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Master thesis

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Simulacije mutageneze K-Ras GTPaze

Viktorija Dujmović

Sveučilišni diplomski studij Fizika; smjer: Biofizika

Sažetak:

K-Ras (eng. *Kirsten rat sarcoma*) protein je signalna GTPaza koja igra važnu ulogu u radu signalnog puta mitogenom-aktiviranih proteinskih kinaza (MAPK). U ovom radu ćemo koristeći molekulsko-dinamičke (MD) simulacije istražiti mogući utjecaj specifične mutageneze K-Ras proteina na njegova signalna svojstva. Promatrana je mutacija G12C koja je predominantna kod ljudi. K-Ras je u aktivnom stanju kad veže molekulu GTP-a te tada signalizira aktivaciju MAPK signalnog puta čija funkcija je među ostalim proliferacija i rast stanica. Hidrolizom GTP-a do molekule GDP-a K-Ras pada u neaktivno stanje. Pokazano je da poremećaji u signaliranju K-Ras proteinom dovode do razvoja karcinoma. Stoga su simulacije njegove mutageneze relevantne da bi se pokazali točni mehanizmi vezanja molekula GTP-a/GDP-a. U ovom radu izračunali smo slobodne energije primjenjujući metodu termodinamičke integracije na MD simulacijama i napravili analizu trajektorija istog skupa simulacija. Istraživanje je pokazalo da se afinitet vezanja molekule GTP-a/GDP-a mijenja ovisno o tome veže li se na protein divljeg tipa ili na njegovu mutaciju. Simulacije su pokazale nedosljednost u rezultatima između identičnih replika sustava i histerezu među simulacijama istog sustava perturbiranog u suprotnim smjerovima. Ove nedosljednosti opravdane su ekstenzivnom analizom trajektorija. Pokazano je da nereproducibilnost replika proizlazi iz nepouzdanog stvaranja vodikove veze između cisteina i molekule GTP-a, dok je histereza posljedica konformacijskih promjena tzv. Switch-petlji K-Ras proteina. Kao glavni rezultat izdvaja se izračun slobodnih energija koji pokazuje da je vezanje molekule GTP-a preferirano stanje mutiranog proteina. Drugim riječima, mutirani protein preferira aktivno stanje čime utječe na aktivnost cijeloga MAPK signalnog puta te dovodi do razvoja karcinoma.

Ključne riječi: K-Ras, MD simulacije, Slobodna energija, Termodinamička integracija

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Master thesis

Simulations of K-Ras GTPase Mutagenesis

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Abstract:

Kirsten rat sarcoma (K-Ras) protein is a signaling GTPase which has an important role in the Mitogen-activated protein kinase (MAPK) pathway. Using the methods of molecular dynamics (MD) simulations, in this thesis we will investigate if and how does the specific mutagenesis of K-Ras protein affect its signaling properties. The mutation studied here is G12C, as it is predominant in humans. K-Ras exists in an active state when it binds to a GTP molecule, and then it signals the activation of MAPK pathway, whose functions include proliferation and growth of cells. By a hydrolysis GTP to GDP molecule, K-Ras gets into an inactive state. As it is observed that irregularities in K-Ras signaling lead to development of cancer, simulations of its mutagenesis become more relevant to show the exact mechanisms of binding GTP/GDP molecules. In this thesis we have calculated the free energy using the method of thermodynamic integration on MD simulations and performed the analysis of the trajectories from the same set of simulations. The results reveal that the binding affinity of GTP/GDP molecule changes depending on whether it binds to the wild type or mutated protein. Apparent inconsistencies in results between identical replicas, as well as a prominent hysteresis between different directions in simulations of the same perturbation system is discussed. In order to understand these inconsistencies, a thorough analysis of simulation trajectories is exploited. It is shown that such a lack of the full reproducibility of the simulation replicas emerges from the inconsistencies in the formation of h-bond between cysteine and GTP molecule, whereas the hysteresis is a consequence of the conformational changes in the Switch loops of K-Ras protein. In other words, a mutated protein favours the active state, which directly influences the activity of the MAPK pathway, which in turn leads to a cancer development.

Keywords: K-Ras, MD simulations, Free energy, Thermodynamic integration

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Contents

1	Introduction	1
2	Theoretical overview	2
2.1	K-Ras in MAPK pathway	2
2.1.1	K-Ras mutagenesis	3
2.2	Molecular dynamics simulations	4
2.3	Free energy calculations	6
2.3.1	Thermodynamic integration	7
3	Methods	7
3.1	Preparing the system	7
3.1.1	Thermodynamic cycle	9
3.1.2	Creating replicas	10
3.2	Setting simulations	10
3.3	Extended thermodynamic integration	11
3.4	Free energy calculations	12
3.5	Data analysis	12
4	Results and discussion	13
4.1	K-Ras mutations occurrence in human cancers	13
4.2	Obtained free energies	13
4.2.1	MBAR-BAR differences	17
4.2.2	λ -point evaluation	18
4.2.3	Problems with perturbations	20
4.3	Trajectory analysis	21
4.3.1	Root mean square distance analysis	21
4.3.2	Analysis of h-bonds	24
4.3.3	Atom-atom distance analysis	25
5	Conclusion	31
A	Appendix: Frequency of K-Ras mutations in human cancers with fatalities - full data	i
B	Appendix: Free energy calculations file preparation code	iv
C	Appendix: H-bond occurrence tables	xiv

1 Introduction

The fast advance in computational systems and algorithm development lead to a vast use of computational methods in many scientific fields. A need to simulate fundamental physical processes in large biological systems, such as membranes and membrane-embedded proteins, caused a whole new branch within biophysics to emerge. Molecular dynamics (MD) simulations have made it possible to explore biological systems at smaller scales than the experiments provided.

This research is based on utilizing the MD simulations, specifically free energy calculations through the method of thermodynamic integration, on a G12C mutation of the Kirsten rat sarcoma (K-Ras) protein. This specific mutation and its mechanisms became crucial since it is known that this protein's mutations lead to cancer development in humans [1, 2, 3]. It is known that the cause of cancerous cell development lies in the over-activation of the Mitogen-activated protein kinase (MAPK) pathway, in which K-Ras plays a prominent role [1, 3, 4]. The preferable binding of GTP $P\gamma$ oxygen to the sulfate on a mutated cysteine leads to excessive amount of phosphorylations [1, 3, 4]. To inspect how the process of activation and over-activation plays out on a molecular level, binding of GTP and GDP molecules to a wild type and a mutated protein were simulated using the method of thermodynamic integration.

In the second chapter of the thesis, a theoretical overview is given on K-Ras protein, its mutations and involvement in MAPK pathway. Additionally, there is a short review of the basis of MD simulations and free energy calculations as used in this work.

The third chapter exposes the methods used in this research, specifically how the system was prepared, which were the simulation settings and how were the energies calculated. Programs and algorithms that have been used are described in a more detail.

In the final chapter, results of the research are presented and discussed. Results of the free energy calculations are revealed, and possible problems within the system and its perturbations are explained, as well as the question how they might relate to apparent energy differences between the same system simulation replicas. Finally, trajectories are directly analysed to show how system conformations affected final results and if there are additional aspects of the system that are important to consider.

Overall, the main goal of this thesis is to show that binding free energies, calculated using MD simulations, can be used to explain the over-activation of a mutated K-Ras protein in MAPK pathway and therefore to explain the molecular mechanism behind the cancer development in these proteins by cystein-GTP binding.

2 Theoretical overview

In this chapter, the significance of K-Ras protein is discussed, as well as the effects of its mutagenesis to cancer development. Secondly, the theoretical overview of the MD simulations is presented, together with the evaluation of free energies using the method of thermodynamic integration.

2.1 K-Ras in MAPK pathway

K-Ras protein is a signaling GTPase which regulates cell proliferation, playing a key role in the mitogen-activated protein kinase (MAPK) pathway [1, 2]. K-Ras protein cycles between an active GTP-bound and an inactive GDP-bound state which are regulated by the presence of guanine nucleotide exchange factors (GEFs). GEFs promote the exchange from GDP to GTP and GTPase-activating proteins (GAPs) which mediate GTP hydrolysis [3].

K-Ras protein is a part of the Ras superfamily which contains 36 proteins in humans. Within the family, H-Ras and N-Ras are phylogenetically close to K-Ras protein that is studied in this thesis [2]. K-Ras is found in two alternative splice variants, K-Ras4a and K-Ras4b, with the change in fourth exons which encode the C-terminal helix $\alpha 5$ of the G-domain (residues 1-166, non-conserved residues being 151, 153, 165, and 166) and the hypervariable region (HVR, residues 167-189/188 of K-Ras4a and K-Ras4b, respectively) involved in the membrane attachment [5].

The structure and sequence of K-Ras4b isoform is shown in Figure 1. The crystal structure shows that GDP molecule binds to the magnesium ion with its PO_3 group, which connects it to the Switch-I loop. The pocket into which the GDP slides is formed by another two loops: Switch-II and P-loop. Switch loops additionally form binding interfaces for Ras regulators, GAPs and GEFs [1]. Loop definitions throughout the literature are quite arbitrary, which means that shown depiction might be expanded by a few residues. This is a result of protein's high intrinsic flexibility, especially taking into consideration the P-loop which can be extended up to residue number 17 [1, 6].

MAPK is a pathway of signal transduction activated downstream of protein kinases with highly conserved regions that play a significant role in signal transduction in eukaryotic cells, specifically being characterised as a part of the extracellular signal-regulated kinase (ERK) family in mammalian cells [7]. Being composed of serine/threonine and tyrosine kinases, most notably Ras, Raf, Mitogen-activated protein kinase kinase (MAPKK or MEK) and ERK, MAPK pathway gets activated by the stimulation of membrane tyrosine kinase receptors [8].

The central role of ERK signaling in mammalian cells came to light from the studies of the Ras proteins, first identified as the oncogenic proteins that cause sarcomas in rats [1, 2, 7, 8].

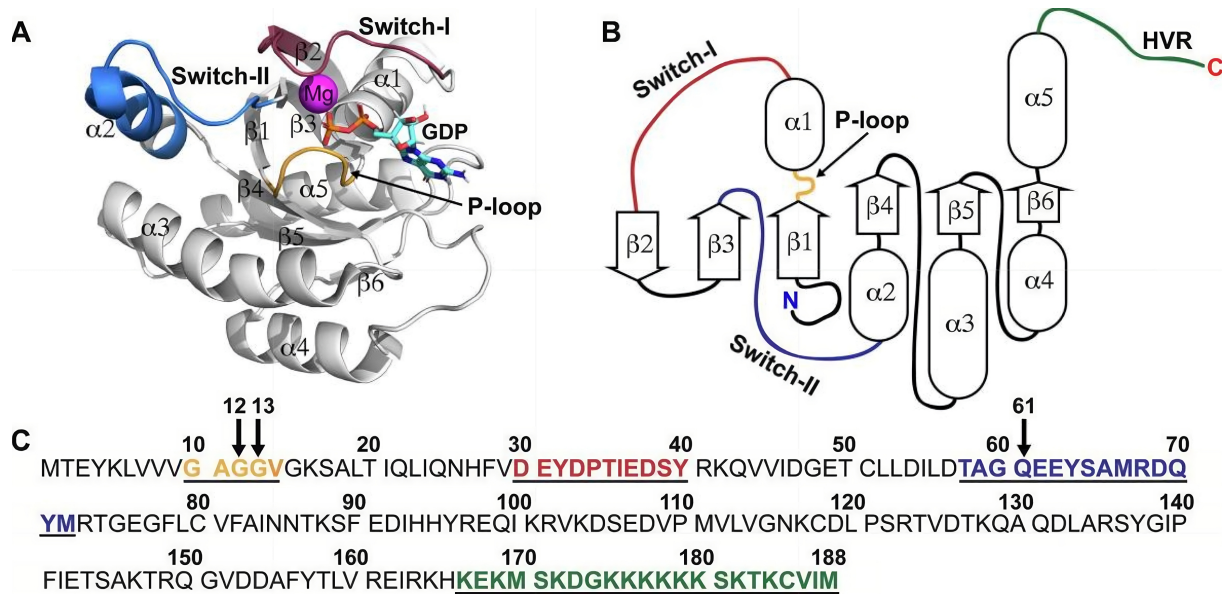


Figure 1: Structure and sequence of K-Ras4b protein. All relevant loops are marked. (A) Wild type crystal structure with magnesium ion and bound GDP molecule. Protein is presented without HVR region. (B) 2D depiction of secondary structure. (C) K-Ras4b sequence with colored loop residues (analog to A and B). Arrows point to most common mutation hotspots. Figure taken from [1].

The first research that connected mutations to cancerous cell growth was made in 1982 [9]. In 1993 the first conclusions on how Ras protein is activated by epidermal growth factor (EGF) tyrosine kinase receptors were made [10].

As it is shown in the MAPK pathway scheme in Figure 2, GEFs bind to the phosphorylated site of the receptor, which enables the exchange of GDP to GTP in membrane-bound Ras and its activation [8]. This activation leads to fixation, dimerization and phosphorylation of Raf, activating MEK by phosphorylation on serines 218 and 222, which finally activates ERK phosphorylating threonine 202 and tyrosine 204 in ERK1 isoform or threonine 185 and tyrosine 187 in ERK2 isoform [7, 8]. Once activated, ERK then phosphorylates a variety of target proteins, and a fraction of them translocates to the cell nucleus, regulating transcription factors [7].

The mutagenesis of MAPK effectors affects the functionality and signaling of the cascade, which leads to development of cancer [11]. Therefore, in the next subsection, the focus will be on the mutagenesis of Ras proteins, specifically K-Ras, and the way they can affect human health.

2.1.1 K-Ras mutagenesis

Concerning MAPK pathway, the majority of tumors are caused by mutations of Raf and Ras families of proteins [1, 2, 3, 11]. Namely, Ras mutations are found in 15-29% cases of melanoma, 5-20% colorectal, and 12-30% lung cancer; Raf mutations are observed in 50-60%

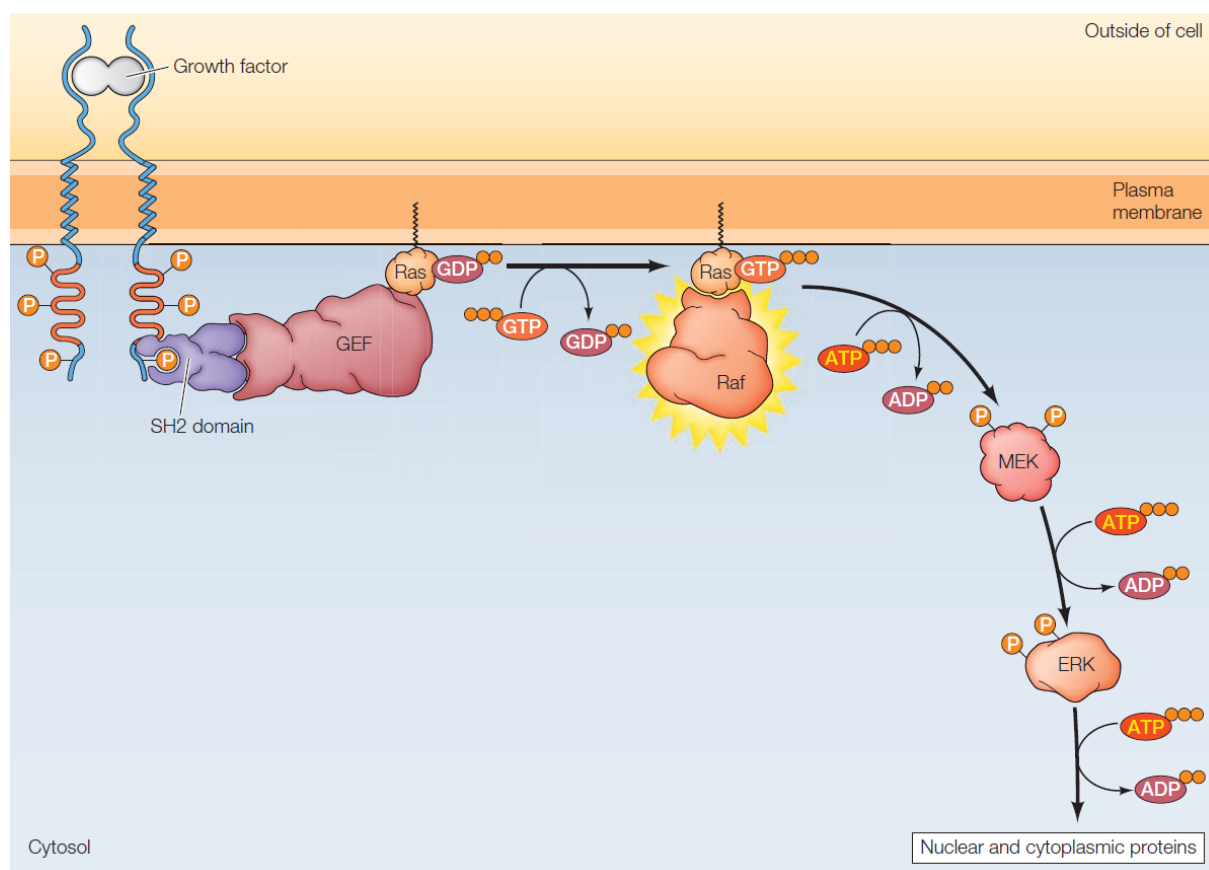


Figure 2: Scheme of MAPK pathway. Activation of Ras, Raf and ERK downstream of receptor tyrosine kinases. Figure taken from [7].

melanoma, 5-20% colorectal, 4% lung cancer cases; mutations in MEK and ERK are found in only 3-8% melanoma and 3% colorectal cases [11]. Specific mutation occurrences in human cancers are shown in Results and discussion chapter.

2.2 Molecular dynamics simulations

Molecular dynamics (MD) simulations are utilized to show the differences between molecular mechanisms of the wild type and mutated protein. They allow one to analyse basic interactions between molecules and solvent described through classical mechanics and thermodynamics, as well as to calculate the physical parameters of the system [15, 16].

