Amino Acids (2010) 39:651–660
DOI 10.1007/s00726-010-0486-6

ORIGINAL ARTICLE

# Exploring the cause of drug resistance by the detrimental missense mutations in KIT receptor: computational approach

**R. Rajasekaran · Rao Sethumadhavan**

Received: 27 May 2009 / Accepted: 15 January 2010 / Published online: 7 February 2010


**Abstract** In this work, we computationally identified the most detrimental missense mutations of KIT receptor causing gastrointestinal stromal tumors and analyzed the drug resistance of these missense mutations. Out of 31 missense mutations, 19 variants were commonly found less stable, deleterious and damaging by I-Mutant 2.0, SIFT and PolyPhen programs, respectively. Subsequently, we performed modeling of these 19 variants to understand their change in conformations with respect to native KIT receptor by computing their RMSD. Further, the native and 19 mutants were docked with the drug 'Imatinib' to explain the drug resistance of these detrimental missense mutations. Among the 19 mutants, we found by docking studies that 12 mutants, namely, F584C, F584L, V654A, L656P, T670I, R804W, D816F, D816V, D816Y, N822K, Y823D and E839K had less binding affinity with Imatinib than the native type. Finally, we analyzed that the loss of binding affinity of these 12 mutants, was due to altered flexibility in their binding amino acids with Imatinib as compared with native type by normal mode analysis. In our work, we found the novel data that the majority of the drug-binding amino acids in those 12 mutants had encountered loss of flexibility, which could be the theoretical basis for the cause of drug insensitivity.

**Keywords** Missense mutation · KIT receptor · Imatinib · Flexibility

## Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. These form a distinct category of tumors characterized by oncogenic mutations of the KIT receptor in a majority of patients (Gupta et al. 2008). KIT is a member of the type III family of RTKs, which also includes PDGF-receptor-$\alpha$ and $\beta$, CSF-1 receptor (also known as M-CSF receptor or FMS), and the FLT3 receptor (also known as FLK2) (Ullrich and Schlessinger 1990; Blume-Jensen and Hunter 2001). KIT is composed of a glycosylated extracellular ligand-binding domain (ectodomain) that is connected to a cytoplasmic region by means of a single transmembrane (TM) domain (Schlessinger 2000). The ectodomain of KIT and other members of type III RTKs all contain five Ig-like domains, in which the second and third membrane distal domains were shown to play a role in ligand recognition (Ullrich and Schlessinger 1990). The cytoplasmic region of KIT contains a protein tyrosine kinase (PTK) domain with a large kinase-insert region, another hallmark of type III RTKs. Binding of SCF to KIT leads to receptor dimerization, intermolecular autophosphorylation, and PTK activation. It was proposed that the fourth Ig-like domain of KIT is responsible for KIT dimerization in response to either monovalent or bivalent SCF binding (Lev et al. 1992; Blechman et al. 1995). However, other studies have demonstrated that ligand-induced dimerization of KIT is driven by bivalent binding of SCF (Philo et al. 1996; Lemmon et al. 1997).

KIT is used not only for diagnosis but also for targeted therapy of GISTs. Imatinib (also known as Gleevec or

**Electronic supplementary material** The online version of this article (doi:10.1007/s00726-010-0486-6) contains supplementary material, which is available to authorized users.

R. Rajasekaran · R. Sethumadhavan (✉)
Bioinformatics Division, School of Biosciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu 632014, India
e-mail: rsethumadhavan@vit.ac.in

STI571), a tyrosine kinase inhibitor, is widely used in the treatment of advanced and metastatic GISTs. Specific mutations in the KIT gene result in GISTs that are relatively unresponsive to the Imatinib treatment (Bauer et al. 2005; Jonathan Fletcher and Brian Rubin 2007; Engelman and Settleman 2008). Identifying the disease-associated missense mutation and drug resistance caused by them is a challenging task for cancer research. So, we have given an attempt to investigate the KIT receptor by our devised computational protocol which we established, implemented and analyzed with HER2 and CDKN2A (Rajasekaran et al. 2008a, b). With this continuation, we undertook and implemented our devised protocol to identify the detrimental missense mutations in KIT protein and proposed a modeled structure for the mutants. Then we docked the inhibitor, Imatinib with both the native and mutants of KIT receptor to find out the binding effect and the nature of flexibility in the binding pockets to understand the cause of drug resistance by these missense mutations.

## Materials and methods

### Data sets

The protein sequence and variants (single amino acid polymorphisms/missense mutations/point mutants) for KIT receptor were obtained from the Swissprot, available at http://www.expasy.ch/sprot/ to find out the detrimental point mutants. The subsection of each Swissprot entry provides information on polymorphic variants, in which some polymorphic variants may be disease(s) associated with defects in a given protein; most of them are nsSNPs (non-synonymous SNPs) in gene sequence and SAPs (single amino acid polymorphisms) in protein sequence (Yip et al. 2004, 2008; Boeckmann et al. 2003). The 3D Cartesian coordinates of KIT receptor and KIT-drug complex were obtained from Protein Data Bank (Berman et al. 2000) for *in silico* mutation modeling and docking studies based on detrimental missense mutations.

### Predicting stability change on single amino acid polymorphism based on support vector machine (I-Mutant 2.0)

We used the program I-Mutant 2.0 available at http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I-Mutant2.0.cgi. The I-Mutant 2.0 is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single-point mutations; I-Mutant 2.0 predictions are performed starting either from the protein structure or, more importantly, from the protein sequence (Capriotti et al. 2005). This program was trained and tested on a data set derived from ProTherm (Bava et al. 2004), which is presently the most comprehensive available database of thermodynamic experimental data of free energy changes of protein stability upon mutation under different conditions. The output file shows the predicted free energy change value or sign ($\Delta\Delta G$), which is calculated from the unfolding Gibbs free energy value of the mutated protein minus the unfolding Gibbs free energy value of the native type (kcal/mol). Positive $\Delta\Delta G$ values mean that the mutated protein possesses high stability and vice versa.

