.

The two-level disorder (intrinsic and extrinsic) provides high conformational variability of KIT and supplies the high adaptability of JMR, KID and C-tail required for the scaffolding (docking sites) and recruitment of different protein partners of KIT, and accomplishes the tight regulation of cellular processes. Consequently, the overall structure of KIT represents a continuous spectrum of conformations with a different degree and depth of disorder, thereby generating a complex protein structural space. It is partially reflected by free energy landscapes lacking a unique global deep minimum as typically observed in ordered proteins. Such energy landscapes, with two local minima joined by a 'flattened plateau' containing the intermediate conformations, show that KIT is extremely sensitive to different environmental changes (e.g. phosphorylation) that can alter its free energy landscape in different ways.
We suggest that JMR, displaced from its packed auto-inhibited position upon the SCF-induced activation of KIT, will achieve higher levels of disorder, and therefore a higher level of adaptability for the recruitment of signalling proteins.

Since ID domains are multiple in RTK KIT, does the disorder/order of one domain depend on the disorder/order of other remote regions? As evidenced by the cross-correlations, the highly coupled motions of distant regions of KIT suggest their functional dependence which is classified as allosteric regulation, phenomenon largely observed in many proteins. In particular, the coupling motions within the TK domain reflect the allosteric regulation of kinase function which is well-described for different non-receptor and receptor tyrosine kinases [1]. The coupled/uncoupled motions of A-loop and JMR were described through their allosteric communication in the wild-type KIT and oncogenic mutants [6, 2].

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