First molecular simulations were introduced in 1949 by Metropolis et al., which proposed a probabilistic approach to approximate properties of a set of particles [17]. This inspired molecular simulations by Alder and Wainwright in 1959 that introduced assigned initial velocities and positions of individual atoms [18]. First simulations of molecules were presented by Rahman and Stillinger in 1971 [19]. These were also first simulations of liquid water. In 1976, Warshel and Levitt integrated quantum mechanics into molecular simulations exchanging classical atom charge with quantum mechanics equations [20]. An enormous

advancement occurred in 1977 when molecular simulations were used to study proteins by setting constraints to reach longer simulation times. This study by Karplus et al. led to the Nobel Prize in Chemistry in 2013 awarded to Warshel, Levitt and Karplus for the development of multiscale models for complex chemical systems [20, 21]. In 1985, Car and Parrinello combined molecular dynamics with density-functional theory [22]. Further advances in technology led to higher computer performance, which meant that MD simulations could be more widely used and performed on larger systems for longer simulation times [16].

The importance of MD simulations in protein research is in the ability to look into dynamic features which are essential for protein function, and can not be observed through available experimental methods [16]. In MD simulations, time evolution of interacting particles motion is given as a solution of Newton's equations:

$$\mathbf{F}_i = m_i \frac{d^2 \mathbf{r}_i(t)}{dt^2}, \quad (2.1)$$

where \mathbf{F}_i is the force acting upon i th particle, m_i the mass of the particle, and $\mathbf{r}_i(t) = (x_i(t), y_i(t), z_i(t))$ is the position vector at time t [23]. Positions \mathbf{r}_i and velocities \mathbf{v}_i define particle trajectories and determine kinetic energy and temperature of the system. To solve equation 2.1 numerically in MD simulations, the Verlet algorithm is most commonly used:

$$\mathbf{r}_i(t + \Delta t) \cong 2\mathbf{r}_i(t) - \mathbf{r}_i(t - \Delta t) + \frac{\mathbf{F}_i(t)}{m_i} \Delta t^2, \quad (2.2)$$

expression being accurate to the fourth power of Δt [23].

To solve former equations, one needs to evaluate the force \mathbf{F}_i on each particle, and for that force fields are used. Force field is a set of mathematical expressions and parameters that describes all the intramolecular and intermolecular forces on each particle of the system [16]. A typical force field expression looks like this:

$$\begin{aligned} U = & \sum_{\text{bonds}} \frac{1}{2} k_b (r - r_0)^2 + \sum_{\text{angles}} \frac{1}{2} k_a (\theta - \theta_0)^2 + \sum_{\text{torsions}} \frac{V_n}{2} [1 + \cos(n\phi - \delta)] + \\ & + \sum_{\text{improper}} V_{\text{imp}} + \sum_{\text{LJ}} 4\epsilon_{ij} \left(\frac{\sigma_{ij}^{12}}{r_{ij}^{12}} - \frac{\sigma_{ij}^6}{r_{ij}^6} \right) + \sum_{\text{elec}} \frac{q_i q_j}{r_{ij}}, \end{aligned} \quad (2.3)$$

where first four terms present intramolecular contributions in the form of bond stretching, angle bending, dihedral and improper torsion, while the last two describe Van der Waals interactions, specifically Lennard-Jones and Coulombic interactions [24]. From this expression, calculating the force \mathbf{F}_i is trivial, using well known expression $\mathbf{F} = -\nabla U$.

All the simulations in this thesis were performed in the GROMOS Software using GROMOS force fields, specifically 54A8, which is an atomistic force field with exception of CH_n hydrogens modelled as united-atoms [25, 26].

Instead of previously proposed Verlet algorithm (eq 2.2), GROMOS uses leapfrog scheme:

$$\mathbf{r}_i(t + \Delta t) \cong \mathbf{r}_i(t) + \mathbf{v}_i(t)\Delta t + \frac{1}{2}\mathbf{a}_i(t)\Delta t^2, \quad (2.4a)$$

$$\mathbf{v}_i(t + \Delta t) \cong \mathbf{v}_i(t) + \frac{1}{2}(\mathbf{a}_i(t) + \mathbf{a}_i(t + \Delta t))\Delta t, \quad (2.4b)$$

and the MD algorithm runs in 13 steps [25]:

- a) saving positions and velocities for later analysis;
- b) removing center of mass motion;
- c) calculating energies, forces, and virial contribution;
- d) enforcing any given position restraints by setting forces and velocities of constrained atoms to zero;
- e) updating velocities using leapfrog scheme (eq 2.4b)
- f) scaling velocities to apply temperature coupling;
- g) updating positions based on leapfrog scheme (eq 2.4a);
- h) enforcing distance constraints (using SHAKE) and calculating corresponding forces and virials;
- i) calculating kinetic energy and temperature;
- j) calculating pressure;
- k) applying pressure scaling by scaling positions;
- l) updating coupling parameter λ for free energy simulations involving λ changes
- m) calculating total energies, averages and fluctuations.

These steps are then repeated for the duration of a simulation.

2.3 Free energy calculations

The estimation of free energy changes is relevant in understanding biomolecular processes. The calculation of conformational free energies aims at evaluating the relative free energies of relevant conformational states, while alchemical free energies calculation aims at evaluating the relative free energies of different molecules [27, 28]. Whether the free energies or free enthalpies are calculated depends on the constraints given to the system: the relative (Helmholtz) free energy ΔF is calculated under isochoric-isothermic conditions (NVT), while the relative (Gibbs) free enthalpy ΔG is calculated under isobaric-isothermic conditions (NpT) [27]. Some of the computational methods used to estimate free energy are Thermodynamic Integration (TI), Umbrella Sampling, and Enveloping Distribution Sampling (EDS). In this thesis, TI is a method of choice.

2.3.1 Thermodynamic integration

Thermodynamic integration is a method in which the λ parameter is set in a way that $\lambda_A = 0$ indicates state A and $\lambda_B = 1$ indicates state B. System is then perturbed between states A and B with user set λ step change, and free energy difference ΔG_{BA} is given by:

$$\Delta G_{BA} = G(\lambda_B) - G(\lambda_A) = \int_{\lambda_A}^{\lambda_B} \left\langle \frac{\partial H(\lambda)}{\partial \lambda} \right\rangle_{\lambda} d\lambda, \quad (2.5)$$

where $H(\lambda)$ is the ensemble average of the system [27]. Given long simulation times and enough λ points sampled, this expression converges.

Once configuration sets have been sampled, various approaches could be used to estimate free energy changes. Besides TI calculations, one can use Bennett Acceptance Ratio (BAR) to estimate free energy differences. BAR formula states that:

$$\Delta G_{A,B}^{(n)} - C = \ln \frac{\langle f[H_A(x) - H_B(x) - C] \rangle_B}{\langle f[H_B(x) - H_A(x) - C] \rangle_A}, \quad (2.6)$$

where $f(x) = 1/(1 + e^x)$ is the Fermi function and $C \approx \Delta G_{A,B}$ is iteratively determined [29, 30]. This expression can be expanded in case of multiple states to calculate MBAR values [28].

3 Methods

After describing the theoretical concepts of the problem, here the methods used in the research will be displayed. All the used programs will be listed, as well as all the simulation settings.

Simulations were done in GROMOS software [25] and analysis was performed using GROMOS built-in programs, customized Python scripts and GROMACS built-in programs [31].

3.1 Preparing the system

Before setting up simulations, starting structures have to be prepared. To check how the mutation G12C in K-Ras protein affects GTP/GDP binding, one wants to prepare structures containing wild type protein or mutated protein, both with either GTP or GDP, or in total 4 structures. Besides those, structures of sole wild type (wt) / mutated protein and sole GTP/GDP molecules are needed.

To obtain those structures, the protein data bank (PDB) should be firstly searched for possible available crystal structures. Thereby, one structure that suits this research can be found. Under

the PDB ID 8afc one can find the mutated K-Ras4b protein with mutation G12C in complex with the compound 12 obtained through x-ray diffraction with resolution of 2.41 Å [32].

Compound 12 is 2-azanyl-4,4-dimethyl-6,7-dihydro-5~H-1-benzothiophene-3-carbonitrile, or LXX ligand for short. Besides K-Ras with G12C mutation and LXX ligand, PDB file contains one magnesium ion, one sulfate ion, guanosine-5'-diphosphate (GDP) and 26 water molecules.

Simulations in this research were performed after removing LXX, sulfate ion and water molecules. It was decided not to make all the structures initially, but to start with G12C-GDP combination and make rest of the structures from the final ones of each perturbation within a thermodynamic cycle. Still, to represent G12C-GDP→G12C-GTP, there are additional four atoms to include in the final structure, and one to be removed. This means that the input PDB file needs to contain both the atoms included and atoms excluded from the final structure.

To resolve this problem, PyMOL molecular graphics system program was used [33]. To add or remove the phosphate group, PyMOL *Builder* option was used. Both GTP and GDP conformations are saved as PDB files, and the H3P β hydrogen is added to the PDB file, so that it contains three phosphate groups in the end, together with the said hydrogen so GDP molecule can be built. Here one can also change the mutated residues. While mutating cysteine to glycine consists of removing the thiol group, which can be done manually by deleting atoms one by one, if the original file contains glycine, thiol group has to be built from scratch to mutate it to cysteine. This can hardly be done by simply adding atoms manually, as it is troublesome to direct their positions. PyMOL wizard *Mutagenesis* makes this process much easier than building individual atoms, as it allows user to select a residue and simply choose to which residue they want to mutate it to.

Besides getting the PDB files, topologies had to be made. To do this, GROMOS program *make_top* was used, giving the protein sequence of K-Ras. Topologies were made for wt and G12C K-Ras, and also for GTP and GDP molecule and magnesium ion. To get topologies of full systems, GROMOS program *com_top* was used, to combine previously prepared topologies.

Previously made structures have to be translated from PDB format to .cnf file format. This may be done using GROMOS *pdb2g96* program. Sometimes atom names in pdb files do not correspond to atom names in GROMOS topologies, which requires additionally stating those cases and how to resolve them. To check the .cnf file content in PyMOL at any step, GROMOS program *frameout* writes out a pdb file from the .cnf file using given topology file.

Before creating simulation box and filling it out with solvent, a minimisation of the system is done using the steepest-descent algorithm in vacuum for 2500 steps with 0.002 dt. Constraints are imposed by SHAKE algorithm. All of those simulation parameters, as well as some not mentioned, are stated in the .imd file, which is given with topology and .cnf files to the GROMOS *md* program which performs minimisation based on those parameters and gives

back a minimized .cnf file.

This file is then given to the *sim_box* GROMOS program. The simulation box is set to the rectangular box with the minimal solute-wall distance of 0.8, and the minimal solute-solvent distance of 0.23. This step places the protein into the solvent within a box preparing it for further simulation steps. To relax this new soluted system, minimisation has to be performed, this time with positional restraints on the solute. This is performed by making two additional files with extensions .por and .rpr by copying .cnf file and making some small changes manually. In .por file POSITION keyword at the beginning of the atom coordinate block has to be exchanged to POSRESSPEC. All the solvent atoms have to also be removed from this file. Similarly, in .rpr file the keyword in the beginning of the block should read REFPOSITION. Now minimisation is performed the same way as before. Changes in the .imd file include setting the number of solvent molecules and changing it from being performed in vacuum to being performed in solvent. POSITIONRES block is added, specifying that position restraints should be taken into account. Simulations were done without any ion atoms in the solvent, which means that systems are now prepared for further equilibration which will be explained in further subsections.

3.1.1 Thermodynamic cycle

Figure 3 shows a scheme of a thermodynamic cycle which is followed in simulations which shall be presented. The starting point is in upper left corner, or specifically mutated K-Ras protein with GDP molecule bound to it. From there, arrows lead in both the direction of G12C-GTP and WT-GDP perturbations. Simulations are performed in a way that the beginning structure of each new simulation is obtained by taking the final structure of the previous simulation and, if needed, adding or subtracting atoms. This is done until the cycle is closed, in both directions.

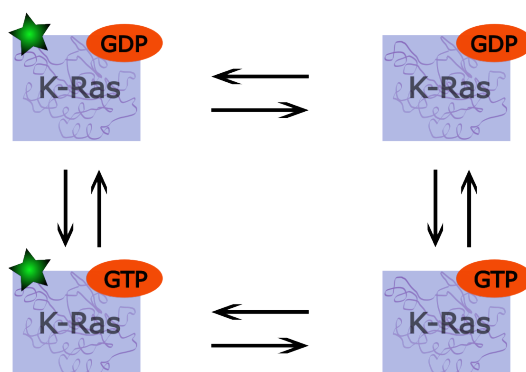


Figure 3: Scheme of a thermodynamic cycle of K-Ras mutation and GTP/GDP binding. The presence of a G12C mutation is represented by a green star.

3.1.2 Creating replicas

To get a better statistical representation, system is simulated in three replicas. Replicas are systems constructed of same number of atoms with a slight change in conformations. This results from using a different random number generator seed in the .imd file in equilibration step, which is to be described. MD simulations are reproducible, meaning that by using the same starting point (all the initial input files) and the same seed and other simulation parameters, one should get the same simulation results, or same trajectories. By changing the seed, the input parameters stay the same, while the initialized velocities are shifted which results in different conformations of atoms.

To set the equilibration steps, a few changes have to be made in the .imd file compared to the minimisation step. Firstly, in INITIALISE block NTIVEL value is set to 1, which means that the velocities were assigned randomly according to Maxwell-Boltzmann distribution. In the same box, IG is the seed value. Also, equilibration is run for a longer simulation time of 10000 steps. As the system is now placed in a box, periodic boundary conditions have to be set in a BOUNDCOND block by setting NTB to 1. Finally, MULBITATH, COMTRANSROT, WRITETRAJ and some other blocks are added to make sure the system remains on the same temperature, without centre of mass motion, and trajectory write out in a file. Most importantly, POSITIONRES block is added, which keeps the volume constant throughout simulation.

To perform equilibration at the temperature of 300 K by slowly incrementing it from 60 K in five steps, GROMOS program *mk_script* is used. This program takes a joblist as an input, where all input parameters that are changed throughout are stated, and as an output gives .imd files and .run files for each equilibration step.

This way, three replicas of the beginning system may be produced. Besides them, equilibration step might be repeated when moving from one simulation to another as number of atoms in the system differs.

3.2 Setting simulations

In contrast to the equilibration step, in the MD sampling simulation, POSITIONRES block is removed and PRESSURESCALE block is added to the .imd file. This means that the system is going from NVT to NpT ensemble. To include thermodynamic integration into MD simulation, PERTURBATION block is also needed. There one can specify whether free energy calculations are performed and set Lennard-Jones and Coulomb soft-core parameters. Those are usually set to 0.5 each. This block controls how the system changes through each λ point.

To reestablish, changing the system from one state to another, it gets slowly perturbed by λ points from 0 to 1 in a way that λ points describe the percentage in which the interactions of

perturbed atoms are perceived by the remaining atoms. To state which atoms are exchanged, besides a regular topology file, there should be a perturbation topology file .ptp that states the exact atoms from topology. There is said which atom goes from *dummy* to *real* atom, and vice versa.

In the initial cycle, all the simulations are set to 21 λ points simulated for 2 ns with 0.002 time step. Before each new lambda point, there is 0.2 ns equilibration run that is excluded from free energy calculations. The other interchangeable thing in those simulations are atom numbers of solute and the whole system. Those range from 1774 to 1779 for solute and 24442 to 24447 for the whole system.

To get the free energy changes in binding of GTP/GDP to G12C/WT, free energies of sole perturbations from GTP to GDP and G12C to WT had to be performed. For that, systems were prepared in the same way as before, and simulated again for 2 ns for 21 λ points. Free energy values taken from here are in the end subtracted from the one of originally set perturbations to get the difference in binding free energies that are of the interest in this research.

3.3 Extended thermodynamic integration

One of the common problems using the method of thermodynamic integration is that the curve that represents the free energies is sometimes too steep between two lambda points, which makes the integration estimate unreliable. For this reason, to make the integration smoother, as shown in Figure 4, the extended thermodynamic integration method is used [34].

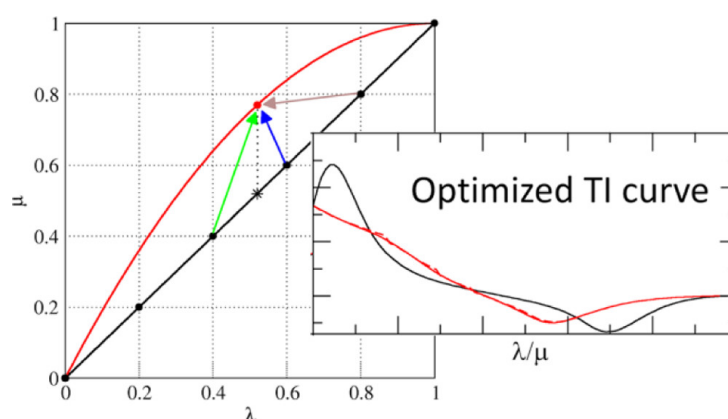


Figure 4: *Extended TI. Graph shows an optimization of the TI curve after performing extended simulations on additional lambda points. Figure taken from [34].*

Extended TI includes prolongation of simulations of selected λ points and inclusion of new λ points in between the existing ones. Also, if some of the λ points do not need to be prolonged, they are excluded from further simulations.

Calculations to determine which λ points are prolonged and for how long can be done in multiple ways, and the convergence conditions can be set freely. In this research, preexisting

Python script written by Assist. Prof. Dr. Dražen Petrov was used. To generate .run and .imd files for new λ points, Python script was written which can be found in Appendix B. The script used SMARt environment [35].

Ideally, after some number of cycle repeats, the free energy results would converge, and there would be no more λ points to simulate. In chapter 4 it is explained how, with this system, that outcome was not reachable and the decision to stop simulating was based on the MBAR and BAR value errors.

3.4 Free energy calculations

As mentioned previously, free energy calculations were performed using a preexisting Python script. Additional ways of estimating free energies from simulations by the thermodynamic integration method are described in section 2.3.1, specifically equations 2.5 and 2.6.

There are many algorithms available that calculate BAR and MBAR values, and the TI values could be calculated using GROMOS. The problem with BAR calculations is to optimise calculation time because of the recursion that is calculated. This makes BAR value slow to calculate, but at the same time more precise.