### Analysis of functional consequences of point mutant by sequence homology based method (SIFT)

We used the program SIFT (Ng and Henikoff 2003), which is specifically to detect the deleterious single amino acid polymorphism, available at http://blocks.fhcrc.org/sift/SIFT.html. SIFT is a sequence homology based tool, which presumes that important amino acids will be conserved in the protein family. Hence, changes at well-conserved positions tend to be predicted as deleterious (Ng and Henikoff 2003). We submitted the query in the form of protein sequences. The underlying principle of this program is that, SIFT takes a query sequence and uses multiple alignment information to predict tolerated and deleterious substitutions for every position of the query sequence. SIFT is a multistep procedure that, given a protein sequence, (a) searches for similar sequences, (b) chooses closely related sequences that may share similar function, (c) obtains the multiple alignment of these chosen sequences, and (d) calculates normalized probabilities for all possible substitutions at each position from the alignment. Substitutions at each position with normalized probabilities less than a chosen cutoff are predicted to be deleterious and greater than or equal to the cutoff are predicted to be tolerated (Ng and Henikoff 2001). The cutoff value in SIFT program is tolerance index of $\geq 0.05$. Higher the tolerance index, less functional impact a particular amino acid substitution is likely to have.

### Simulation for functional change in point mutant by structure homology based method (PolyPhen)

Analyzing the damaged point mutations at the structural level is considered to be very important to understand the functional activity of the concerned protein. We used the server PolyPhen (Ramensky et al. 2002) which is available at http://coot.embl.de/PolyPhen/ for this purpose. The input options for the PolyPhen server are protein sequence or SWALL database ID or accession number together with sequence position with two amino acid variants. We submitted the query in the form of protein sequence with

mutational position and two amino acid variants. Sequence-based characterization of the substitution site, profile analysis of homologous sequences and mapping of the substitution site to known protein three-dimensional structures are the parameters taken into account by the PolyPhen server to calculate the score. It calculates position-specific independent count (PSIC) scores for each of the two variants, and then computes the PSIC scores' difference between them. Higher the PSIC score difference, higher is the functional impact a particular amino acid substitution is likely to have.

### Modeling the effect of mutations on protein flexibility

Structure analysis was performed for evaluating the structural deviation between native type and mutant types by means of RMSD (root mean square deviation). We used the web resource Protein Data Bank (Berman et al. 2000) and single amino acid polymorphism database (SAAPdb) (Cavallo and Martin 2005) to identify the 3D structure of KIT receptor (PDB ID: 1t45). We also confirmed the mutation position and the mutation residue in PDB ID: 1t45. The mutation was performed by using SWISSPDB viewer and energy minimization for 3D structures was performed by NOMAD-Ref server (Lindahl et al. 2006). This server use Gromacs as default force field for energy minimization based on the methods of steepest descent, conjugate gradient and L-BFGS methods (Delarue and Dumas 2004). We used the conjugate gradient method to minimize the energy of the 3D structure of KIT protein. In order to optimize the 3D structure of KIT protein, we used the ifold server (Sharma et al. 2006) for simulated annealing, which is based on discrete molecular dynamics and is one of the fastest strategies for simulating protein dynamics. This server is also efficient in sampling the vast conformation space of biomolecules in both length and time scales. Divergence in mutant structure with native structure is due to mutations, deletions, and insertions (Han et al. 2006) and the deviation between the two structures could alter the functional activity (Varfolomeev et al. 2002) with respect to binding efficiency of the inhibitor which is evaluated by their RMSD values.

### Identification of binding sites and computation of atomic contact energy (ACE) between KIT receptor and inhibitor

In order to compute the atomic contact energy (ACE) between KIT receptor and with inhibitor, we submitted the PDB ID: 1t46, a complex of KIT receptor with Imatinib, into the ligand contact tool (LCT) program available at http://firedb.bioinfo.cnio.es/Php/Contact.php (López et al. 2007). This server calculates contacts between the binding residues of KIT receptor with Imatinib with default parameters. Then, we unbound the Imatinib from the KIT receptor of the PDB ID: 1t46 to perform point mutations on KIT protein by the SWISSPDB viewer, energy minimization by NomadRef and simulated annealing by ifold. Finally, we used the program PatchDock for docking the native and mutants of KIT receptor with the Imatinib to compute ACE by using additional option of binding residue parameter. The underlying principle of this server is based on molecular shape representation, surface patch matching plus filtering and scoring (Duhovny et al. 2002). It is aimed at finding docking transformations that yield good molecular shape complementarity. Such transformations, when applied, induce both wide interface areas and small amounts of steric clashes. A wide interface is ensured to include several matched local features of the docked molecules that have complementary characteristics. The PatchDock algorithm divides the Connolly dot surface representation (Connolly 1983a, b) of the molecules into concave, convex and flat patches. Then, complementary patches are matched in order to generate candidate transformations. Each candidate transformation is further evaluated by a scoring function that considers both geometric fit and atomic desolvation energy (Schneidman-Duhovny et al. 2005; Zhang et al. 1997). Finally, an RMSD (root mean square deviation) clustering is applied to the candidate solutions to discard redundant solutions. The main reason behind PatchDock's high efficiency is its fast transformational search, which is driven by local feature matching rather than brute force searching of the six-dimensional transformation space. It further speeds up the computational processing time by utilizing advanced data structures and spatial pattern detection techniques, such as geometric hashing and pose clustering.

### Exploring the flexibility of binding pocket by normal mode analysis

A quantitative measure of the atomic motions in proteins can be obtained from the mean square fluctuations of the atoms relative to their average positions. These can be related to the *B*-factor (Yuan et al. 2005; Ringe and Petsko 1986). Analysis of *B*-factors, therefore, is likely to provide newer insights into protein dynamics, flexibility of amino acids, and protein stability (Parthasarathy and Murthy 2000). It is to be noted that protein flexibility is important for protein function and for rational drug design (Carlson and McCammon 2000). Also flexibility of certain amino acids in protein is useful for various types of interactions. Moreover, flexibility of amino acids in drug binding pocket is considered to be a significant parameter to understand the binding efficiency. In fact, loss of flexibility impairs the binding effect (Hinkle and Tobacman 2003) and vice versa

(Rajasekaran et al. 2008a). Hence, this can be analyzed by the *B*-factor, which is computed from the mean-square displacement <*R*2> of the lowest-frequency normal mode using the ElNémo server (Suhre and Sanejouand 2004).

## Results and discussion

### Single amino acid polymorphism data set from Swissprot

The KIT receptor and a total of 31 variants, namely, V532I, M541L, K550I, V559A, V559D, E583K, F584C, F584L, G601R, V654A, L656P, G664R, T670I, C691S, S715N, D737N, R791G, R796G, R804W, G812V, D816F, D816H, D816V, D816Y, D820G, D820Y, N822K, Y823D, A829P, E839K and T847P investigated in this work were retrieved from the Swissprot database (Yip et al. 2004, 2008; Boeckmann et al. 2003).

### Identification of functional variants by I-Mutant 2.0

Out of 31 variants, we obtained 29 variants found to be less stable from the I-Mutant 2.0 server (Capriotti et al. 2005) as shown in Table 1. Out of such 29 variants, 7 variants, viz., F584C, F584L, G664R, D737N, G812V, D816V and D820G showed a $\Delta\Delta G$ value of $> -2.0$. The other 15 variants, viz., V532I, M541L, E583K, G601R, V654A, L656P, S715N, D816F, D816H, D816Y, D820Y, N822K, A829P, E839K and T847P showed a $\Delta\Delta G$ value of $> -1.0$ and the remaining 7 variants showed a $\Delta\Delta G$ value of $< -1.0$ as depicted in Table 1.

Out of 29 variants which showed negative $\Delta\Delta G$, 4 variants, namely, E583K, G664R, D816H and E839K changed their amino acid from negatively charged to positively charged amino acid; 2 variants, L656P and A829P changed from non-polar to polar; 2 variants, S715N and N822K changed from polar to positively charged; 2 variants, R791G and R796G from positively charged to non-polar; 2 variants, D816V and D820G changed from negatively charged to non-polar; 2 variants, D816Y and D820Y changed from negatively charged to aromatic, followed by 1 variant each, viz., F584C, F584L, G601R, T670I, D737N, R804W, D816F and Y823D changed from aromatic to polar, aromatic to non-polar, non-polar to positively charged, polar to non-polar, negatively charged to polar, positively charged to aromatic, non-polar to aromatic and aromatic amino acid to negatively charged amino acid, respectively. It is also to be noted that five other variants, V532I, M541I, V559A, V654A and G812V which retained their non-polar properties, and one other variant, T847P which retained its polar property were found to be less stable by I-Mutant 2.0. The above point portrays that preserving amino acid physico-chemical properties does not necessary result in harmless mutation. Indeed considering only amino acid substitution based on physico-chemical properties could not be able to identify the detrimental effect rather than considering the sequence conservation along with the above said properties could have more advantages and reliable to find out the detrimental effect of missense mutations (Teng et al. 2009).

**Table 1** List of variants that were predicted to be functionally significant by I-Mutant 2.0, SIFT and PolyPhen

| AA change | ΔΔG | Tolerance index | PSIC SD |
|---|---|---|---|
| V532I | −1.10 | 1.00 | 0.115 |
| M541L | −1.03 | 0.56 | 0.563 |
| K550I | 0.45 | 0.07 | 3.168 |
| V559A | −0.70 | 0.92 | 2.094 |
| V559D | −0.80 | 0.34 | 2.686 |
| **E583K** | **−1.62** | **0.00** | **2.256** |
| **F584C** | **−2.17** | **0.00** | **2.853** |
| **F584L** | **−2.53** | **0.05** | **1.667** |
| **G601R** | **−1.60** | **0.00** | **2.790** |
| **V654A** | **−1.56** | **0.02** | **1.681** |
| **L656P** | **−1.54** | **0.00** | **2.871** |
| G664R | −2.14 | 0.25 | 2.790 |
| **T670I** | **−0.27** | **0.02** | **2.692** |
| C691S | 0.07 | 0.79 | 1.176 |
| S715N | −1.29 | 0.57 | 1.061 |
| D737N | −3.31 | 0.13 | 1.089 |
| R791G | −0.43 | 0.55 | 3.136 |
| **R796G** | **−0.18** | **0.00** | **3.136** |
| **R804W** | **−0.79** | **0.03** | **2.823** |
| **G812V** | **−3.42** | **0.00** | **3.015** |
| **D816F** | **−1.43** | **0.00** | **3.130** |
| D816H | −1.91 | 0.02 | 1.132 |
| **D816V** | **−2.08** | **0.00** | **3.185** |
| **D816Y** | **−1.56** | **0.00** | **2.995** |
| D820G | −2.82 | 0.27 | 2.789 |
| **D820Y** | **−1.69** | **0.01** | **3.239** |
| **N822K** | **−1.44** | **0.04** | **2.569** |
| **Y823D** | **−0.39** | **0.00** | **2.307** |
| **A829P** | **−1.44** | **0.02** | **1.983** |
| **E839K** | **−1.39** | **0.00** | **2.256** |
| **T847P** | **−1.03** | **0.00** | **2.557** |

*AA change* amino acid change

Letters in bold indicate less stable, deleterious and damaging by I-Mutant, SIFT and PolyPhen, respectively

### Deleterious single point mutant by SIFT program

Protein sequences of 31 variants were submitted independently to the SIFT program (Ng and Henikoff 2003) to

check its tolerance index. Among the 31 variants, 20 variants were found to be deleterious having the tolerance index score of ≤0.05. The results are shown in Table 1. We observed that out of 20 such deleterious variants, 12 variants, E583K, F584C, G601R, L656P, R796G, G812V, D816F, D816V, D816Y, Y823D, E839K and T847P showed a highly deleterious tolerance index score of 0.00, 1 variant, D820Y with a tolerance index score of 0.01, 4 variants, V654A, T670I, D816H and A829P with a tolerance index score of 0.02, 1 variant, R804W with a tolerance index score of 0.03, 1 variant, N822K with a tolerance index score of 0.04 and 1 variant, F584L with a tolerance index score of 0.05. It was interesting to observe that all the deleterious variants according to SIFT also were seen to be less stable by the I-Mutant 2.0 server.

## Damaged single point mutant by PolyPhen server

Protein sequence with mutational position and amino acid variants associated with 31 single point mutants, investigated in this work were submitted as inputs to the PolyPhen server (Ramensky et al. 2002) and the results are shown in Table 1. A PSIC score difference of 1.5 and above is considered to be damaging. It can be seen that, out of 31 variants, 25 variants were considered to be damaging by PolyPhen. Also these 25 variants exhibited a PSIC score difference between 1.667 and 3.239. It is to be noted that all the variants except K550I which were considered to be damaging by PolyPhen also were seen to be less stable according to the I-Mutant 2.0 server. Similarly, all the variants except K550I, V559A, V559D, G664R, R791G and D820G which were considered to be damaging by PolyPhen also were seen to be deleterious according to the SIFT server.

## Rational consideration of detrimental point mutations

We rationally considered the most 19 potential detrimental point mutations for further course of investigations which were E583K, F584C, F584L, G601R, V654A, L656P, T670I, R796G, R804W, G812V, D816F, D816V, D816Y, D820Y, N822K, Y823D, A829P, E839K and T847P as they were commonly found less stable, deleterious and damaging by the I-Mutant 2.0, SIFT and PolyPhen servers, respectively. Among these, 9 variants, V654A, T670I, G812V, D816V, D820Y, N822K, Y823D A829P and E839K showed very good agreement with experimental observation performed elsewhere (Kim et al. 2005; Nishida et al. 2008; Ciccarelli et al. 2000; Ma et al. 2002; Weisberg and Griffin 2003; Fletcher and Rubin 2007; Kemmer et al. 2004; Chen et al. 2005; Roberts et al. 2007; McLean et al. 2005; Chen et al. 2004; Growney et al. 2005; Gajiwala et al. 2009; Debiec-Rychter et al. 2005).