The above mentioned script was used not only to calculate the free energies, but to estimate which λ points to simulate. Reading all the simulation output trajectories and energies and estimating TI and BAR values, based on set of parameters to estimate convergence of a particular region, a new set of λ points is given to the user, together with new simulation times based on the total time of the full cycle that is given by the user.

3.5 Data analysis

Besides energy calculations, a detailed analysis was performed on obtained trajectories. In the following chapters will the motivation for performing these exact analysis become transparent.

As the simulations performed in total were at most in tenths of microseconds and there was a lot of noise, root-mean-square deviation (RMSD) calculations were performed using GROMOS program *rmsd* on these systems compared to the clusters obtained from simulations provided by Dr. Edgar Galicia Andrés, which had much longer simulation times. RMSD was performed on the first two (highest) clusters for Switch-I and Switch-II loop.

Secondly, it was relevant to look into hydrogen bonds forming between GTP $P\gamma$ oxygen and sulfate in mutated cysteine, as this bond is crucial in K-Ras activation. This was done using GROMOS program *hbond*.

Finally, to look more deeply into the role of magnesium ion, and interaction of GTP/GDP with

specific loops, GROMOS files were translated into GROMACS format. This way GROMACS program *mdmat* could be used to get all the distances between residues.

4 Results and discussion

4.1 K-Ras mutations occurrence in human cancers

Looking into Ras protein family, K-Ras isoform has the highest occurrence of human cancers related to its mutations, as it is shown in Table 1. It is notable that K-Ras seems to show a mutual exclusivity with both H-Ras and N-Ras, while H-Ras and N-Ras show co-occurrence tendencies on this specific data set [12, 13, 14].

As it is shown in the further analysis of cancer cases possibly affected by K-Ras mutations shown in Table 2, cancer type is not consistent with a specific mutation, neither is one mutation limited to the specific cancer type. That being said, mutations with high cancer comorbidities and fatalities are G12A, G12C, G12D, G12R, G12S, G12V, G13C, G13D, Q61H, and A146T. Full data can be found in the Appendix A.

All the data used in the work that follows is obtained from cBioPortal database for cancer genomics [12, 13, 14], specifically TCGA PanCancer Atlas Studies (as of August 2nd 2024) and sorted for the purpose of this thesis. The mutation that is specifically investigated in this thesis is G12C, which has been shown to have a significant effect in lung cancer development with high fatality rates.

4.2 Obtained free energies

As mentioned before, free energies were calculated using a Python script. Results for individual replicas and combined results are shown in tables 3, 4, and 5. Results for perturbation from GDP to GTP, WT to G12C, and other ways around, are shown in Table 6. Error values in Table 6 are as high because only 2 ns per λ point was performed, or just the zero-th TI cycle, without extension.

To evaluate binding energies, as discussed before, energies (MBAR) from Table 6 are subtracted from Total MBAR values in Table 4. This gives results shown in Figure 5. First obvious thing is that there are double values in GDP/GTP perturbations in mutated type. This is due to the fact that energies in the first cycle compared to ones in second and third are off (or, as will be deduced and explained later, the other way around). For this reason, the total binding energy is shown in red, while the one taking only the first cycle into account is shown in green.

There is slight hysteresis in results going from mutated to wild type (-3.05 kJ/mol for GDP

Table 1: Frequency of mutations in Ras isoforms in human cancers. Data were compiled from cBioPortal database for cancer genomics, TCGA PanCancer Atlas Studies (as of August 2nd 2024) [12, 13, 14].

Cancer type (c stands for cancer)	Ras mutation frequency	Sample size	K-Ras mut. frequency	H-Ras mut. frequency	N-Ras mut. frequency
Pancreatic c.	65.36%	179	65.36%	0.56%	0%
Colorectal c.	46.82%	534	40.82%	0.75%	6.18%
Melanoma	31.82%	440	2.27%	2.05%	28.41%
Seminoma	26.98%	63	19.05%	0%	7.94%
Endometrial c.	22.13%	574	18.47%	1.39%	3.83%
Non-small cell lung c.	18.38%	1050	16.67%	0.95%	0.95%
Leukemia	13%	200	5%	0%	8%
Thyroid c.	12.04%	490	0.82%	3.27%	7.96%
Pheochromocytoma	11.56%	147	0%	11.56%	0%
Thymic epithelial tumor	11.38%	123	0.81%	8.13%	2.44%
Bladder c.	10%	410	3.66%	4.39%	1.95%
Cholangiocarcinoma	8.33%	36	5.56%	0%	2.78%
Esophagogastric c.	7.61%	618	6.8%	0.16%	0.81%
Head and neck c.	6.41%	515	0.19%	6.02%	0.19%
Cervical c.	6.19%	291	5.15%	0.34%	1.03%
Mature B-cell neoplasms	4.88%	41	4.88%	0%	0%
Miscellaneous neuroepithelial tumor	3.23%	31	0%	3.23%	0%
Hepatobiliary c.	2.73%	366	1.37%	0%	1.37%

Table 2: Frequency of K-Ras mutations in human cancers with fatalities. Data corresponds to Table 1, while numbers presented are number of cancer cases with the specific mutation / number of fatalities / ratio of fatalities in all cases with said mutation. Only cancer types highly impacted by K-Ras mutations from Table 1 are shown. Data were compiled from cBioPortal database for cancer genomics, TCGA PanCancer Atlas Studies (as of August 2nd 2024) [12, 13, 14].

	G12A	G12C	G12D	G12R	G12S
Cervical c.	- / - / -	2 / 1 / 0.5	4 / 1 / 0.25	- / - / -	- / - / -
Colorectal c.	10 / 3 / 0.3	15 / 3 / 0.2	58 / 9 / 0.16	2 / 1 / 0.50	8 / 1 / 0.13
Endometrial c.	9 / 2 / 0.22	7 / 2 / 0.29	34 / 2 / 0.06	- / - / -	3 / - / 0
Esophagogastric c.	- / - / -	2 / - / 0	12 / - / 0	- / - / -	4 / 1 / 0.25
Non-smallcell lung c.	18 / 3 / 0.17	70 / 15 / 0.21	20 / 3 / 0.15	- / - / -	5 / - / 0
Pancreatic c.	1 / 1 / 1	1 / - / 0	49 / 25 / 0.51	25 / 9 / 0.36	1 / - / 0
Seminoma	1 / - / 0	- / - / -	- / - / -	2 / - / 0	1 / - / 0
	G12V	G13C	G13D	Q61H	A146T
Cervical c.	2 / 1 / 0.5	- / - / -	4 / 3 / 0.75	- / - / -	2 / - / 0
Colorectal c.	49 / 5 / 0.1	2 / - / 0	37 / 4 / 0.11	4 / - / 0	16 / 2 / 0.13
Endometrial c.	22 / 1 / 0.05	3 / - / 0	11 / - / 0	4 / - / 0	1 / - / 0
Esophagogastric c.	3 / 1 / 0.33	- / - / -	11 / - / 0	2 / - / 0	3 / - / 0
Non-smallcell lung c.	41 / 8 / 0.2	8 / 3 / 0.38	3 / 1 / 0.33	2 / - / 0	- / - / -
Pancreatic c.	33 / 17 / 0.52	1 / 1 / 1	- / - / -	6 / 3 / 0.5	- / - / -
Seminoma	3 / 1 / 0.33	- / - / -	- / - / -	- / - / -	- / - / -

Table 3: Free energy BAR values. All values are expressed in kJ/mol.

	1 st Replica	2 nd Replica	3 rd Replica	Total	Error
G12C-GDP→WT-GDP	7.66	5.09	4.97	7.25	0.05
WT-GDP→G12C-GDP	-4.36	-11.33	-9.75	-6.2	0.1
WT-GDP→WT-GTP	-10.16	-3.85	-0.68	-5.4	0.2
WT-GTP→WT-GDP	38.41	38.91	32.35	38.9	0.2
WT-GTP→G12C-GTP	-18.48	-14.76	-9.36	-15.4	0.1
G12C-GTP→WT-GTP	20.94	13.08	13.91	15.24	0.09
G12C-GTP→G12C-GDP	60.22	14.00	13.64	26.6	0.2
G12C-GDP→G12C-GTP	-67.91	-21.12	-7.47	-41.4	0.2

Table 4: Free energy MBAR values. All values are expressed in kJ/mol.

	1 st Replica	2 nd Replica	3 rd Replica	Total	Error
G12C-GDP→WT-GDP	6.71	5.09	3.68	6.43	0.05
WT-GDP→G12C-GDP	-3.04	-10.84	-9.62	-6.96	0.09
WT-GDP→WT-GTP	-10.27	-2.84	-1.91	-5.8	0.2
WT-GTP→WT-GDP	38.80	38.16	31.31	38.4	0.2
WT-GTP→G12C-GTP	-18.30	-14.39	-9.45	-15.1	0.1
G12C-GTP→WT-GTP	20.30	12.51	13.92	15.03	0.07
G12C-GTP→G12C-GDP	60.36	13.09	13.55	27.0	0.2
G12C-GDP→G12C-GTP	-66.98	-20.95	-7.08	-37.2	0.2

Table 5: Free energy TI values. All values are expressed in kJ/mol.

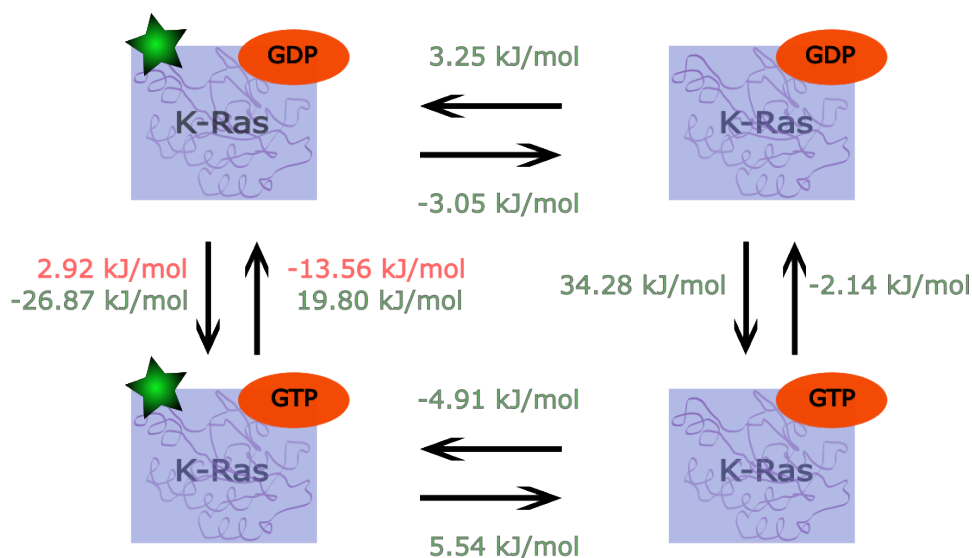
	1 st Replica	2 nd Replica	3 rd Replica	Total
G12C-GDP→WT-GDP	8.39	4.78	4.71	7.19
WT-GDP→G12C-GDP	-4.28	-12.04	-8.34	-7.86
WT-GDP→WT-GTP	-9.33	-3.81	1.52	-4.74
WT-GTP→WT-GDP	36.04	40.22	33.04	37.10
WT-GTP→G12C-GTP	-17.16	-15.05	-9.98	-14.75
G12C-GTP→WT-GTP	21.22	12.62	13.86	14.99
G12C-GTP→G12C-GDP	58.83	14.17	13.54	29.04
G12C-GDP→G12C-GTP	-68.49	-20.78	-5.98	-42.44

and 5.54 kJ/mol for GTP) and the other way around (3.25 kJ/mol for GDP and -4.91 kJ/mol for GTP), but the specific differences are not considered to be significant. On the other hand, there is significant hysteresis (for an order of magnitude) in GDP/GTP perturbations (-26.87 kJ/mol and 19.80 kJ/mol for the mutated protein), especially in the wild type protein (34.28 kJ/mol and -2.14 kJ/mol).

Taking into account results that are consistent, they show a biological relevance. Specifically, they show that the preferable state in case of the wild type is GDP binding, and in the case of mutated type GTP binding. This goes together with the assumption that GTP over-binding in mutated type is the main cause of cancer development [1, 3, 4].

Table 6: Free energy values for ligand and protein perturbations in water. All values are expressed in kJ/mol.

	1 st Replica	2 nd Replica	3 rd Replica	Total	Error
G12C→WT BAR	7.90	7.23	12.77	9.3	0.2
G12C→WT MBAR	7.91	7.34	13.30	9.5	0.2
G12C→WT TI	7.87	6.88	12.94	9.23	-
WT→G12C BAR	-8.79	-9.02	-12.54	-10.1	0.2
WT→G12C MBAR	-8.81	-9.02	-12.85	-10.2	0.2
WT→G12C TI	-9.15	-9.32	-12.59	-10.36	-
GDP→GTP BAR	-40.19	-41.25	-39.41	-40.3	0.5
GDP→GTP MBAR	-39.93	-41.05	-39.13	-40.1	0.5
GDP→GTP TI	-40.19	-41.63	-39.32	-40.38	-
GTP→GDP BAR	41.16	40.17	40.92	40.8	0.5
GTP→GDP MBAR	41.01	40.06	40.59	40.6	0.5
GTP→GDP TI	40.98	40.96	41.65	41.19	-


Figure 5: Free energies in thermodynamic cycle. Energies in red inconsistent with biological implications.

4.2.1 MBAR-BAR differences

First thing to realize from this data is that there is a significant difference from BAR to MBAR data. While it is not common to be so, because of the slight algorithm difference, it is possible when the data is not nicely converging. As this is the case with K-Ras system, the difference in BAR and MBAR data is somewhat justified, even though still highly unwanted.

Another thing to look at is the comparison to TI data. The difference here is more prominent, and one specific result to show so is the WT-GDP→WT-GTP perturbation, replica 3, where the

energy value becomes positive, which, compared to other results and biological background, is clearly the consequence of the algorithm inadequacies.

4.2.2 λ -point evaluation

To show the convergence of individual λ points, unconverged (or simulated) λ points are shown in green, newly converged in pink and previously converged in yellow in figures 6-13. Left side numbers indicate λ points with the step of 0.025, or 41 λ points in total. The numbers on the top represent the number of cycle performed, zeroth being the starting TI cycle, and one and higher repeats for the extended TI. Blue colour represents full convergence.

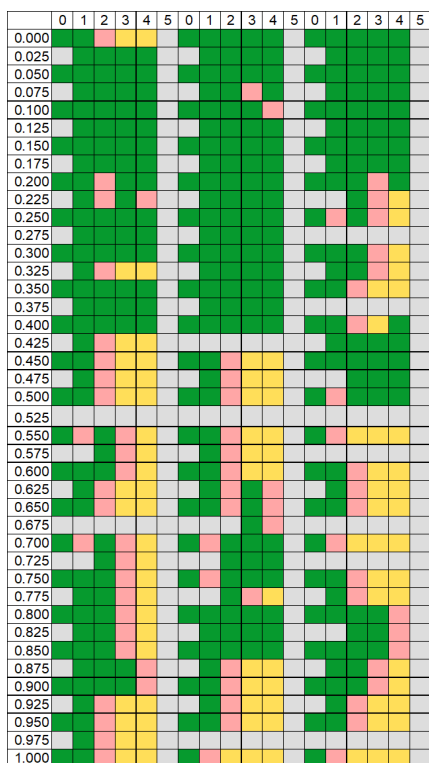


Figure 6: Convergence regions of $G12C-GDP \rightarrow WT-GDP$ perturbation, replicas 1-3.

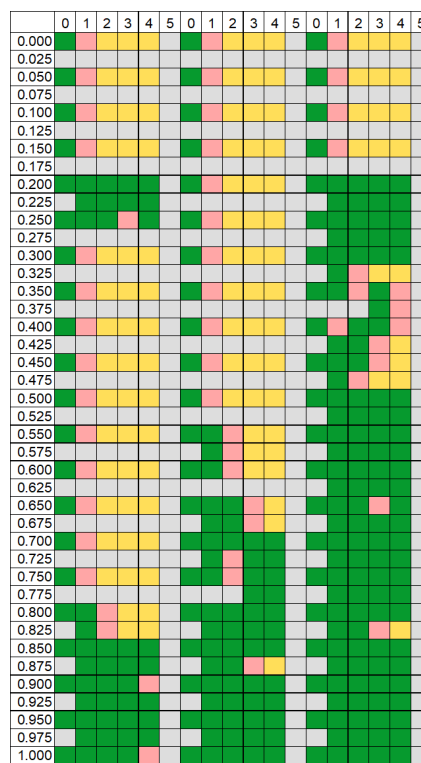


Figure 7: Convergence regions of $WT-GDP \rightarrow G12C-GDP$ perturbation, replicas 1-3.

As one can realize from the figures above, some perturbations converged to some point, while the others hardly at all. The only successful convergence is shown in Figure 11 for the first replica. It is also notable that convergence moves on more smoothly for WT to G12C and G12C to WT perturbation than for GTP and GDP molecule ones.

The difference in convergence patterns may also justify energy differences between replicas, while the difference in convergence patterns between same perturbations in different directions might explain hysteresis in results between those.

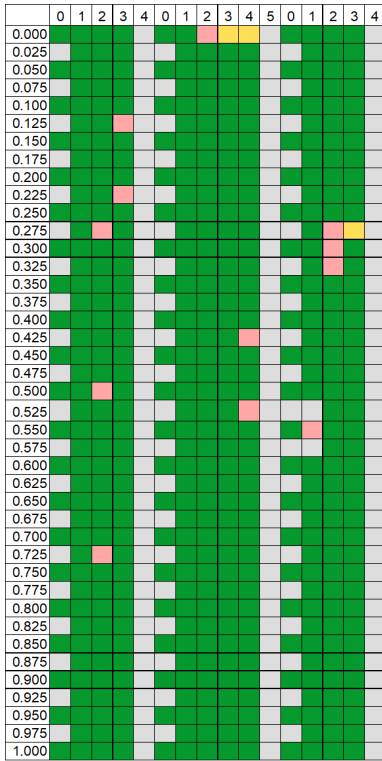


Figure 8: Convergence regions of WT-GDP→WT-GTP perturbation, replicas 1-3.