## Computing RMSD by modeling of mutant structures

Mapping the 19 potential detrimental variants into protein structure information was obtained from SAAPdb (Cavallo and Martin 2005). Mutation at specified position was performed by SWISSPDB viewer independently to get modeled structures. Then, energy minimizations and simulated annealing were performed by NOMAD-Ref server (Lindahl et al. 2006) and ifold server (Sharma et al. 2006), respectively, for both the native structure (PDB 1t45) and mutant modeled structures (E583K, F584C, F584L, G601R, V654A, L656P, T670I, R796G, R804W, G812V, D816F, D816V, D816Y, D820Y, N822K, Y823D, A829P, E839K and T847P).

In order to find out the deviation between the two structures, we superimposed the energy refined native structure (PDB 1t45) with all the energy refined mutant modeled structures to get RMSD. The higher the RMSD value, the more is the deviation between the native and the mutant structure, which in turn changes their binding efficiency with inhibitors due to deviation in the 3D space of the binding residues of KIT receptor. Table 2 shows the RMSD for native structure with all the mutant modeled structures. It can be seen from Table 2 that, four mutants, V654A, T670I, Y823D and E839K exhibited a high RMSD value >3.00 Å; two mutants, D816F and D816V with RMSD >2.00 Å; six mutants, F584C, F584L, L656P,

**Table 2** RMSD and ACE for native and mutants

| KIT receptor | RMSD (Å) | ACE (Kcal/mol) |
|---|---|---|
| Native | 0.00 | −261.73 |
| E583K | 0.06 | −293.98 |
| **F584C** | **1.69** | **−207.98** |
| **F584L** | **1.33** | **−213.86** |
| G601R | 0.91 | −276.75 |
| **V654A** | **3.11** | **−147.43** |
| **L656P** | **1.41** | **−224.34** |
| **T670I** | **3.69** | **−121.48** |
| R796G | 0.98 | −279.71 |
| **R804W** | **1.32** | **−230.78** |
| G812V | 0.79 | −299.67 |
| **D816F** | **2.57** | **−167.43** |
| **D816V** | **2.92** | **−137.15** |
| **D816Y** | **1.67** | **−246.58** |
| D820Y | 0.60 | −290.19 |
| **N822K** | **1.45** | **−233.18** |
| **Y823D** | **3.14** | **−198.85** |
| A829P | 0.31 | −310.64 |
| **E839K** | **3.06** | **−113.31** |
| T847P | 0.68 | −267.40 |

Letters in bold indicate mutants with less binding affinity than native

R804W, D816Y and N822K with RMSD >1.00 Å; the remaining seven variants exhibited a low RMSD value ≤0.98 Å. Figure 1a shows the superimposed structure of native KIT with the mutant D816V having the RMSD of 2.92 Å as an illustrative example of this work.

## Rationale of binding efficiency for native and mutant KIT receptor with its inhibitor

In order to understand the binding efficiency of KIT protein with its inhibitor, we selected the PDB ID: 1t46 which has KIT complex with Imatinib. The LCT program was used to calculate contacts between the binding residues of KIT and inhibitor. It can be seen from Table 3 that a total of 29 amino acids, viz., Leu(595), Val(603), Ala(621), Val(622), Lys(623), Glu(640), Val(643), Leu(644), Leu(647), Ile(653), Val(654), Val(668), Ile(669), Thr(670), Glu(671), Tyr(672), Cys(673), Gly(676), Asp(677), Leu(783), Cys(788), Ile(789), His(790), Arg(791), Leu(799), Ile(808), Cys(809), Asp(810) and Phe(811) act as binding residues for KIT complex with Imatinib. It was interesting to observe that some of these amino acids include Lys(623), Glu(640), Thr(670), Cys(673), Asp(810) and Phe(811) that act as key binding residues of KIT with Imatinib which was also proved experimentally by other groups (Mol et al. 2004; Roskoski 2005). To understand the binding efficiency of Imatinib with both native and mutants of KIT structure, we unbound the Imatinib from KIT from the PDB ID: 1t46. We once again performed the point mutation by SWISSPDB viewer for the 19 variants. Energy minimization was performed by Nomad-Ref (Lindahl et al. 2006) followed by simulated annealing with ifold server (Sharma et al. 2006) to get optimized structures for both native and mutants.

Docking was performed using the PatchDock server between Imatinib with both native and mutant modeled structures of KIT to find out the binding efficiency in the form of atomic contact energy (ACE) as can be seen from Table 2. By this analysis, the ACE between Imatinib and native KIT was found to be −261.73, whereas with mutants, the ACE was found to be between the ranges −113.31 and −310.64. It can be seen from Table 2 that 7 mutants, E583K, G601R, R796G, G812V, D820Y, A829P and T847P established high binding affinity with Imatinib than native KIT; the remaining 12 mutants were found to have less binding affinity with Imatinib than native KIT in terms of ACE. These data clearly portray that Imatinib is considered to be the most favorable inhibitor not only for native but also for the former seven mutants. Also we emphasize that the maximum binding effect of Imatinib with these mutants might be due to the 3D conformation of Imatinib which exclusively made comfortable fit with high ACE into the 3D space of the binding residues of these former mutants as compared to the later mutants. But the aim of our study was to find out the most detrimental point mutants causing drug resistance. Hence the later 12 mutants, F584C, F584L, V654A, L656P, T670I, R804W, D816F, D816Y, D816Y, N822K, Y823D and E839K, which had less binding affinity with Imatinib in terms of ACE, as they have the RMSD of about ≥1.32 to ≤3.69 Å, were considered to be rationally significant missense mutations with respect to drug resistance as can be seen from Table 2. This analysis reveals that the some of these mutants, viz., V654A, T670I, D816V, N822K, Y823D and E839K that render resistance with Imatinib are also in good agreement with clinical observations (Ma et al. 2002; Weisberg and Griffin 2003; Fletcher and Rubin 2007; Kemmer et al. 2004; Chen et al. 2005; Roberts et al. 2007; McLean et al. 2005; Chen et al. 2004; Growney et al. 2005; Gajiwala et al. 2009; Debiec-Rychter et al. 2005; Tan et al. 2006; Guo et al. 2007). Figure 1b shows the superimposed structure of binding region of native complex (i.e., native KIT receptor and Imatinib) with mutant D816V complex (i.e., mutant D816V and Imatinib) as an another illustrative example of this work.

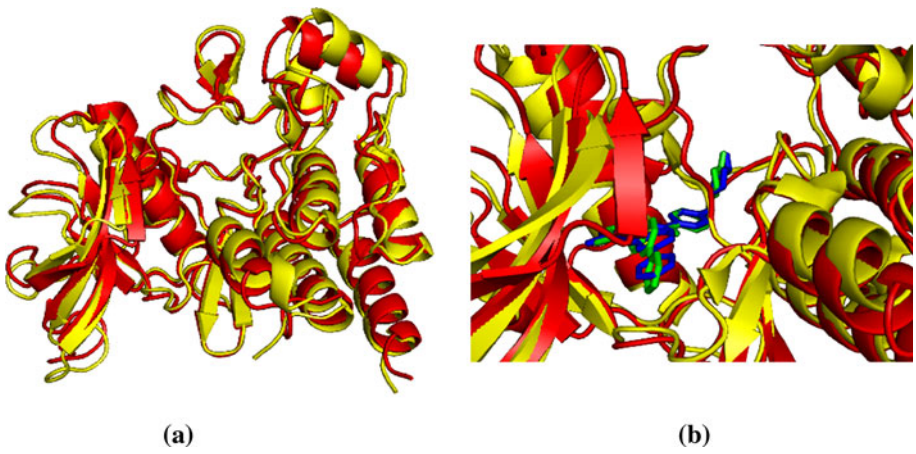


**Fig. 1** **a** Superimposed structure of native with mutant D816V **b** Superimposed structure of binding region of native complex (native KIT receptor and imatinib) with mutant D816V complex (mutant D816V and imatinib)

**Table 3** Comparison of normalized mean square displacement of drug binding amino acids of native and mutants

| Binding residues | Normalized mean square displacement $<R^2>$ | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Native | F584C | F584L | V654A | L656P | T670I | R804W | D816F | D816V | D816Y | N822K | Y823D | E839K |
| Leu(595) | 0.0070 | 0.0067* | 0.0071 | 0.0064* | **0.0070** | **0.0070** | 0.0068* | 0.0075 | 0.0055* | 0.0069* | 0.0065* | 0.0021* | 0.0066* |
| Val(603) | 0.0037 | **0.0037** | 0.0039 | 0.0036* | 0.0036* | 0.0035* | **0.0037** | 0.0042 | 0.0022* | 0.0038 | 0.0034* | 0.0047 | 0.0036* |
| Ala(621) | 0.0041 | 0.0040* | 0.0044 | **0.0041** | 0.0044 | 0.0044 | 0.0040* | 0.0043 | 0.0039* | 0.0043 | 0.0040* | 0.0032* | 0.0042 |
| Val(622) | 0.0012 | 0.0014 | 0.0014 | 0.0019 | 0.0015 | 0.0016 | **0.0012** | 0.0013 | 0.0014 | 0.0016 | 0.0013 | 0.0060 | 0.0017 |
| Lys(623) | 0.0009 | 0.0014 | **0.0009** | 0.0015 | **0.0009** | 0.0010 | 0.0010 | 0.0016 | 0.0021 | 0.0013 | **0.0009** | 0.0060 | 0.0013 |
| Glu(640) | 0.0026 | 0.0037 | **0.0026** | 0.0040 | 0.0025* | 0.0032 | **0.0026** | 0.0040 | 0.0047 | 0.0047 | 0.0027 | 0.0157 | 0.0032 |
| Val(643) | 0.0009 | 0.0030 | 0.0005* | 0.0040 | 0.0013 | 0.0010 | 0.0016 | 0.0027 | 0.0019 | 0.0029 | 0.0016 | 0.0151 | 0.0033 |
| Leu(644) | 0.0015 | 0.0032 | 0.0011* | 0.0036 | 0.0020 | 0.0012* | 0.0021 | 0.0023 | 0.0011* | 0.0023 | 0.0020 | 0.0115 | 0.0034 |
| Leu(647) | 0.0027 | 0.0036 | 0.0024* | 0.0045 | 0.0031 | 0.0024* | 0.0030 | **0.0027** | 0.0022* | 0.0023* | 0.0030 | 0.0147 | 0.0041 |
| Ile (653) | 0.0043 | 0.0037* | 0.0042* | 0.0035* | 0.0042* | 0.0038* | 0.0040* | 0.0037* | 0.0041* | 0.0042* | 0.0042* | 0.0093 | 0.0037* |
| Val(654) | 0.0035 | 0.0031* | **0.0035** | 0.0032* | **0.0035** | 0.0033* | 0.0034* | 0.0029* | 0.0032* | 0.0032* | 0.0036 | 0.0080 | **0.0035** |
| Val(668) | 0.0038 | 0.0043 | 0.0035* | 0.0029* | **0.0038** | 0.0037* | 0.0035* | 0.0040 | **0.0038** | 0.0036* | 0.0034* | 0.0063 | 0.0033* |
| Ile (669) | 0.0045 | **0.0045** | 0.0043* | 0.0032* | 0.0046 | 0.0042* | 0.0042* | 0.0044* | 0.0040* | 0.0046 | 0.0042* | 0.0048 | 0.0039* |
| Thr(670) | 0.0043 | 0.0046 | 0.0038* | 0.0040* | 0.0047 | **0.0043** | 0.0044 | 0.0042* | 0.0042* | 0.0041* | **0.0043** | 0.0036* | 0.0045 |
| Glu(671) | 0.0053 | 0.0054 | 0.0050* | 0.0052* | 0.0055 | 0.0050* | **0.0053** | 0.0052* | 0.0048* | 0.0049* | **0.0053** | 0.0046* | 0.0057 |
| Tyr(672) | 0.0066 | 0.0065* | 0.0065* | 0.0063* | 0.0068 | 0.0062* | 0.0065* | 0.0067 | 0.0061* | 0.0063* | 0.0062* | 0.0054* | 0.0067 |
| Cys(673) | 0.0065 | 0.0062* | 0.0063* | 0.0060* | 0.0068 | 0.0058* | 0.0063* | **0.0065** | 0.0051* | 0.0063* | 0.0063* | 0.0052* | **0.0065** |
| Gly(676) | 0.0071 | 0.0067* | 0.0072 | 0.0061* | 0.0074 | 0.0067* | 0.0070* | **0.0071** | 0.0062* | 0.0070 | 0.0070* | 0.0092 | 0.0070* |
| Asp(677) | 0.0071 | 0.0064* | 0.0070* | 0.0053* | 0.0070* | 0.0065* | 0.0066* | 0.0065* | **0.0071** | 0.0065* | 0.0067* | 0.0069* | 0.0063* |
| Leu(783) | 0.0053 | 0.0044* | 0.0051* | 0.0031* | 0.0048* | 0.0049* | 0.0046* | 0.0049* | 0.0049* | 0.0052* | 0.0047* | 0.0050* | 0.0036* |
| Cys(788) | 0.0045 | 0.0033* | **0.0045** | 0.0017* | 0.0040* | 0.0041* | 0.0037* | 0.0039* | 0.0034* | 0.0042* | 0.0038* | 0.0041* | 0.0023* |
| Ile (789) | 0.0057 | 0.0049* | 0.0055* | 0.0026* | 0.0052* | 0.0053* | 0.0050* | 0.0055* | 0.0051* | 0.0056* | 0.0050* | 0.0049* | 0.0036* |
| His(790) | 0.0055 | 0.0047* | 0.0053* | 0.0027* | 0.0050* | 0.0051* | 0.0048* | 0.0051* | 0.0053* | **0.0055** | 0.0049* | 0.0051* | 0.0037* |
| Arg(791) | 0.0057 | 0.0050* | 0.0056* | 0.0028* | 0.0052* | 0.0054* | 0.0051* | 0.0055* | 0.0052* | 0.0059 | 0.0051* | 0.0047* | 0.0038* |
| Leu(799) | 0.0058 | 0.0052* | **0.0058** | 0.0046* | 0.0059 | 0.0053* | 0.0055* | 0.0054* | 0.0043* | 0.0055* | 0.0056* | 0.0052* | 0.0054* |
| Ile (808) | 0.0049 | 0.0040* | 0.0048* | 0.0034* | 0.0047* | 0.0043* | 0.0044* | 0.0043* | 0.0042* | 0.0047* | 0.0046* | 0.0031* | 0.0040* |
| Cys(809) | 0.0042 | 0.0033* | **0.0042** | 0.0025* | 0.0039* | 0.0038* | 0.0037* | 0.0037* | 0.0041* | **0.0042** | 0.0038* | 0.0039* | 0.0032* |
| Asp(810) | 0.0041 | 0.0032* | 0.0042 | 0.0018* | 0.0037* | 0.0038* | 0.0035* | 0.0037* | 0.0040* | 0.0044 | 0.0036* | 0.0027* | 0.0027* |
| Phe(811) | 0.0045 | 0.0037* | 0.0046 | 0.0022* | 0.0040* | 0.0044* | 0.0040* | 0.0047 | 0.0060 | 0.0050 | 0.0040* | 0.0041* | 0.0030* |