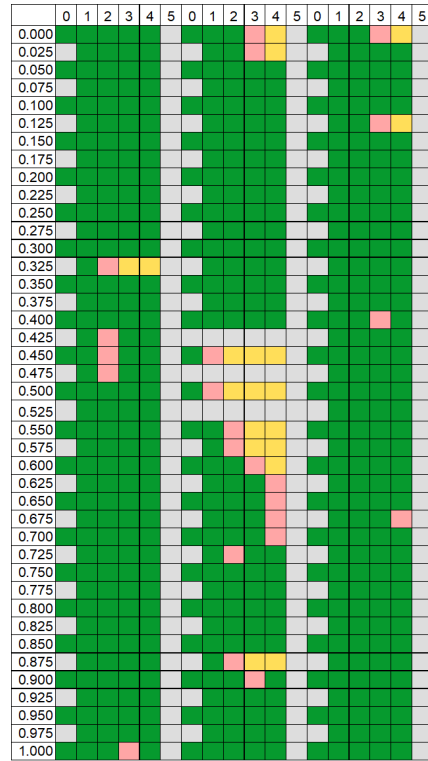


Figure 9: Convergence regions of WT-GTP→WT-GDP perturbation, replicas 1-3.

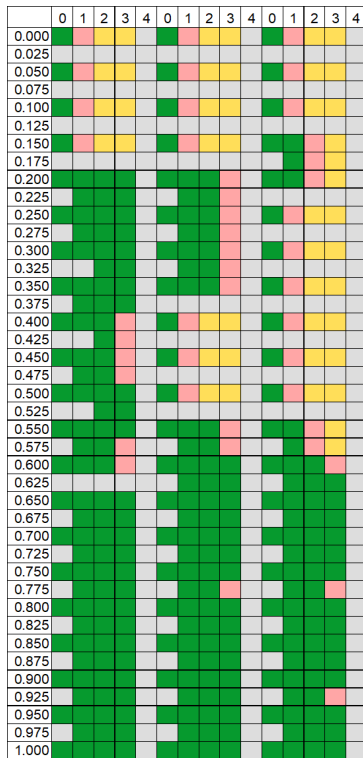


Figure 10: Convergence regions of WT-GTP→G12C-GTP perturbation, replicas 1-3.

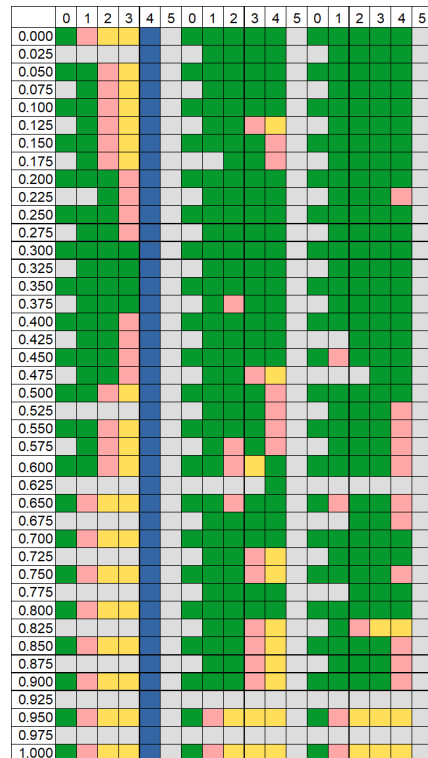


Figure 11: Convergence regions of G12C-GTP→WT-GTP perturbation, replicas 1-3.

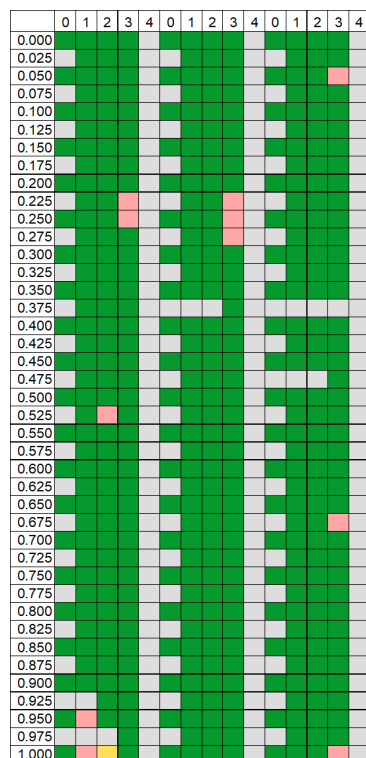


Figure 12: Convergence regions of $G12C-GTP \rightarrow G12C-GDP$ perturbation, replicas 1-3.

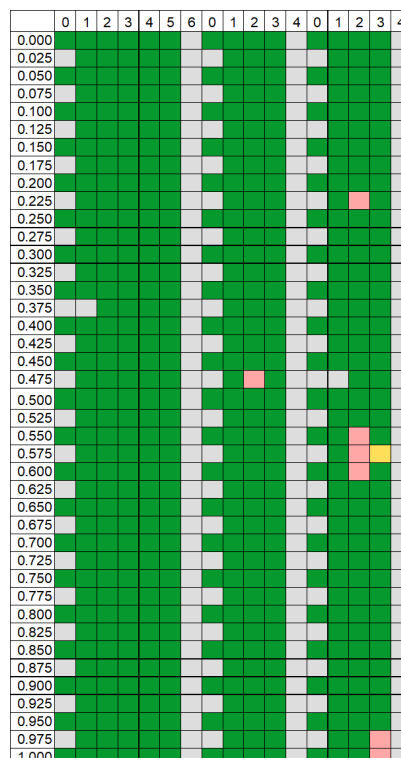


Figure 13: Convergence regions of $G12C-GDP \rightarrow G12C-GTP$ perturbation, replicas 1-3.

4.2.3 Problems with perturbations

As observed, there is an obvious difference in convergence between simulations where protein is perturbed and where GDP or GTP is perturbed. This is due to the charge change while going from one molecule to another. PO_3 group has a triple negative charge. Such a change in the protein pocket leads to highly disturbed system that takes a while to converge.

Besides the charge change, there is also a question of size of the binding molecule, but this change is not as prominent as the one mentioned before. To solve this, one could go for longer simulation times, but looking into convergence figures, there was no sign of it converging at some short distance point.

Another reason not to proceed with simulations were BAR and MBAR error values and value changes between the two cycles. As those errors and changes were small enough, simulations were stopped at that point to save resources, as it was not sure if simulating for double the time would make a change.

It is important to mention that even the glycine perturbation is problematic. It is shown that perturbations to and from glycine yield high degree hysteresis due to poor sampling of dihedral angles [36]. This basically means that any change from glycine to another aminoacid might take a while to settle as glycine is too flexible, or that other way around glycine doesn't get to

be as flexible as it would be. This might also influence results in WT protein going from GDP to GTP and vice versa, where a huge hysteresis is seen in tables 4-6. Due to the flexibility of glycine and its poor binding to GDP/GTP, there might be some influence on final values.

Another obvious problem is a difference in energies between replicas. While slight differences are expected and contribute to final result statistics, perturbations of GTP and GDP in G12C mutated protein show by far different results in the first replica compared to the second and the third. To have a better look into why it is like that, it was an imperative to look into trajectory files and look for answers there.

4.3 Trajectory analysis

In an attempt to explain the hysteresis observed between pairs of simulations going in different directions as well as the difference in energies between replicas in GTP/GDP perturbations in mutated type, trajectories were thoroughly analyzed.

4.3.1 Root mean square distance analysis

First analysis performed on trajectories were RMSD calculations. Structures from simulations performed by Dr. Edgar Galicia Andrés were taken as reference structures. This is because doing clustering on simulations as short as these would not be effective, and the ones used for reference were simulated for around 1 microsecond or more. These simulations were clustered based on the movement of Switch-I and Switch-II loops.

As those loops are quite flexible, results from these RMSD calculations, taking into account only the movement of those loops, gave some interesting results. RMSD for the Switch-II loop was performed on the first two clusters, while for the Switch-I loop it was performed on the first three clusters. Results are shown in tables 7-11. Average values are colored based on the value.

From the Switch-II tables 7 and 8 it is seen that the system stayed in cluster 2 for most of the simulation time. This means it was not in the most preferable state detected by Dr. Galicia's simulations. Besides that, RMSD values seem consistent between simulations.

Switch-I RMSD results are more versatile, showing a major difference in RMSD values between same system simulations in different directions for all the clusters and replicas. As simulations in the one direction take the previous state as a starting point, this could mean that an initial perturbation already experiences a huge shift in the structure. With regard to the free energy calculations, this might explain the hysteresis between same system perturbations, as the Switch-I loop is not in the same state in both directions. Its proximity to both the magnesium ion and the GTP/GDP molecule means that changes in the RMSD influence the free energy values.

Table 7: RMSD values for Switch-II loop, cluster 01. All the values are in nm.

		min	max	avg	dev
1 st Replica	G12C-GDP→WT-GDP	0.14	0.35	0.28	0.03
	WT-GDP→G12C-GDP	0.19	0.33	0.27	0.03
	WT-GDP→WT-GTP	0.15	0.31	0.23	0.02
	WT-GTP→WT-GDP	0.15	0.34	0.27	0.03
	WT-GTP→G12C-GTP	0.12	0.25	0.18	0.02
	G12C-GTP→WT-GTP	0.20	0.33	0.26	0.02
	G12C-GTP→G12C-GDP	0.12	0.21	0.16	0.02
	G12C-GDP→G12C-GTP	0.16	0.40	0.29	0.04
2 nd Replica	G12C-GDP→WT-GDP	0.13	0.31	0.20	0.03
	WT-GDP→G12C-GDP	0.15	0.24	0.20	0.01
	WT-GDP→WT-GTP	0.13	0.24	0.18	0.02
	WT-GTP→WT-GDP	0.14	0.25	0.19	0.02
	WT-GTP→G12C-GTP	0.12	0.22	0.16	0.02
	G12C-GTP→WT-GTP	0.13	0.25	0.18	0.02
	G12C-GTP→G12C-GDP	0.11	0.20	0.16	0.01
	G12C-GDP→G12C-GTP	0.16	0.35	0.24	0.04
3 rd Replica	G12C-GDP→WT-GDP	0.18	0.33	0.26	0.02
	WT-GDP→G12C-GDP	0.07	0.25	0.19	0.03
	WT-GDP→WT-GTP	0.18	0.32	0.25	0.02
	WT-GTP→WT-GDP	0.11	0.26	0.18	0.02
	WT-GTP→G12C-GTP	0.16	0.28	0.22	0.02
	G12C-GTP→WT-GTP	0.14	0.31	0.24	0.03
	G12C-GTP→G12C-GDP	0.13	0.30	0.23	0.03
	G12C-GDP→G12C-GTP	0.14	0.32	0.26	0.02

Table 8: RMSD values for Switch-II loop, cluster 02. All the values are in nm.

		min	max	avg	dev
1 st Replica	G12C-GDP→WT-GDP	0.07	0.22	0.14	0.02
	WT-GDP→G12C-GDP	0.08	0.19	0.13	0.02
	WT-GDP→WT-GTP	0.05	0.18	0.12	0.02
	WT-GTP→WT-GDP	0.07	0.23	0.14	0.03
	WT-GTP→G12C-GTP	0.04	0.20	0.09	0.02
	G12C-GTP→WT-GTP	0.06	0.20	0.12	0.03
	G12C-GTP→G12C-GDP	0.06	0.18	0.11	0.02
	G12C-GDP→G12C-GTP	0.06	0.29	0.17	0.04
2 nd Replica	G12C-GDP→WT-GDP	0.06	0.22	0.12	0.02
	WT-GDP→G12C-GDP	0.06	0.13	0.09	0.01
	WT-GDP→WT-GTP	0.05	0.18	0.10	0.02
	WT-GTP→WT-GDP	0.05	0.16	0.09	0.02
	WT-GTP→G12C-GTP	0.07	0.22	0.13	0.03
	G12C-GTP→WT-GTP	0.05	0.15	0.09	0.02
	G12C-GTP→G12C-GDP	0.05	0.18	0.11	0.02
	G12C-GDP→G12C-GTP	0.06	0.24	0.12	0.03
3 rd Replica	G12C-GDP→WT-GDP	0.07	0.19	0.12	0.02
	WT-GDP→G12C-GDP	0.05	0.25	0.12	0.04
	WT-GDP→WT-GTP	0.06	0.21	0.12	0.03
	WT-GTP→WT-GDP	0.06	0.23	0.12	0.03
	WT-GTP→G12C-GTP	0.06	0.21	0.14	0.03
	G12C-GTP→WT-GTP	0.07	0.22	0.13	0.02
	G12C-GTP→G12C-GDP	0.07	0.25	0.13	0.02
	G12C-GDP→G12C-GTP	0.07	0.21	0.13	0.02

Table 9: RMSD values for Switch-I loop, cluster 01. All the values are in nm.

		min	max	avg	dev
1 st Replica	G12C-GDP→WT-GDP	0.07	0.22	0.13	0.02
	WT-GDP→G12C-GDP	0.09	0.17	0.13	0.01
	WT-GDP→WT-GTP	0.09	0.19	0.14	0.02
	WT-GTP→WT-GDP	0.08	0.18	0.14	0.02
	WT-GTP→G12C-GTP	0.12	0.24	0.17	0.02
	G12C-GTP→WT-GTP	0.13	0.21	0.16	0.01
	G12C-GTP→G12C-GDP	0.10	0.29	0.16	0.02
	G12C-GDP→G12C-GTP	0.07	0.24	0.15	0.03
2 nd Replica	G12C-GDP→WT-GDP	0.08	0.19	0.13	0.02
	WT-GDP→G12C-GDP	0.15	0.29	0.21	0.02
	WT-GDP→WT-GTP	0.07	0.22	0.14	0.03
	WT-GTP→WT-GDP	0.17	0.26	0.21	0.01
	WT-GTP→G12C-GTP	0.09	0.20	0.14	0.02
	G12C-GTP→WT-GTP	0.17	0.26	0.21	0.01
	G12C-GTP→G12C-GDP	0.07	0.21	0.13	0.02
	G12C-GDP→G12C-GTP	0.07	0.24	0.16	0.04
3 rd Replica	G12C-GDP→WT-GDP	0.09	0.28	0.14	0.03
	WT-GDP→G12C-GDP	0.11	0.22	0.17	0.02
	WT-GDP→WT-GTP	0.07	0.26	0.16	0.03
	WT-GTP→WT-GDP	0.09	0.20	0.15	0.02
	WT-GTP→G12C-GTP	0.12	0.35	0.18	0.02
	G12C-GTP→WT-GTP	0.08	0.21	0.14	0.02
	G12C-GTP→G12C-GDP	0.11	0.23	0.18	0.01
	G12C-GDP→G12C-GTP	0.08	0.20	0.13	0.02

Table 10: RMSD values for Switch-I loop, cluster 02. All the values are in nm.

		min	max	avg	dev
1 st Replica	G12C-GDP→WT-GDP	0.11	0.24	0.17	0.02
	WT-GDP→G12C-GDP	0.13	0.23	0.18	0.02
	WT-GDP→WT-GTP	0.11	0.27	0.16	0.02
	WT-GTP→WT-GDP	0.11	0.22	0.18	0.02
	WT-GTP→G12C-GTP	0.17	0.31	0.23	0.02
	G12C-GTP→WT-GTP	0.14	0.23	0.19	0.02
	G12C-GTP→G12C-GDP	0.17	0.37	0.23	0.03
	G12C-GDP→G12C-GTP	0.10	0.29	0.17	0.02
2 nd Replica	G12C-GDP→WT-GDP	0.09	0.24	0.16	0.03
	WT-GDP→G12C-GDP	0.16	0.30	0.21	0.03
	WT-GDP→WT-GTP	0.07	0.23	0.14	0.03
	WT-GTP→WT-GDP	0.16	0.23	0.20	0.01
	WT-GTP→G12C-GTP	0.08	0.24	0.13	0.02
	G12C-GTP→WT-GTP	0.15	0.23	0.19	0.01
	G12C-GTP→G12C-GDP	0.07	0.24	0.13	0.02
	G12C-GDP→G12C-GTP	0.11	0.23	0.17	0.02
3 rd Replica	G12C-GDP→WT-GDP	0.13	0.34	0.19	0.03
	WT-GDP→G12C-GDP	0.08	0.21	0.16	0.02
	WT-GDP→WT-GTP	0.10	0.33	0.20	0.03
	WT-GTP→WT-GDP	0.08	0.22	0.16	0.02
	WT-GTP→G12C-GTP	0.16	0.39	0.23	0.02
	G12C-GTP→WT-GTP	0.10	0.25	0.17	0.02
	G12C-GTP→G12C-GDP	0.15	0.30	0.24	0.02
	G12C-GDP→G12C-GTP	0.14	0.25	0.18	0.02

Table 11: RMSD values for Switch-I loop, cluster 03. All the values are in nm.

		min	max	avg	dev
1 st Replica	G12C-GDP→WT-GDP	0.09	0.25	0.14	0.03
	WT-GDP→G12C-GDP	0.09	0.18	0.13	0.02
	WT-GDP→WT-GTP	0.10	0.22	0.16	0.02
	WT-GTP→WT-GDP	0.07	0.19	0.13	0.03
	WT-GTP→G12C-GTP	0.09	0.25	0.16	0.02
	G12C-GTP→WT-GTP	0.13	0.23	0.17	0.02
	G12C-GTP→G12C-GDP	0.10	0.26	0.15	0.02
	G12C-GDP→G12C-GTP	0.08	0.29	0.16	0.04
2 nd Replica	G12C-GDP→WT-GDP	0.06	0.24	0.14	0.03
	WT-GDP→G12C-GDP	0.19	0.31	0.24	0.02
	WT-GDP→WT-GTP	0.09	0.24	0.16	0.03
	WT-GTP→WT-GDP	0.19	0.29	0.24	0.02
	WT-GTP→G12C-GTP	0.10	0.25	0.18	0.02
	G12C-GTP→WT-GTP	0.19	0.29	0.24	0.02
	G12C-GTP→G12C-GDP	0.09	0.26	0.17	0.03
	G12C-GDP→G12C-GTP	0.09	0.28	0.18	0.04
3 rd Replica	G12C-GDP→WT-GDP	0.09	0.32	0.14	0.04
	WT-GDP→G12C-GDP	0.12	0.25	0.20	0.02
	WT-GDP→WT-GTP	0.10	0.29	0.17	0.03
	WT-GTP→WT-GDP	0.11	0.24	0.18	0.02
	WT-GTP→G12C-GTP	0.14	0.40	0.19	0.03
	G12C-GTP→WT-GTP	0.08	0.23	0.16	0.03
	G12C-GTP→G12C-GDP	0.12	0.26	0.18	0.02
	G12C-GDP→G12C-GTP	0.07	0.24	0.14	0.03

4.3.2 Analysis of h-bonds

To look more into the problem of energy differences between replicas in the GTP/GDP perturbations in mutated type protein, analysis of h-bonds between protein residues and GTP/GDP molecule was performed. Results for those simulations in the P-loop region are shown in figures 14 and 15. Additionally, all the h-bonds for all of the simulations can be found in the Appendix C.

Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%			
12	CYSH	-	1	GTP	110	SG	O1PG	72	13.46	12	CYSH	-	1	GTP	110	SG	O1PG	52	8.51	12	CYSH	-	1	GTP	110	SG	O1PB	32	5.32
12	CYSH	-	1	GTP	110	SG	O3PG	6	1.12	12	CYSH	-	1	GTP	110	SG	O2PG	39	6.38	12	CYSH	-	1	GTP	110	SG	O3PB	1	0.17
13	GLY	-	1	GTP	114	N	O3PA	2	0.37	13	GLY	-	1	GTP	114	N	O3PA	15	2.45	12	CYSH	-	1	GTP	110	SG	O1PG	15	2.5
13	GLY	-	1	GTP	114	N	O1PB	29	5.42	13	GLY	-	1	GTP	114	N	O1PB	3	0.49	13	GLY	-	1	GTP	114	N	O3PA	20	3.33
13	GLY	-	1	GTP	114	N	O3PB	279	52.15	13	GLY	-	1	GTP	114	N	O3PB	396	64.81	13	GLY	-	1	GTP	114	N	O1PB	456	75.87
13	GLY	-	1	GTP	114	N	O1PG	41	7.66	13	GLY	-	1	GTP	114	N	O1PG	36	5.89	13	GLY	-	1	GTP	114	N	O3PB	69	11.48
13	GLY	-	1	GTP	114	N	O2PG	5	0.93	13	GLY	-	1	GTP	114	N	O2PG	46	7.53	13	GLY	-	1	GTP	114	N	O1PG	6	1
13	GLY	-	1	GTP	114	N	O3PG	25	4.67	13	GLY	-	1	GTP	114	N	O3PG	34	5.56	13	GLY	-	1	GTP	114	N	O2RG	3	0.5
14	VAL	-	1	GTP	119	N	O3PA	1	0.19	14	VAL	-	1	GTP	119	N	O1PB	107	17.51	14	VAL	-	1	GTP	119	N	O1PB	44	7.32
14	VAL	-	1	GTP	119	N	O1PB	231	43.18	14	VAL	-	1	GTP	119	N	O1PG	8	1.31	15	GLY	-	1	GTP	127	N	O2'	3	0.5
14	VAL	-	1	GTP	119	N	O1PG	1	0.19	14	VAL	-	1	GTP	119	N	O2PG	2	0.33	15	GLY	-	1	GTP	127	N	O3'	57	9.48
15	GLY	-	1	GTP	127	N	O4'	1	0.19	14	VAL	-	1	GTP	119	N	O3PG	1	0.16	16	LYSH	-	1	GTP	132	N	O1PB	1	0.17

Figure 14: Occurrence of h-bonds in G12C-GTP→G12C-GDP perturbation P-loop. Colored in yellow is P-loop, and in orange the h-bond between sulfur in cysteine and P γ /P β oxygen. Three colours represent three different replicas, numerated from left to right.

Comparing the occurrence of h-bonds between cysteine sulfur and P γ oxygen in replicas 1-3, it can be concluded that in replica 1 h-bond is present for a significant amount of time, while in replicas 2-3, it is not seen. One must take into account that only for a certain time of the simulation are P γ oxygens visible to the full or close to full extent to the sulfur atom, as

Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%			
12	CYSH	-	1	GTP	110	SC	O3PB	1	0.09	12	CYSH	-	1	GTP	110	SC	O1PB	36	6.81	12	CYSH	-	1	GTP	110	SC	O1PG	1	0.19
12	CYSH	-	1	GTP	110	SC	O1PG	220	20.39	12	CYSH	-	1	GTP	110	SC	O1PG	14	2.65	12	CYSH	-	1	GTP	110	SC	O2PG	6	1.13
12	CYSH	-	1	GTP	110	SC	O3PG	2	0.19	12	CYSH	-	1	GTP	110	SC	O2PG	3	0.57	12	CYSH	-	1	GTP	110	SC	O3PG	1	0.19
13	GLY	-	1	GTP	114	N	O3PA	10	0.93	13	GLY	-	1	GTP	110	SC	O3PG	4	0.76	13	GLY	-	1	GTP	114	N	O3PB	41	7.72
13	GLY	-	1	GTP	114	N	O1PB	34	3.15	13	GLY	-	1	GTP	114	N	O1PA	3	0.57	13	GLY	-	1	GTP	114	N	O1PG	26	4.9
13	GLY	-	1	GTP	114	N	O3PB	459	42.54	13	GLY	-	1	GTP	114	N	O3PA	20	3.78	13	GLY	-	1	GTP	114	N	O2PG	152	28.63
13	GLY	-	1	GTP	114	N	O1PG	146	13.53	13	GLY	-	1	GTP	114	N	O1PB	179	33.84	13	GLY	-	1	GTP	114	N	O3PG	168	31.64
13	GLY	-	1	GTP	114	N	O2PG	74	6.86	13	GLY	-	1	GTP	114	N	O3PB	14	2.65	14	VAL	-	1	GTP	119	N	O1PB	12	2.26
13	GLY	-	1	GTP	114	N	O3PG	172	15.94	13	GLY	-	1	GTP	114	N	O1PG	23	4.35	14	VAL	-	1	GTP	119	N	O1PG	141	26.55
14	VAL	-	1	GTP	119	N	O1PB	112	10.38	13	GLY	-	1	GTP	114	N	O2PG	21	3.97	14	VAL	-	1	GTP	119	N	O2PG	6	1.13
14	VAL	-	1	GTP	119	N	O1PG	20	1.85	13	GLY	-	1	GTP	114	N	O3PG	11	2.08	14	VAL	-	1	GTP	119	N	O3PG	2	0.38
14	VAL	-	1	GTP	119	N	O2PG	9	0.83	14	VAL	-	1	GTP	119	N	O1PA	15	2.84	15	GLY	-	1	GTP	127	N	O3PA	15	2.82
14	VAL	-	1	GTP	119	N	O3PG	12	1.11	14	VAL	-	1	GTP	119	N	O2PA	1	0.19	15	GLY	-	1	GTP	127	N	O1PB	240	45.2
15	GLY	-	1	GTP	127	N	O1PA	3	0.28	14	VAL	-	1	GTP	119	N	O1PB	4	0.76	15	GLY	-	1	GTP	127	N	O1PG	136	25.61
15	GLY	-	1	GTP	127	N	O3PA	78	7.23	14	VAL	-	1	GTP	119	N	O3PG	2	0.38	15	GLY	-	1	GTP	127	N	O3PG	7	1.32

Figure 15: Occurrence of h-bonds in $G12C-GDP \rightarrow G12C-GTP$ perturbation P-loop. Colored in yellow is P-loop, and in orange the h-bond between sulfur in cysteine and $P\gamma/P\beta$ oxygen. Three colours represent three different replicas, numerated from left to right.

perturbation moves on through λ points.

4.3.3 Atom-atom distance analysis

As RMSD hinted that there is a significant difference in how Switch-I loop moves, more analysis was done in that direction. The position of the Switch-I loop can be observed in Figure 1. There one can see that it interacts with GTP/GDP molecule through bound magnesium ion. For this reason, atom-atom distance analysis of magnesium ion and GTP/GDP molecule was performed. Figures 16-23 show once again an inconsistency in binding between the two directions.

In Figures 21 and 22, distances of the C12 residue, as well as surrounding residues, are a bit smaller compared to the distances with G12 present or with the GDP molecule involved. The distance of the magnesium ion from P-loop residues is consistent regardless of the perturbation or mutation.

There is also a consistency in magnesium ion binding to D57 and T58 residues of the Switch-II loop. Its interaction with the GTP/GDP molecule also relies on interactions with G60, Q61 and R68 residues. Looking into Tables 12-14 in Appendix A, those are other possible mutation hotspots.

The problematic Switch-I loop does not have a clear binding scheme as the Switch-I loop, but it can be seen in Figures 16-23 that the GTP/GDP interacts with different aminoacid residues based on the perturbation and replica. Residues with the highest interaction rate (lowest distance from the GTP/GDP molecule) are F28, D30, E31, Y32, D33, P34, I36, and Y40. A lack of the pattern in presented pictures confirms that the Switch-I loop lacks consistency within different perturbations and replicas, which leads to inconsistent binding free energy results.

Differences between replicas for the beginning to the end of a simulation can also be observed. Distances of specific residues of the P-loop and Switch-II loop seem consistent throughout, which is shown as light colored squares. In Figures 24-25 it is seen once more that the Switch-I loop is the most flexible one. The distance change within single simulation is prominent in Switch-I region, difference being the most prominent in the exact residues that

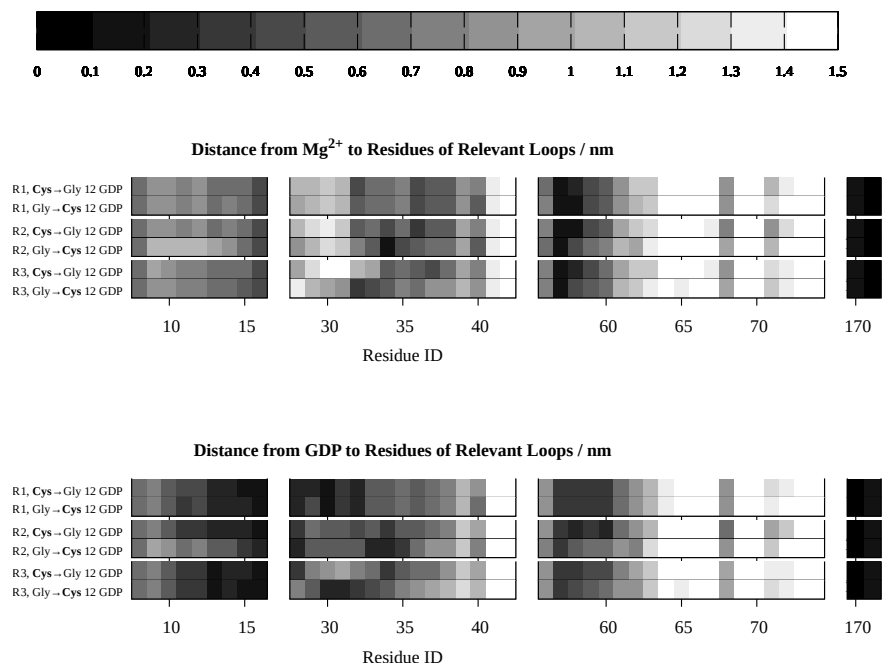


Figure 16: Distance between residues in $G12C-GDP \rightarrow WT-GDP$ perturbation at $\lambda=0$ and $WT-GDP \rightarrow G12C-GDP$ perturbation at $\lambda=1$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

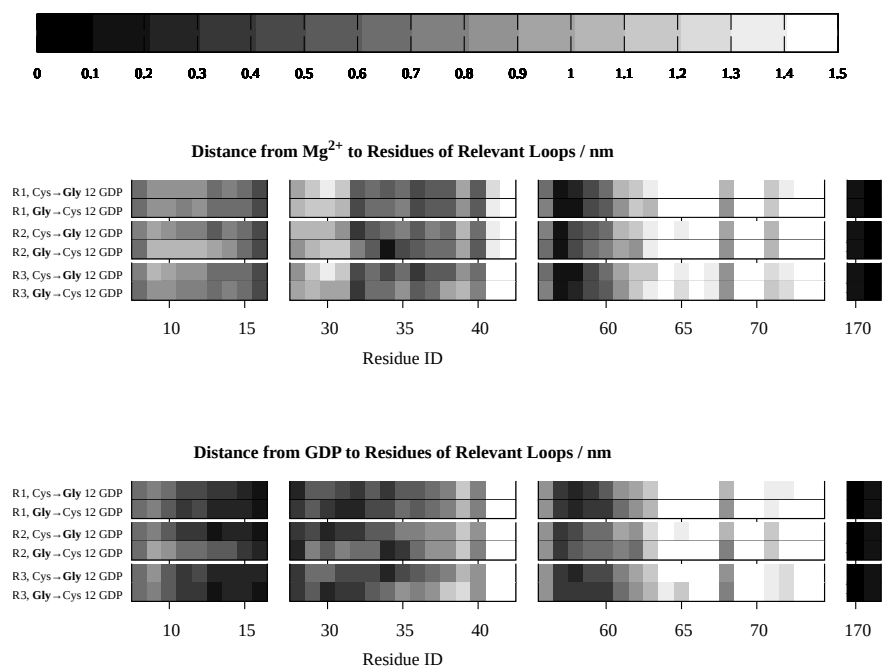


Figure 17: Distance between residues in $G12C-GDP \rightarrow WT-GDP$ perturbation at $\lambda=1$ and $WT-GDP \rightarrow G12C-GDP$ perturbation at $\lambda=0$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

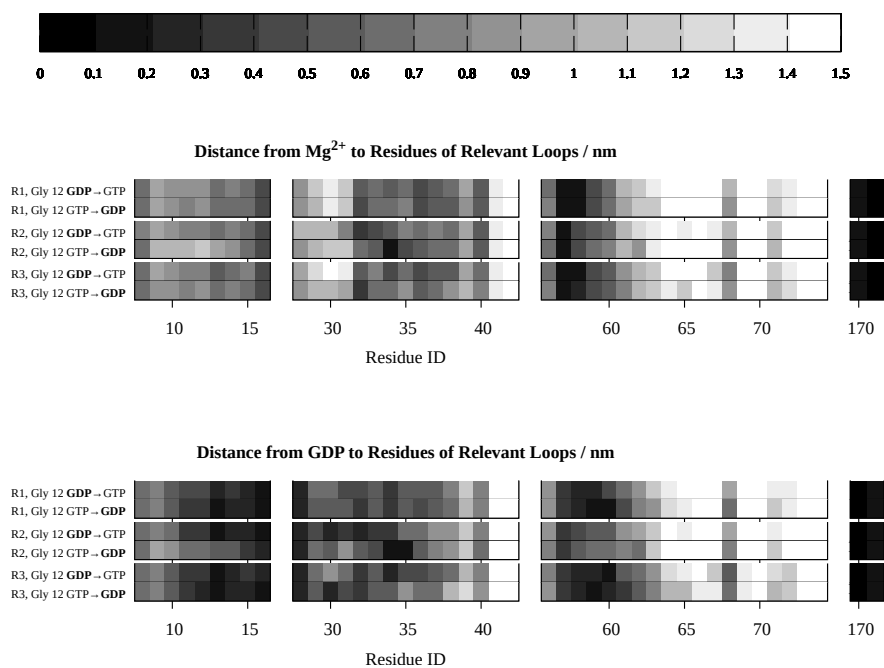


Figure 18: Distance between residues in WT-GDP→WT-GTP perturbation at $\lambda=0$ and WT-GTP→WT-GDP perturbation at $\lambda=1$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

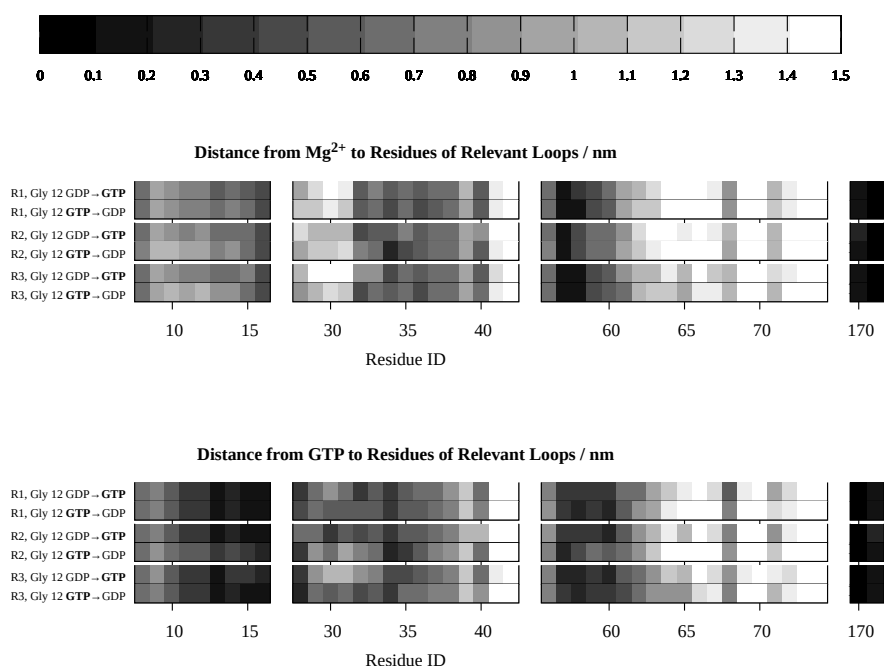


Figure 19: Distance between residues in WT-GDP→WT-GTP perturbation at $\lambda=1$ and WT-GTP→WT-GDP perturbation at $\lambda=0$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

are interacting the most with the GTP/GDP molecule based on Figures 16-23.