Bold number indicates amino acids with identical flexibility of both native and mutants

* Indicates amino acids with decreased flexibility of mutants than native

## Majority of amino acids in drug binding pocket show loss of flexibility

In order to understand the cause of drug insensitivity by these 12 detrimental missense mutations, we used the program Elnemo (Suhre and Sanejouand 2004) to compare the flexibility of amino acids of both native and mutants, which are involved in binding with Imatinib. Table 3 depicts the flexibility of amino acids in drug binding pocket of both native and mutants by means of normalized mean-square displacement $<R2>$. We further sorted out these data into three different ranges of flexibility. One is the $<R2>$ of amino acids in drug binding pocket of mutants which is exactly the same as $<R2>$ of the amino acids in drug binding pocket of native named as 'identical flexibility'. The other is the $<R2>$ of amino acids in drug binding pocket of mutants which is higher than $<R2>$ of the amino acids in drug binding pocket of native named as 'increased flexibility'. And the last is the $<R2>$ of amino acids in drug binding pocket of mutants which is lesser than $<R2>$ of amino acids in drug binding pocket of native named as 'decreased flexibility'. Table 4 depicted that the three different ranges of flexibility of amino acids in drug binding pocket for 12 mutants. From this analysis, we found that the number of drug binding amino acids of these 12 mutants having the range of identical flexibility is lesser than those of increased and decreased flexibility. On the other hand the number of drug binding amino acids having the range of increased flexibility is lesser than those of decreased flexibility as could be seen from Table 4. This

**Table 4** Drug binding amino acids of mutants with different ranges of flexibility based on <R2>

| Mutants | A <R2> | B | C |
|---|---|---|---|
| F584C | 18 | 2 | 9 |
| F584L | 16 | 6 | 7 |
| V654A | 22 | 1 | 6 |
| L656P | 13 | 4 | 12 |
| T670I | 22 | 2 | 5 |
| R804W | 20 | 4 | 5 |
| D816F | 15 | 3 | 11 |
| D816V | 22 | 2 | 5 |
| D816Y | 15 | 2 | 12 |
| N822K | 20 | 3 | 6 |
| Y823D | 17 | 0 | 12 |
| E839K | 17 | 2 | 10 |

Where the letter 'A' denotes amino acids with decreased flexibility of mutants than native; 'B' denotes amino acids with identical flexibility of both native and mutants; 'C' denotes amino acids with increased flexibility of both native and mutants

evidently exemplified that majority of amino acids participated in drug binding pocket of these mutants lost their flexibility due to their occurrence in the range of 'decreased flexibility' which signify the loss of binding efficiency with the inhibitor, Imatinib. The supplementary tables are given for all the 31 mutants with RMSD, ACE, flexibility of binding amino acids and possible citations for drug resistant mutants to support our work.