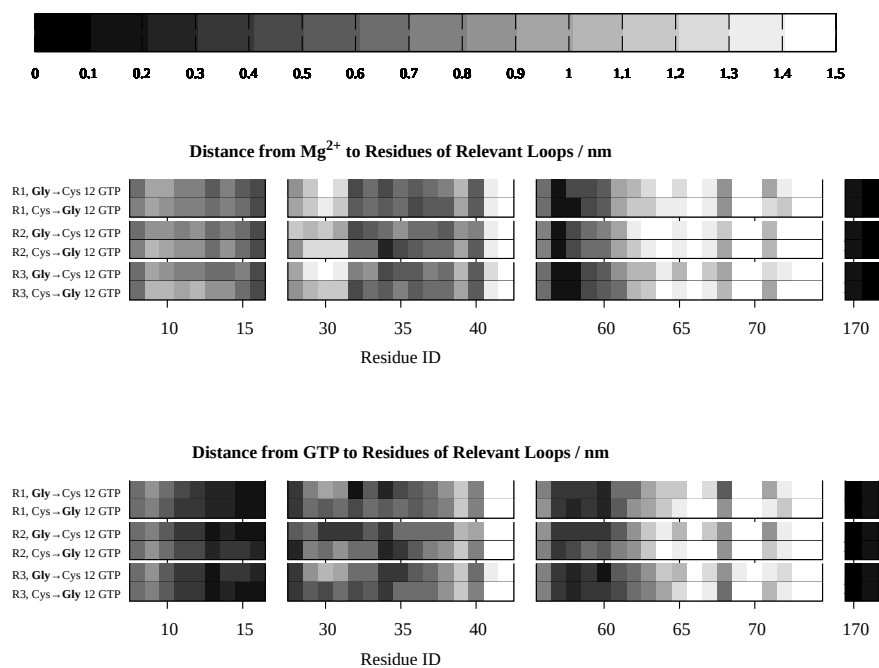


Figure 20: Distance between residues in WT-GTP→G12C-GTP perturbation at $\lambda=0$ and G12C-GTP→WT-GTP perturbation at $\lambda=1$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

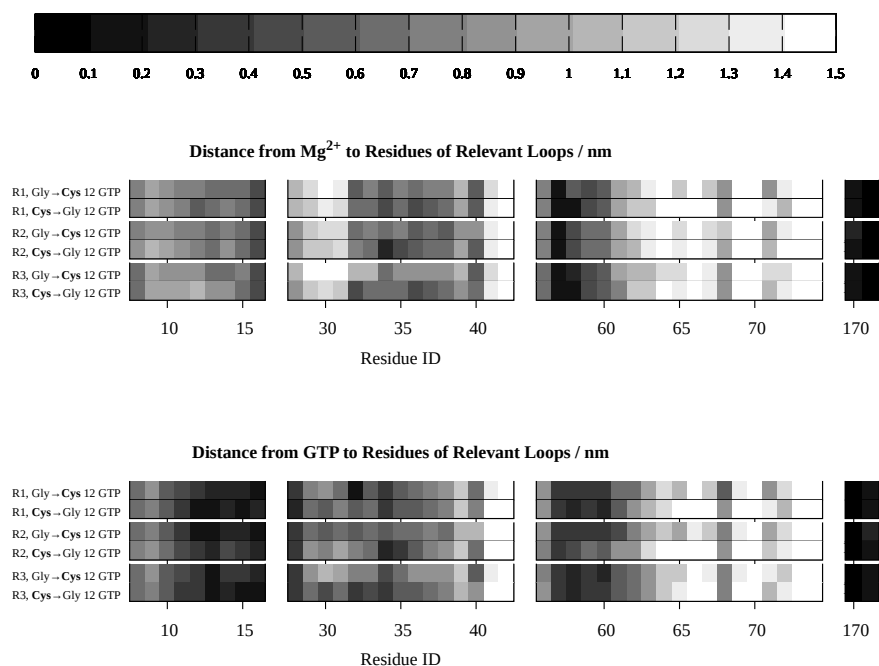


Figure 21: Distance between residues in WT-GTP→G12C-GTP perturbation at $\lambda=1$ and G12C-GTP→WT-GTP perturbation at $\lambda=0$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

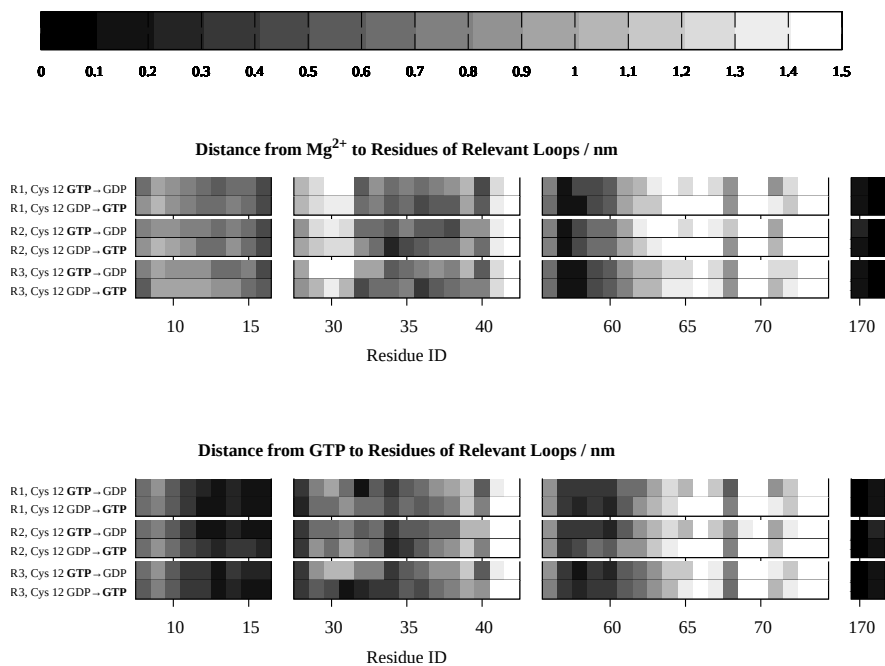


Figure 22: Distance between residues in $G12C-GTP \rightarrow G12C-GDP$ perturbation at $\lambda=0$ and $G12C-GDP \rightarrow G12C-GTP$ perturbation at $\lambda=1$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

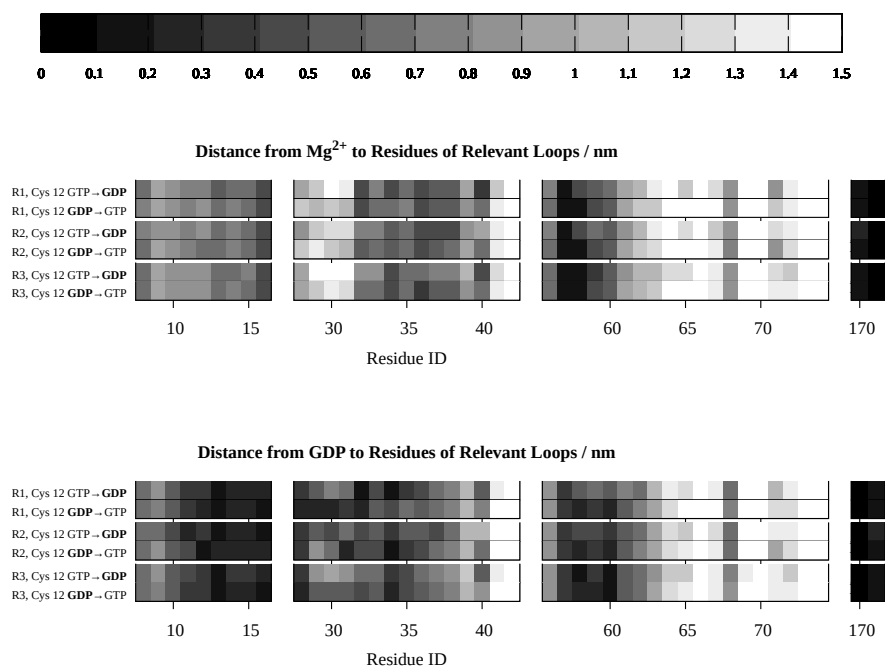


Figure 23: Distance between residues in $G12C-GTP \rightarrow G12C-GDP$ perturbation at $\lambda=1$ and $G12C-GDP \rightarrow G12C-GTP$ perturbation at $\lambda=0$. Residue number 170 is GTP/GDP molecule and 171 is magnesium ion.

Looking into a pattern on Figures 24 and 25, it can also be seen that the distance changes more in one of the simulation directions. This is best observed in Figure 25, third set of perturbations, but is also quite visible in the same set of simulations in Figure 24. Looking into results in Figure 5, there is no significant hysteresis there, but energy values are also quite low. More of the inconsistency in the pattern is seen in the first and fourth block, which correlates with the prominent hysteresis and the differences in replica's energies shown in Figure 5, respectively.

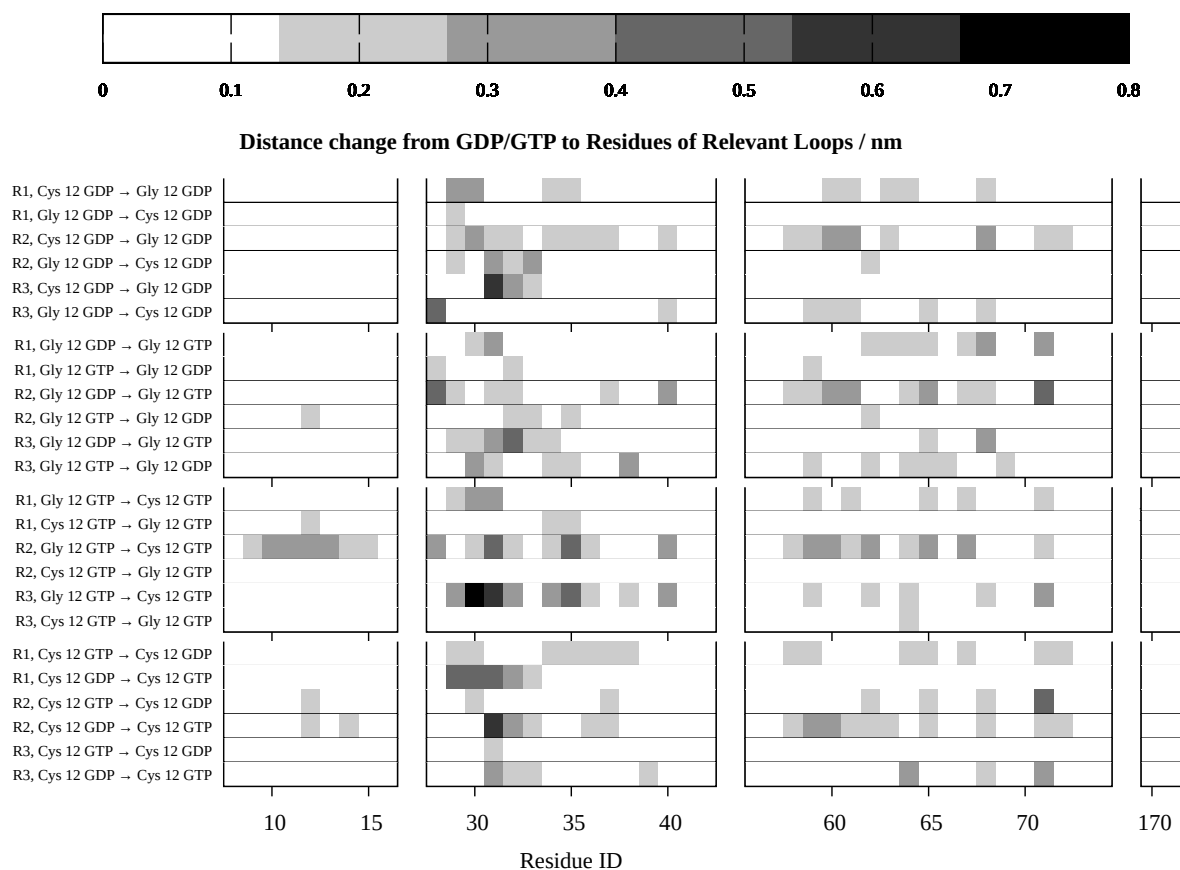


Figure 24: Difference in starting and final position of GTP/GDP molecule.

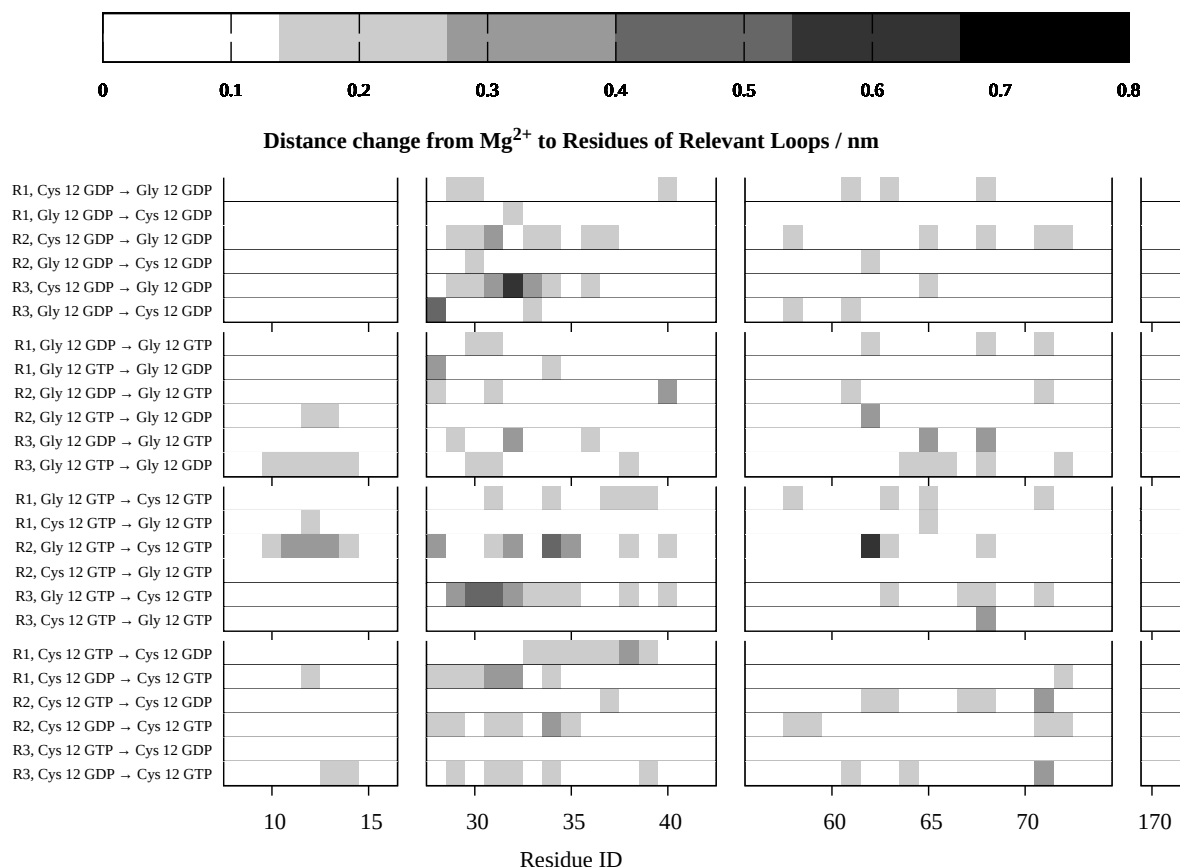


Figure 25: *Difference in starting and final position of magnesium ion.*

5 Conclusion

K-Ras protein is one of the significant players in cancer research as there are multiple mutations involved which show correlation with cancer development. For this reason, it was taken as the topic of the research presented in this thesis.

In the final analysis of the results obtained in researching the molecular dynamics and free energy binding of GTP/GDP molecule in K-Ras G12C mutation, a starting hypothesis was confirmed: Binding free energies showed the preference in binding of GTP molecule in the mutated type which goes together with the activity of mutated protein in MAPK pathway. This concludes that G12C mutation has a predisposition to cause cancer.

While performing simulations, there were obvious inconsistencies in results between replicas. Main reason for those turned out to be the lack of h-bond forming between sulfur on mutated cysteine and $P\gamma$ oxygen of GTP molecule. For this reason, simulation replicas that do not form h-bonds are not considered in free energy calculations. These simulations are unstable and require higher simulation time to converge and produce relevant h-bond sampling. A reason that there is no forming of the h-bond in some replicas is not yet concluded.

Other problem that was encountered was a significant hysteresis between same system simulations going in different perturbation directions. RMSD and atom-atom distance analysis showed inconsistencies between those simulations in Switch-II loop.

There are two main ways for improving obtained results. First one would be prolonging simulations to get better sampling. This solution is not preferable, as it requires a lot of time and resources, and is not as significant as there are existing MD simulations done of this system. Another idea would be moving from the chosen apo structure of the protein to the holo structure, which might be much less flexible.

Either way, a direction to look into would also be to work on full automatization of extended TI simulations in a way that after each TI cycle analysis is performed and the system is set for the next cycle, repeating the proces until a condition is met. This will allow simulations to run smoothly without human intervention which would make the whole process faster.

6 Bibliography

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A Appendix: Frequency of K-Ras mutations in human cancers with fatalities - full data

Table 12: Frequency of K-Ras mutations in human cancers, full table. Data were compiled from cBioPortal database for cancer genomics, TCGA PanCancer Atlas Studies (as of August 2nd 2024) [12, 13, 14].