## Conclusion

As several missense mutations like V560G, D820A and K641E of Kit receptor showed drug resistance (Fletcher and Rubin 2007; Heinrich et al. 2006; Bauer et al. 2006), in this work, we also found quite a few drug-resistant mutations by our computational approach. Based on our investigation, we concluded that out of 31 variants which were retrieved from Swissprot, 29 variants were found less stable by I-Mutant 2.0; 20 variants were found deleterious by SIFT and 25 variants were considered damaging by PolyPhen. We rationally considered that the 19 variants were considered to be the potential detrimental point mutations as they were commonly found to be less stable, deleterious and damaging by the I-Mutant 2.0, SIFT and PolyPhen servers, respectively. Further we modeled the mutants based on these 19 variants. The RMSD for mutants with native was computed which showed the range between 0.06 and 3.69 Å. Moreover docking was performed between Imatinib with native and mutants which showed the ACE between −113.31 and −310.64. Finally we conclude that the less binding affinity of 12 mutants, viz., F584C, F584L, V654A, L656P, T670I, R804W, D816F, D816Y, D816Y, N822K, Y823D and E839K with Imatinib when compared with native KIT structure in terms of ACE, as they have the RMSD between ≥1.32 and ≤3.69 Å, were persuasively considered to be significant for the cause of drug resistance. We also elucidated the cause of drug resistance of these 12 mutants in terms of normalized mean square displacement $\langle R2 \rangle$ by normal mode analysis. From this analysis, we concluded that the majority of amino acids of these mutants participated as binding residues with Imatinib had 'decreased flexibility' which could be the cause for drug insensitivity. The overall scope of our result is (1) to consider computationally a suitable protocol for missense mutation (point mutation/single amino acid polymorphism) analysis before wet lab experimentation, (2) to provide an optimal path for further clinical and experimental studies to characterize KIT mutants in depth, (3) to consider an appropriate protocol for identifying the drug resistance by point mutations, (4) to validate the potency of drug molecules and (5) to select the potential drug based on point mutation associated cancer targets.

**Acknowledgment** The authors thank the management of Vellore Institute of Technology for providing the facilities to carry out this work.

## References

Bauer S, Hartmann JT, de WM, Lang H, Grabellus F, Antoch G, Niebel W, Erhard J, Ebeling P, Zeth M (2005) Resection of residual disease in patients with metastatic gastrointestinal stromal tumors responding to treatment with imatinib. Int J Cancer 117:316–325

Bauer S, Yu LK, Demetri GD, Fletcher JA (2006) Heat shock protein 90 inhibition in imatinib-resistant gastrointestinal stromal tumor. Cancer Res 66(18):9153–9161

Bava KA, Gromiha MM, Uedaira H, Kitajima K, Sarai A (2004) ProTherm, version 4.0: thermodynamic database for proteins and mutants. Nucleic Acids Res 32:120–121

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE (2000) The Protein Data Bank. Nucleic Acids Res 28(1):235–242

Blechman JM, Lev S, Barg J, Eisenstein M, Vaks B, Vogel Z, Givol D, Yarden Y (1995) The fourth immunoglobulin domain of the stem cell factor receptor couples ligand binding to signal transduction. Cell 80:103–113

Blume-Jensen P, Hunter T (2001) Oncogenic kinase signaling. Nature 411:355–365

Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M (2003) The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res 31(1):365–370

Capriotti E, Fariselli P, Casadio R (2005) I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res 33:306–310

Carlson HA, McCammon JA (2000) Accommodating protein flexibility in computational drug design. Mol Pharmacol 57:213–218
Cavallo A, Martin AC (2005) Mapping SNPs to protein sequence and structure data. Bioinformatics 21(8):1443–1450
Chen LL, Trent JC, Wu EF, Fuller GN, Ramdas L, Zhang W, Raymond AK, Prieto VG, Oyedeji CO, Hunt KK, Pollock RE, Feig BW, Hayes KJ, Choi H, Macapinlac HA, Hittelman W, Velasco MA, Patel S, Burgess MA, Benjamin RS, Frazier ML (2004) A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. Cancer Res 64(17):5913–5919
Chen LL, Sabripour M, Andtbacka RH, Patel SR, Feig BW, Macapinlac HA, Choi H, Wu EF, Frazier ML, Benjamin RS (2005) Imatinib resistance in gastrointestinal stromal tumors. Curr Oncol Rep 7(4):293–299
Ciccarelli FD, Acciarito A, Alberti S (2000) Large and diverse numbers of human diseases with HIKE mutations. Hum Mol Genet 9(6):1001–1007
Connolly ML (1983a) Solvent-accessible surfaces of proteins and nucleic acids. Science 221(4612):709–713
Connolly ML (1983b) Analytical molecular surface calculation. J Appl Cryst 16:548–558
Debiec-Rychter M, Cools J, Dumez H, Sciot R, Stul M, Mentens N, Vranckx H, Wasag B, Prenen H, Roesel J, Hagemeijer A, Van Oosterom A, Marynen P (2005) Mechanisms of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 128(2):270–279
Delarue M, Dumas P (2004) On the use of low-frequency normal modes to enforce collective movements in refining macromolecular structural models. Proc Natl Acad Sci USA 101(18): 6957–6962
Duhovny D, Nussinov R, Wolfson HJ (2002) Efficient unbound docking of rigid molecules. In: Gusfield D et al (eds) Proceedings of the second workshop on algorithms in bioinformatics (WABI) Lecture Notes in Computer Science, vol 2452, Springer, Rome, Italy, pp 185–200
Engelman JA, Settleman J (2008) Acquired resistance to tyrosine kinase inhibitors during cancer therapy. Curr Opin Genet Dev 18:73–79
Fletcher JA, Rubin BP (2007) KIT mutations in GIST. Curr Opin Genet Dev 17(1):3–7
Gajiwala KS, Wu JC, Christensen J, Deshmukh GD, Diehl W, Dinitto JP, English JM, Greig MJ, He YA, Jacques SL, Lunney EA, McTigue M, Molina D, Quenzer T, Wells PA, Yu X, Zhang Y, Zou A, Emmett MR, Marshall AG, Zhang HM, Demetri GD (2009) KIT kinase mutants show unique mechanisms of drug resistance to imatinib and sunitinib in gastrointestinal stromal tumor patients. Proc Natl Acad Sci USA 106(5):1542–1547
Growney JD, Clark JJ, Adelsperger J, Stone R, Fabbro D, Griffin JD, Gilliland DG (2005) Activation mutations of human c-KIT resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. Blood 106(2):721–724
Guo T, Agaram NP, Wong GC, Hom G, D'Adamo D, Maki RG, Schwartz GK, Veach D, Clarkson BD, Singer S, DeMatteo RP, Besmer P, Antonescu CR (2007) Sorafenib inhibits the imatinib-resistant KITT670I gatekeeper mutation in gastrointestinal stromal tumor. Clin Cancer Res 13(16):4874–4881
Gupta P, Tewari M, Shukla HS (2008) Gastrointestinal stromal tumor. Surg Oncol 17(2):129–138
Han JH, Kerrison N, Chothia C, Teichmann SA (2006) Divergence of interdomain geometry in two-domain proteins. Structure 14(5):935–945
Heinrich MC, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, Eisenberg BL, von Mehren M, Fletcher CD, Sandau K, McDougall K, Ou WB, Chen CJ, Fletcher JA (2006) Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 24(29):4764–4774
Hinkle A, Tobacman LS (2003) Folding and function of the troponin tail domain. Effects of cardiomyopathic troponin T mutations. J Biol Chem 278(1):506–513
Jonathan Fletcher A, Brian Rubin P (2007) KIT mutations in GIST. Curr Opin Genet Dev 17:3–7
Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, Cummings OW, Wait C, Town A, Heinrich MC (2004) KIT mutations are common in testicular Seminomas. Am J Pathol 164(1):305–313
Kim HJ, Lim SJ, Park K, Yuh YJ, Jang SJ, Choi J (2005) Multiple gastrointestinal stromal tumors with a germline c-kit mutation. Pathol Int 55(10):655–659
Lemmon MA, Pinchasi D, Zhou M, Lax I, Schlessinger J (1997) Kit receptor dimerization is driven by bivalent binding of stem cell factor. J Biol Chem 272:6311–6317
Lev S, Yarden Y, Givol D (1992) Dimerization and activation of the kit receptor by monovalent and bivalent binding of the stem cell factor. J Biol Chem 267:15970–15977
Lindahl E, Azuara C, Koehl P, Delarue M (2006) NOMAD-Ref: visualization, deformation and refinement of macromolecular structures based on all-atom normal mode analysis. Nucleic Acids Res 34:52–56
López G, Valencia A, Tress ML (2007) Firestar—prediction of functionally important residues using structural templates and alignment reliability. Nucleic Acids Res 35:573–577
Ma Y, Zeng S, Metcalfe DD, Akin C, Dimitrijevic S, Butterfield JH, McMahon G, Longley BJ (2002) The c-KIT mutation causing human mastocytosis is resistant to STI571 and other KIT kinase inhibitors; kinases with enzymatic site mutations show different inhibitor sensitivity profiles than wild-type kinases and those with regulatory-type mutations. Blood 99(5):1741–1744
McLean SR, Gana-Weisz M, Hartzoulakis B, Frow R, Whelan J, Selwood D, Boshoff C (2005) Imatinib binding and cKIT inhibition is abrogated by the cKIT kinase domain I missense mutation Val654Ala. Mol Cancer Ther 4(12):2008–2015
Mol CD, Dougan DR, Schneider TR, Skene RJ, Kraus ML, Scheibe DN, Snell GP, Zou H, Sang BC, Wilson KP (2004) Structural basis for the autoinhibition and STI-571 inhibition of c-Kit tyrosine kinase. J Biol Chem 279(30):31655–31663
Ng PC, Henikoff S (2001) Predicting deleterious amino acid substitutions. Genome Res 11(5):863–874
Ng PC, Henikoff S (2003) SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 31(13):3812–3814
Nishida T, Kanda T, Nishitani A, Takahashi T, Nakajima K, Ishikawa T, Hirota S (2008) Secondary mutations in the kinase domain of the KIT gene are predominant in imatinib-resistant gastrointestinal stromal tumor. Cancer Sci 99(4):799–804
Parthasarathy S, Murthy MR (2000) Protein thermal stability: insights from atomic displacement parameters (B values). Protein Eng 13:9–13
Philo JS, Wen J, Wypych J, Schwartz MG, Mendiaz EA, Langley KE (1996) Human stem cell factor dimer forms a complex with two molecules of the extracellular domain of its receptor, Kit. J Biol Chem 271:6895–6902
Rajasekaran R, George Priya Doss C, Sudandiradoss C, Ramanathan K, Purohit R, Sethumadhavan R (2008a) Effect of deleterious nsSNP on the HER2 receptor based on stability and binding affinity with herceptin: a computational approach. C R Biol 331(6):409–417
Rajasekaran R, Priya Doss CG, Sudandiradoss C, Ramanathan K, Sethumadhavan R (2008b) In silico analysis of structural and functional consequences in p16INK4A by deleterious nsSNPs associated CDKN2A gene in malignant melanoma. Biochimie 90(10):1523–1529

Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30(17):3894–3900

Ringe D, Petsko GA (1986) Study of protein dynamics by X-ray diffraction. Methods Enzymol 131:389–433

Roberts KG, Odell AF, Byrnes EM, Baleato RM, Griffith R, Lyons AB, Ashman LK (2007) Resistance to c-KIT kinase inhibitors conferred by V654A mutation. Mol Cancer Ther 6(3):1159–1166

Roskoski R Jr (2005) Structure and regulation of Kit protein-tyrosine kinase—the stem cell factor receptor. Biochem Biophys Res Commun 338(3):1307–1315

Schlessinger J (2000) Cell signaling by receptor tyrosine kinases. Cell 103:211–225

Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res 33:363–367

Sharma S, Ding F, Nie H, Watson D, Unnithan A, Lopp J, Pozefsky D, Dokholyan NV (2006) iFold: a platform for interactive folding simulations of proteins. Bioinformatics 22(21):2693–2694

Suhre K, Sanejouand YH (2004) ElNémo: a normal mode web-server for protein movement analysis and the generation of templates for molecular replacement. Nucleic Acids Res 32:610–614

Tan A, Westerman D, McArthur GA, Lynch K, Waring P, Dobrovic A (2006) Sensitive detection of KIT D816V in patients with mastocytosis. Clin Chem 52(12):2250–2257

Teng S, Madej T, Panchenko A, Alexov E (2009) Modeling effects of human single nucleotide polymorphisms on protein–protein interactions. Biophys J 96(6):2178–2188

Ullrich A, Schlessinger J (1990) Signal transduction by receptors with tyrosine kinase activity. Cell 61:203–212

Varfolomeev SD, Uporov IV, Fedorov EV (2002) Bioinformatics and molecular modeling in chemical enzymology. Active sites of hydrolases. Biochemistry (Mosc) 67(10):1099–1108

Weisberg E, Griffin JD (2003) Resistance to imatinib (Glivec): update on clinical mechanisms. Drug Resist Updat 6(5):231–238

Yip YL, Scheib H, Diemand AV, Gattiker A, Famiglietti LM, Gasteiger E, Bairoch A (2004) The Swiss-Prot variant page and the ModSNP database: a resource for sequence and structure information on human protein variants. Hum Mutat 23(5):464–470

Yip YL, Famiglietti M, Gos A, Duek PD, David FP, Gateau A, Bairoch A (2008) Annotating single amino acid polymorphisms in the UniProt/Swiss-Prot knowledgebase. Hum Mutat 29(3):361–366

Yuan Z, Bailey TL, Teasdale RD (2005) Prediction of protein B-factor profiles. Proteins 58(4):905–912

Zhang C, Vasmatzis G, Cornette JL, DeLisi C (1997) Determination of atomic desolvation energies from the structures of crystallized proteins. J Mol Biol 267(3):707–726