	Bladder c.	Breast c.	Cervical c.	Cholangiocarcinoma	Colorectal c.	Endometrial c.	Esophagogastric c.	Glioblastoma	Glioma	Head and neck c.	Hepatobiliary c.	Leukemia	Mature B-cell neoplasms	Melanoma	Non-small cell lung c.	Ovarian epithelial tumor	Pancreatic c.	Pleural mesothelioma	Prostate c.	Renal clear cell carcinoma	Renal non-clear cell carcinoma	Sarcoma	Stomach	Thymic epithelial tumor	Thyroid cancer
E3K	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
K5E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G10dup	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A11_G12dup	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G12A	-	-	-	-	10	9	-	-	1	-	-	-	-	-	18	-	1	-	-	-	-	-	-	1	-
G12C	2	1	2	-	15	7	2	-	-	1	-	-	-	70	-	1	1	-	-	1	-	-	-	-	
G12D	6	1	4	-	58	34	12	2	-	2	2	2	2	20	-	49	-	1	-	-	3	-	-	1	
G12R	1	-	-	1	2	-	-	-	-	-	-	-	1	-	1	25	-	1	-	-	-	-	2	-	
G12S	-	-	-	-	8	3	4	-	-	-	-	-	-	5	1	-	-	-	-	-	-	-	1	-	
G12V	2	-	2	-	49	22	3	-	-	-	2	-	-	41	4	33	-	-	-	-	2	-	3	-	
G13C	-	-	-	-	2	3	-	-	-	-	-	-	-	8	-	1	-	-	-	-	1	-	-	1	
G13D	1	-	4	-	37	11	11	-	-	1	2	-	1	3	-	-	-	-	-	-	-	-	-	-	
G13V	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
V14I	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
S17T	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
L19F	2	-	-	-	1	-	-	-	-	-	-	1	-	3	-	-	-	-	-	-	-	-	-	-	
I21R	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q22K	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
I24N	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
D33E	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
P34L	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
I36M	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
D38Y	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
T58K	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A59E	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
A59G	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	
A59T	-	-	-	-	1	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q61E	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q61H	1	-	-	-	4	4	2	-	-	-	1	-	-	2	-	6	-	-	-	-	-	1	-	-	
Q61K	-	-	-	1	3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	
Q61L	-	-	-	-	1	1	-	-	-	-	-	-	1	3	1	-	-	-	-	-	-	-	2	-	
Q61P	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q61R	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	2	-	-	-	-	-	-	1	1	
E62K	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	
E63K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	
R68M	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R68S	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Y71C	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
K88*	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
D92Y	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
E98*	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
P110S	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
K117N	-	-	-	-	4	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
R123*	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
A130V	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R135T	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A146P	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
A146T	-	-	2	-	16	1	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
A146V	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	
R151T	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A155D	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
T158A	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R164Q	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
I171Nfs*14	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
K176Q	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
?	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Table 13: Frequency of K-Ras mutation fatalities in human cancers, full table. Data were compiled from cBioPortal database for cancer genomics, TCGA PanCancer Atlas Studies (as of August 2nd 2024) [12, 13, 14].

	Bladder c.	Breast c.	Cervical c.	Cholangiocarcinoma	Colorectal c.	Endometrial c.	Esophagogastric c.	Glioblastoma	Glioma	Head and neck c.	Hepatobiliary c.	Leukemia	Mature B-cell neoplasms	Melanoma	Non-small cell lung c.	Ovarian epithelial tumor	Pancreatic c.	Pleural mesothelioma	Prostate c.	Renal clear cell carcinoma	Renal non-clear cell carcinoma	Sarcoma	Sminoma	Thymic epithelial tumor	Thyroid cancer
E3K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
K5E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G10dup	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A11_G12dup	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G12A	-	-	-	-	3	2	-	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	-	-	-
G12C	1	1	-	-	3	2	-	-	-	-	1	-	-	1	15	-	1	-	-	1	-	-	-	-	-
G12D	4	1	-	-	9	2	-	1	-	-	-	-	-	2	3	25	-	-	-	-	-	-	-	-	-
G12R	1	-	-	-	1	-	-	-	-	-	-	-	-	-	1	9	-	-	-	-	-	-	-	-	-
G12S	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G12V	-	-	1	-	5	1	1	-	-	-	-	-	-	-	8	2	17	-	-	-	-	-	1	-	-
G13C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	-	-	-
G13D	-	-	3	-	4	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-
G13V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V14I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S17T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L19F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I21R	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q22K	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I24N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D33E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P34L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I36M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D38Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T58K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A59E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A59G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A59T	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q61E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q61H	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	1	-	-	-
Q61K	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q61L	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-
Q61P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q61R	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	2	-	-	-	-	-	-	-	-	-
E62K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E63K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R68M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R68S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y71C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K88*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D92Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E98*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P110S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K117N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R123*	-	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
A130V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R135T	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A146P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A146T	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A146V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R151T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A155D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T158A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R164Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I171Nfs*14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K176Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 14: Ratio of K-Ras mutation fatalities to comorbidities in human cancers, full table. Data were compiled from cBioPortal database for cancer genomics, TCGA PanCancer Atlas Studies (as of August 2nd 2024) [12, 13, 14].

	Bladder c.	Breast c.	Cervical c.	Cholangiocarcinoma	Colorectal c.	Endometrial c.	Esophagogastric c.	Glioblastoma	Glioma	Head and neck c.	Hepatobiliary c.	Leukemia	Mature B-cell neoplasms	Melanoma	Non-small cell lung c.	Ovarian epithelial tumor	Pancreatic c.	Pleural mesothelioma	Prostate c.	Renal clear cell carcinoma	Renal non-clear cell carcinoma	Sarcoma	Stimoma	Thymic epithelial tumor	Thyroid cancer
E3K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
K5E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G10dup	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A11_G12dup	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G12A	-	-	-	-	.3	.22	-	-	-	-	-	-	-	-	.17	-	-	-	-	-	-	-	-	-	-
G12C	.5	0	.5	-	.2	.29	0	-	-	-	0	0	-	.21	-	0	-	-	-	-	-	-	0	-	
G12D	.67	0	.25	-	.16	.06	0	.5	-	-	0	0	-	.15	-	0	-	-	-	-	-	-	0	-	
G12R	1	-	-	0	.5	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	0	-	
G12S	-	-	-	-	.13	0	.25	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	0	-	
G12V	0	-	.5	-	.1	.05	.33	-	-	-	0	0	-	.2	.5	-	-	-	-	-	-	0	0	0	
G13C	-	-	-	-	0	0	0	-	-	-	-	-	-	.38	-	1	-	-	-	-	-	0	-	0	
G13D	0	-	.75	-	.11	0	0	-	-	-	1	0	-	.33	-	-	-	-	-	-	-	-	-	-	
G13V	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
V14I	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	
S17T	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
L19F	0	-	-	-	0	-	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	-	-	-	
I21R	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q22K	-	-	-	-	1	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	
I24N	-	-	-	-	-	0	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	
D33E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
P34L	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
I36M	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	
D38Y	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	
T58K	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A59E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A59G	-	-	-	-	0	0	-	-	-	-	-	-	0	-	-	-	-	-	-	-	0	-	-	-	
A59T	-	-	-	-	0	-	.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q61E	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q61H	1	-	-	-	0	0	0	-	-	-	0	-	-	0	-	.5	-	-	-	-	-	1	-	-	
Q61K	-	-	-	0	.33	0	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	0	
Q61L	-	-	-	-	0	0	-	-	-	-	-	-	1	.33	0	-	-	-	-	-	-	-	0	-	
Q61P	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Q61R	-	-	-	-	0	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	0	0	
E62K	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
E63K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R68M	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R68S	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Y71C	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
K88*	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	
D92Y	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
E98*	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
P110S	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
K117N	-	-	-	-	.5	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
R123*	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	
A130V	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R135T	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
A146P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	
A146T	-	-	0	-	-	0	0	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	
A146V	-	-	-	-	.13	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R151T	-	-	-	-	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	
A155D	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
T158A	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
R164Q	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
I171Nfs*14	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
K176Q	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
?	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

B Appendix: Free energy calculations file preparation code

```
1 import os
2 import shutil
3 import numpy as np
4 import SMart
5 from SMart.md import pipeline
6 from SMart.md.data_st import MD_Parameters
7
8 SMart.incl.set_print_warnings(False)
9
10 run_file_EQ_template = '''#!/bin/sh
11 #SBATCH --ntasks=1
12 #SBATCH --cpus-per-task=4
13 #SBATCH --gres=mps:33
14 #SBATCH --mem=4096
15 #SBATCH --partition NGN
16 #SBATCH --time=24:00:00
17
18 NAME='whoami'
19 PROGRAM={md_path}
20
21 export LD_LIBRARY_PATH=$LD_LIBRARY_PATH:/usr/lib:/usr/local/cuda/lib64:\
22 /usr/local/cuda/lib:/usr/lib/nvidia-current:\
23 /home/oostenbrink/programs/mmslogin/cukernellib_8
24
25 SYS_NAME={sys_name}
26 LAM_NAME={lam_name}
27 PREV_LAM={prev_lam}
28 NEXT_LAM={next_lam}
29 LAM_SYS_NAME=${SYS_NAME}_$LAM_NAME_EQ
30 PREV_SYS_NAME=${SYS_NAME}_$PREV_LAM_EQ
31
32 OLD={old}
33 FIRST={first}
34 LAST={last}
35 CNF_FILE={cnf_file}
36 OLD_CYCLE={old_cycle}
37
38 DIR={work_dir}
39 SIMULDIR=${DIR}$LAM_NAME
40 PREV_DIR=${DIR}$PREV_LAM
41 NEXT_DIR=${DIR}$NEXT_LAM
42 if `test $OLD_CYCLE -eq 0`; then
43     LAST_CONF=${DIR}/../$SYS_NAME/$LAM_NAME/$SYS_NAME\
44     _$LAM_NAME.cnf
45 else
```



```

46         LAST_CONF=${{DIR}}/../../${{SYS_NAME}}_${{OLD_CYCLE}}_post_analysis/\
47     ${{LAM_NAME}}/${{SYS_NAME}}_${{LAM_NAME}}.cnf
48 fi
49
50 #if there are no old eqs and tis performed for this lambda point do eq,
51 ↪ else use last ti
51 if `test ${{OLD}} -eq 0`; then
52     #create temporary directory;
53     WORKDIR=/scratch/${{SLURM_JOBID}};
54     mkdir -p ${{WORKDIR}};
55     cd      ${{WORKDIR}};
56
57     #input;
58     TOPO={topo};
59     PTTOP={pttop};
60     #if this is the first one, use original cnf (changed for later cycles)
61     if `test ${{OLD_CYCLE}} -eq -1`; then
62         if `test ${{FIRST}} -eq 1`; then
63             INPUTCRD=${{CNF_FILE}};
64         else
65             INPUTCRD=${{PREV_DIR}}/${{PREV_SYS_NAME}}.cnf;
66         fi
67     else
68         INPUTCRD=${{LAST_CONF}};
69     fi
70     IUNIT=${{SIMULDIR}}/EQ_ext_TI_${{LAM_NAME}}.imd;
71
72     #output;
73     OUTPUTCRD=${{LAM_SYS_NAME}}.cnf;
74     OUTPUTTRX=${{LAM_SYS_NAME}}.trc;
75     OUTPUTTRE=${{LAM_SYS_NAME}}.tre;
76     OUTPUTTRG=${{LAM_SYS_NAME}}.trg;
77     OUNIT=${{LAM_SYS_NAME}}.omd;
78
79     export OMP_NUM_THREADS=${{SLURM_CPUS_PER_TASK}};
80
81     MDOK=1;
82
83     ${{PROGRAM}} \
84         @topo  ${{TOPO}} \
85         @conf  ${{INPUTCRD}} \
86         @input ${{IUNIT}} \
87         @pttopo ${{PTTOP}} \
88         @fin   ${{OUTPUTCRD}} \
89         @trc   ${{OUTPUTTRX}} \
90         @tre   ${{OUTPUTTRE}} \
91         @trg   ${{OUTPUTTRG}} \

```

```

92         >          ${OUNIT}};
93     grep "finished successfully" ${OUNIT}} > /dev/null || MDOK=0;
94
95     uname -a >> ${OUNIT}};
96
97     #compress some files;
98     gzip ${OUTPUTTRX}};
99     gzip ${OUTPUTTRE}};
100    gzip ${OUTPUTTRG}};
101
102    #copy files back;
103    OK=1;
104    cp ${OUNIT}}          ${SIMULDIR}} || OK=0;
105    cp ${OUTPUTCRD}}      ${SIMULDIR}} || OK=0;
106    cp ${OUTPUTTRX}}.gz   ${SIMULDIR}} || OK=0;
107    cp ${OUTPUTTRE}}.gz   ${SIMULDIR}} || OK=0;
108    cp ${OUTPUTTRG}}.gz   ${SIMULDIR}} || OK=0;
109
110    #clean up after successful run;
111    if `test ${OK}} -eq 0`; then
112        uname -a > mess;
113        echo 'cp failed for EDSR_lig_bmax2_search, run 1' >> mess;
114        MAIL -s "ERROR" ${NAME}} < mess:
115        cd ${SIMULDIR}};
116    else
117        cd ${SIMULDIR}};
118        rm ${WORKDIR}}/*;
119        rmdir ${WORKDIR}};
120    fi
121
122    #stop if MD was not successful
123    if `test ${MDOK}} -eq 0`; then
124        exit
125    fi
126
127 else
128     cp ${LAST_CONF}} ${SIMULDIR}}/${LAM_SYS_NAME}}.cnf;
129
130 fi
131
132 #perform last command (usually submit next job)
133
134 cd ${SIMULDIR}}

```

```

135 sbatch --ntasks ${SLURM_NTASKS} --cpus-per-task ${SLURM_CPUS_PER_TASK}
    ↪ --gres=mps:$(scontrol show job ${SLURM_JOB_ID} | grep gres/mps | sed
    ↪ "s/^.*gres\/mps=//" ) --mem ${SLURM_MEM_PER_NODE} --partition
    ↪ ${SLURM_JOB_PARTITION} --time $(scontrol show job ${SLURM_JOB_ID} |
    ↪ grep TimeLimit | sed "s/^.*TimeLimit=//" | cut -d" " -f1)
    ↪ run_TI_${LAM_NAME}.run
136
137 if `test ${LAST} -eq 0`; then
138     cd ${NEXT_DIR}/
139     ./run_EQ_${NEXT_LAM}.run
140 fi
141
142 '''
143
144 run_file_TI_template = '''#!/bin/sh
145
146 NAME='whoami'
147 PROGRAM={md_path}
148
149 export LD_LIBRARY_PATH=$LD_LIBRARY_PATH:/usr/lib:/usr/local/cuda/lib64:\
150 /usr/local/cuda/lib:/usr/lib/nvidia-current:\
151 /home/oostenbrink/programs/mmslogin/cukernellib_8
152
153 SYS_NAME={sys_name}
154 LAM_NAME={lam_name}
155 LAM_SYS_NAME=${SYS_NAME}_${LAM_NAME}
156
157 DIR={work_dir}
158 SIMULDIR=${DIR}/${LAM_NAME}
159
160 #create temporary directory
161 WORKDIR=/scratch/${SLURM_JOBID}
162 mkdir -p ${WORKDIR}
163 cd      ${WORKDIR}
164
165 #input
166 TOPO={topo}
167 INPUTCRD=${SIMULDIR}/${SYS_NAME}_${LAM_NAME}_EQ.cnf;
168 IUNIT=${SIMULDIR}/ext_TI_${LAM_NAME}.imd
169 PTOPO={ptop}
170
171 #output
172 OUTPUTCRD=${LAM_SYS_NAME}.cnf
173 OUTPUTTRX=${LAM_SYS_NAME}.trc
174 OUTPUTTRE=${LAM_SYS_NAME}.tre
175 OUTPUTTRG=${LAM_SYS_NAME}.trg
176 OUNIT=${LAM_SYS_NAME}.omd

```

```

177
178 export OMP_NUM_THREADS=${{SLURM_CPUS_PER_TASK}}
179
180 MDOK=1
181
182 ${{PROGRAM}} \
183     @topo  ${{TOPO}} \
184     @conf  ${{INPUTCRD}} \
185     @input ${{IUNIT}} \
186     @pttopo ${{PTTOPO}} \
187     @fin   ${{OUTPUTCRD}} \
188     @trc   ${{OUTPUTTRX}} \
189     @tre   ${{OUTPUTTRE}} \
190     @trg   ${{OUTPUTTRG}} \
191     >      ${{OUNIT}}
192 grep "finished successfully" ${{OUNIT}} > /dev/null || MDOK=0
193
194 uname -a >> ${{OUNIT}}
195
196 #compress some files
197 gzip ${{OUTPUTTRX}}
198 gzip ${{OUTPUTTRE}}
199 gzip ${{OUTPUTTRG}}
200
201 #copy files back
202 OK=1
203 cp ${{OUNIT}}                ${{SIMULDIR}} || OK=0
204 cp ${{OUTPUTCRD}}            ${{SIMULDIR}} || OK=0
205 cp ${{OUTPUTTRX}}.gz         ${{SIMULDIR}} || OK=0
206 cp ${{OUTPUTTRE}}.gz         ${{SIMULDIR}} || OK=0
207 cp ${{OUTPUTTRG}}.gz         ${{SIMULDIR}} || OK=0
208
209 #clean up after successful run
210 if `test ${{OK}} -eq 0`; then
211     uname -a > mess;
212     echo 'cp failed for EDSR_lig_bmax2_search, run 1' >> mess;
213     MAIL -s "ERROR" ${{NAME}} < mess:
214     cd ${{SIMULDIR}};
215 else
216     cd ${{SIMULDIR}};
217     rm ${{WORKDIR}}/*;
218     rmdir ${{WORKDIR}};
219 fi
220
221 #stop if MD was not successful
222 if `test ${{MDOK}} -eq 0`; then
223     exit

```

```

224 fi
225
226 '''
227
228 def make_imd_f_EQ(lp, lam_name, lam_work_dir, eq_time, eq_step_dt,
↳ traj_print_eq, en_print_eq):
229     imd.change_imd(dict(PERTURBATION={2:lp}))
230     imd.change_imd(dict(STEP={0:int(eq_time*1000/eq_step_dt)}))
231     imd.change_imd(dict(STEP={2:eq_step_dt}))
232     imd.change_imd(dict(PRINTOUT={0:traj_print_eq}))
233     imd.change_imd(dict(WRITETRAJ={0:traj_print_eq}))
234     imd.change_imd(dict(WRITETRAJ={4:en_print_eq}))
235     imd.change_imd(dict(WRITETRAJ={5:en_print_eq}))
236     imd_f_eq=os.path.join(lam_work_dir, 'EQ_ext_TI_'+lam_name+'.imd')
237     imd.write_imd(imd_f_eq)
238     pass
239
240 def make_imd_f_TI(lp, lam_name, lam_work_dir, prod_time, prod_step_dt,
↳ traj_print_ti, en_print_ti):
241     imd.change_imd(dict(PERTURBATION={2:lp}))
242     imd.change_imd(dict(STEP={0:int(prod_time*1000/prod_step_dt)}))
243     imd.change_imd(dict(STEP={2:prod_step_dt}))
244     imd.change_imd(dict(PRINTOUT={0:traj_print_ti}))
245     imd.change_imd(dict(WRITETRAJ={0:traj_print_ti}))
246     imd.change_imd(dict(WRITETRAJ={4:en_print_ti}))
247     imd.change_imd(dict(WRITETRAJ={5:en_print_ti}))
248     imd_f=os.path.join(lam_work_dir, 'ext_TI_'+lam_name+'.imd')
249     imd.write_imd(imd_f)
250     pass
251
252 def make_run_f_EQ(lam_name, md_path, sys_name, prev_lam, next_lam, old,
↳ first, last, cnf_file, old_cycle, work_dir, topo, ptop):
253     return run_file_EQ_template.format(lam_name = lam_name, md_path =
↳ md_path, sys_name = sys_name, prev_lam = prev_lam, next_lam =
↳ next_lam, old = old, first = first, last = last, cnf_file =
↳ cnf_file, old_cycle = old_cycle, work_dir = work_dir, topo =
↳ topo, ptop = ptop)
254
255 def make_run_f_TI(lam_name, md_path, sys_name, old_cycle, work_dir, topo,
↳ ptop):
256     return run_file_TI_template.format(lam_name = lam_name, md_path =
↳ md_path, sys_name = sys_name, old_cycle = old_cycle, work_dir =
↳ work_dir, topo = topo, ptop = ptop)
257
258 def check_for_old(cycle, sim_dir, sys_name, lam_name):
259     i = cycle - 1
260     while i >= 0:

```

```
261         if (i == 0):
262             old_dir = os.path.join(sim_dir,
                ↪ sys_name+'/' + lam_name)
263         else:
264             old_dir =
                ↪ os.path.join(sim_dir, sys_name+'_'+str(i)+'_post_analysis/' + lam_name)
265         if os.path.isdir(old_dir):
266             return i
267         else:
268             i -= 1
269     return -1
270
271
272 if __name__ == '__main__':
273     import argparse
274     parser = argparse.ArgumentParser(fromfile_prefix_chars='@')
275     parser.convert_arg_line_to_args = lambda arg_line: arg_line.split()
276
277     #run file arguments
278     parser.add_argument('-c', '--cnf_file', help = 'path to
                ↪ configuration file of the system', type = str, required = True)
279     parser.add_argument('-md', '--md_path', help = 'path to md file
                ↪ version', type = str, required = True)
280     parser.add_argument('-d', '--sim_dir', help = 'simulation
                ↪ directory', type = str, required = True)
281     parser.add_argument('-i', '--imd_template', help = 'path to
                ↪ parameter input file', type = str, required = True)
282     parser.add_argument('-p', '--ptop', help = 'path to perturbation
                ↪ topology file', type = str, required = True)
283     parser.add_argument('-t', '--topo', help = 'path to topology file',
                ↪ type = str, required = True)
284     parser.add_argument('-s', '--sys_name', help = 'system name', type
                ↪ = str, required = True)
285     parser.add_argument('-n', '--sys_num', help = 'number of an
                ↪ additional cycle (0 for initial)', type = int, required = True)
286     #additional arguments
287     parser.add_argument('-eq_time', help='equilibration time in ns',
                ↪ type=float, default=0.2)
288     parser.add_argument('-prod_time', help='production time in ns',
                ↪ type=float, default=2.0)
289     parser.add_argument('-eq_step_dt', help='equilibration step time dt
                ↪ in ns', type=float, default=0.002)
290     parser.add_argument('-prod_step_dt', help='production step time dt
                ↪ in ns', type=float, default=0.002)
291     parser.add_argument('-traj_print_eq', help='equilibration
                ↪ trajectory printout', type=int, default=100000)
```

```
292     parser.add_argument('-en_print_eq', help='equilibration energy
    ↪ printout', type=int, default=100000)
293     parser.add_argument('-traj_print_ti', help='production trajectory
    ↪ printout', type=int, default=50000)
294     parser.add_argument('-en_print_ti', help='production energy
    ↪ printout', type=int, default=500)
295     parser.add_argument('-N_LPs', help='number of lambda points for the
    ↪ first cycle', type=int, default=21)
296
297     args = parser.parse_args()
298
299     cnf_file = args.cnf_file
300     md_path = args.md_path
301     sim_dir = args.sim_dir
302     imd_template = args.imd_template
303     ptop = args.ptop
304     topo = args.topo
305     sys_name = args.sys_name
306     sys_num = args.sys_num
307     eq_time = args.eq_time
308     prod_time = args.prod_time
309     eq_step_dt = args.eq_step_dt
310     prod_step_dt = args.prod_step_dt
311     traj_print_eq = args.traj_print_eq
312     en_print_eq = args.en_print_eq
313     traj_print_ti = args.traj_print_ti
314     en_print_ti = args.en_print_ti
315     N_LPs = args.N_LPs
316
317     imd = MD_Parameters(imd_template)
318
319     work_dir = os.path.join(sim_dir + sys_name + '/')
320     if (sys_num != 0):
321         work_dir = os.path.join(sim_dir+sys_name + '_' +
    ↪ str(sys_num) + '_post_analysis/')
322     if not os.path.isdir(work_dir):
323         os.mkdir(work_dir)
324
325     #setting paths
326     if (sys_num == 0):
327         new_lambda = 0
328         new_times = 0
329     if (sys_num == 1):
330         new_lambda = os.path.join(sim_dir + sys_name +
    ↪ '/new_lambda.txt')
331         new_times = os.path.join(sim_dir + sys_name +
    ↪ '/new_times.txt')
```

```

332     else:
333         new_lambda = os.path.join(sim_dir + sys_name + '_' +
334             ↪ str(sys_num-1) + '_post_analysis/new_lambda.txt')
335         new_times = os.path.join(sim_dir + sys_name + '_' +
336             ↪ str(sys_num-1) +
337             ↪ '_post_analysis/new_times.txt')
338
339     #setting variables
340     first=1
341     last=0
342     old=0
343     cycle=0
344     old_cycle=-1
345     prev_lam='0'
346     next_lam='0'
347     LPs=[]
348     time=[]
349
350     #setting lambda points and times depending on the system number
351     if (sys_num == 0):
352         LPs = SMARt.md.ana.incl.get_lset(np.linspace(0,1,N_LPs))
353     else:
354         f = open(new_lambda)
355         for line in f.readlines():
356             LPs.append(float(line))
357         f.close()
358         f = open(new_times)
359         for line in f.readlines():
360             time.append(float(line))
361         f.close()
362
363     #simulating through lambda points
364     for i, lp in enumerate(LPs):
365         cycle = sys_num
366
367         #making lambda point directories
368         lam_name = 'l_{:.3f}'.format(lp)
369         lam_work_dir = os.path.join(work_dir, lam_name)
370         if not os.path.isdir(lam_work_dir):
371             os.mkdir(lam_work_dir)
372         f_EQ_run = os.path.join(lam_work_dir, 'run_EQ_' + lam_name
373             ↪ + '.run')
374         f_TI_run = os.path.join(lam_work_dir, 'run_TI_' + lam_name
375             ↪ + '.run')
376
377     #checking if last lambda
378     if i == len(LPs)-1:

```



```
374         last = 1
375     else:
376         next_lam = 'l_{:.3f}'.format(LPs[i+1])
377
378     old=0 #resets for every lambda, checks if there is same
379     ↪ lambda in old cycles
380     old_cycle=check_for_old(cycle, sim_dir, sys_name, lam_name)
381     if old_cycle != -1:
382         old=1
383
384     if (sys_num != 0):
385         prod_time = time[i]
386
387     #writing .imd and .run files
388     make_imd_f_EQ(lp, lam_name, lam_work_dir, eq_time,
389     ↪ eq_step_dt, traj_print_eq, en_print_eq)
390     make_imd_f_TI(lp, lam_name, lam_work_dir, prod_time,
391     ↪ prod_step_dt, traj_print_ti, en_print_ti)
392     f = open(f_EQ_run, 'w')
393     f.write(make_run_f_EQ(lam_name, md_path, sys_name,
394     ↪ prev_lam, next_lam, old, first, last, cnf_file,
395     ↪ old_cycle, work_dir, topo, ptop))
396     f.close()
397     os.system('chmod a+x '+f_EQ_run)
398     f = open(f_TI_run, 'w')
399     f.write(make_run_f_TI(lam_name, md_path, sys_name,
400     ↪ old_cycle, work_dir, topo, ptop))
401     f.close()
402     os.system('chmod a+x '+f_TI_run)
403
404     first = 0
405     prev_lam = lam_name
```


Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%			
13	GLY	-	1	GDP	114	N	O3PB	113	28.9	13	GLY	-	1	GDP	114	N	O3PB	1	0.23	12	CYSH	-	1	GDP	110	SG	O3PB	2	0.3
14	VAL	-	1	GDP	119	N	O1PB	36	9.21	14	VAL	-	1	GDP	119	N	O1PB	1	0.23	13	GLY	-	1	GDP	114	N	O3PA	2	0.3
15	GLY	-	1	GDP	127	N	O3PA	12	3.07	15	GLY	-	1	GDP	127	N	O3PA	2	0.45	13	GLY	-	1	GDP	114	N	O1PB	13	1.95
15	GLY	-	1	GDP	127	N	O1PB	303	77.49	15	GLY	-	1	GDP	127	N	O1PB	18	4.08	13	GLY	-	1	GDP	114	N	O3PB	538	80.54
16	LYSH	-	1	GDP	132	N	O1PB	390	99.74	16	LYSH	-	1	GDP	132	N	O1PA	17	3.85	14	VAL	-	1	GDP	119	N	O1PB	103	15.42
17	SER	-	1	GDP	145	N	O1PA	248	63.43	16	LYSH	-	1	GDP	132	N	O3PA	180	40.82	15	GLY	-	1	GDP	127	N	O5*	7	1.05
17	SER	-	1	GDP	145	N	O3PA	1	0.25	16	LYSH	-	1	GDP	132	N	O1PB	107	24.26	15	GLY	-	1	GDP	127	N	O3PA	71	10.63
17	SER	-	1	GDP	145	N	O1PB	1	0.25	16	LYSH	-	1	GDP	132	N	O2PB	150	34.01	15	GLY	-	1	GDP	127	N	O1PB	483	72.31
17	SER	-	1	GDP	145	N	O2PB	47	12.02	17	SER	-	1	GDP	145	N	O1PA	394	89.34	16	LYSH	-	1	GDP	132	N	O1PA	2	0.3
17	SER	-	1	GDP	149	OC	O2PA	150	38.38	17	SER	-	1	GDP	145	N	O2PA	5	1.13	16	LYSH	-	1	GDP	132	N	O3PA	2	0.3
17	SER	-	1	GDP	149	OC	O3PA	4	1.02	17	SER	-	1	GDP	145	N	O2PB	1	0.23	16	LYSH	-	1	GDP	132	N	O1PB	660	98.8
18	ALA	-	1	GDP	153	N	O1PA	379	96.93	17	SER	-	1	GDP	145	N	O3PA	19	4.31	16	LYSH	-	1	GDP	139	NZ	O1PB	1	0.15
28	PHE	-	1	GDP	262	CE	O3*	1	0.25	17	SER	-	1	GDP	149	OC	O1PA	56	12.7	16	LYSH	-	1	GDP	139	NZ	O3PB	2	0.3
28	PHE	-	1	GDP	262	CE	O5*	1	0.25	17	SER	-	1	GDP	149	OC	O2PA	380	86.17	16	LYSH	-	1	GDP	139	NZ	O1PB	3	0.45
28	PHE	-	1	GDP	264	CE	O2*	2	0.51	18	ALA	-	1	GDP	153	N	O1PA	396	89.8	16	LYSH	-	1	GDP	139	NZ	O3PB	3	0.45
28	PHE	-	1	GDP	266	CZ	O6	1	0.25	18	ALA	-	1	GDP	153	N	O2PA	2	0.45	16	LYSH	-	1	GDP	139	NZ	O1PB	2	0.3
28	PHE	-	1	GDP	266	CZ	O3*	5	1.28	28	PHE	-	1	GDP	266	CZ	O3*	6	1.36	16	LYSH	-	1	GDP	139	NZ	O3PB	2	0.3
28	PHE	-	1	GDP	266	CZ	O2PA	5	1.28	117	LYSH	-	1	GDP	1184	NZ	O4*	1	0.23	17	SER	-	1	GDP	145	N	O1PA	581	86.98
31	GLU	-	1	GDP	287	N	O2*	4	1.02	145	SER	-	1	GDP	1463	OC	O6	2	0.45	17	SER	-	1	GDP	145	N	O3PA	4	0.6
32	TYR	-	1	GDP	297	N	O3*	90	23.02	146	ALA	-	1	GDP	1467	N	O6	12	2.72	17	SER	-	1	GDP	145	N	O2PB	58	8.68
32	TYR	-	1	GDP	297	N	O2PA	1	0.25	146	ALA	-	1	GDP	1467	N	N7	4	0.91	17	SER	-	1	GDP	149	OC	O1PA	120	17.96
32	TYR	-	1	GDP	304	CD	O5*	2	0.51	147	LYSH	-	1	GDP	1473	N	O6	355	80.5	17	SER	-	1	GDP	149	OC	O2PA	518	77.54
32	TYR	-	1	GDP	304	CD	O2PA	22	5.63	147	LYSH	-	1	GDP	1473	N	N7	75	17.01	18	ALA	-	1	GDP	153	N	O1PA	66	9.88
116	ASN	-	1	GDP	1172	NC	N7	221	56.52	147	LYSH	-	1	GDP	1480	NZ	O2*	1	0.23	28	PHE	-	1	GDP	262	CE	O2*	1	0.15
117	LYSH	-	1	GDP	1177	N	O6	6	1.53	148	THR	-	1	GDP	1486	N	O6	44	9.98	28	PHE	-	1	GDP	264	CE	O2*	1	0.15
117	LYSH	-	1	GDP	1184	NZ	O4*	1	0.25										28	PHE	-	1	GDP	266	CZ	N7	3	0.45	
117	LYSH	-	1	GDP	1184	NZ	O4*	3	0.77										28	PHE	-	1	GDP	266	CZ	O2*	2	0.3	
117	LYSH	-	1	GDP	1184	NZ	O4*	1	0.25										32	TYR	-	1	GDP	297	N	O2PA	3	0.45	
146	ALA	-	1	GDP	1467	N	O6	138	35.29										32	TYR	-	1	GDP	304	CD	O2PA	6	0.9	
147	LYSH	-	1	GDP	1473	N	O6	27	6.91										60	GLY	-	1	GDP	571	N	O3PB	1	0.15	
147	LYSH	-	1	GDP	1480	NZ	N2	1	0.26										116	ASN	-	1	GDP	1172	NC	N7	185	27.69	
																			116	ASN	-	1	GDP	1172	NC	O6	2	0.3	
																			116	ASN	-	1	GDP	1172	NC	N7	23	3.44	
																			117	LYSH	-	1	GDP	1177	N	O6	37	5.54	
																			117	LYSH	-	1	GDP	1184	NZ	O4*	5	0.75	
																			117	LYSH	-	1	GDP	1184	NZ	O4*	4	0.6	
																			117	LYSH	-	1	GDP	1184	NZ	O4*	5	0.75	
																			146	ALA	-	1	GDP	1467	N	O6	236	35.33	
																			147	LYSH	-	1	GDP	1473	N	O6	295	44.16	
																			147	LYSH	-	1	GDP	1473	N	N7	1	0.15	
																			147	LYSH	-	1	GDP	1480	NZ	O2*	4	0.6	
																			147	LYSH	-	1	GDP	1480	NZ	O2*	2	0.3	
																			147	LYSH	-	1	GDP	1480	NZ	O2*	4	0.6	
																			148	THR	-	1	GDP	1486	N	O6	1	0.15	

Figure 27: Occurrence of h-bonds in WT-GDP→G12C-GDP perturbation. Colored in yellow is P-loop, in red Switch-I loop, in blue Switch-II loop, and in orange the h-bond between sulfur in cysteine and P γ /P β oxygen. Three colours represent three different replicas, numerated from left to right.

Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%
12	CYS	1	GTP	110	SG	O3PB	2	0.54	12	CYS	1	GTP	110	SG	O1PB	4	0.63	12	CYS	1	GTP	110	SG	O2PG	3	0.45
12	CYS	1	GTP	110	SG	O1PG	74	19.95	12	CYS	1	GTP	110	SG	O3PB	1	0.16	13	GLY	1	GTP	114	N	O2PG	651	96.88
13	GLY	1	GTP	114	N	O3PA	14	3.77	13	GLY	1	GTP	114	N	O3PA	22	3.49	13	GLY	1	GTP	114	N	O3PG	12	1.79
13	GLY	1	GTP	114	N	O1PB	11	2.95	13	GLY	1	GTP	114	N	O1PB	389	61.65	14	VAL	1	GTP	119	N	O1PG	311	46.28
13	GLY	1	GTP	114	N	O3PB	241	64.96	13	GLY	1	GTP	114	N	O3PB	40	6.34	14	VAL	1	GTP	119	N	O2PG	2	0.3
14	VAL	1	GTP	119	N	O3PA	3	0.81	15	GLY	1	GTP	127	N	O1PA	4	0.63	15	GLY	1	GTP	127	N	O1PG	648	96.43
14	VAL	1	GTP	119	N	O1PB	168	45.28	16	LYSH	1	GTP	132	N	O1PA	149	23.61	15	GLY	1	GTP	127	N	O3PG	25	3.72
15	GLY	1	GTP	127	N	O3PA	68	18.33	16	LYSH	1	GTP	132	N	O3PA	220	34.87	16	LYSH	1	GTP	132	N	O1PB	127	18.9
15	GLY	1	GTP	127	N	O1PB	309	83.29	16	LYSH	1	GTP	132	N	O1PB	25	3.96	16	LYSH	1	GTP	132	N	O3PB	2	0.3
16	LYSH	1	GTP	132	N	O1PB	329	88.68	16	LYSH	1	GTP	132	N	O2PB	3	0.48	16	LYSH	1	GTP	132	N	O1RG	529	78.72
16	LYSH	1	GTP	132	N	O2PB	67	18.06	16	LYSH	1	GTP	139	NZ	O1PB	1	0.16	16	LYSH	1	GTP	139	NZ	O3PB	16	2.38
16	LYSH	1	GTP	139	NZ	O1PB	78	21.02	17	SER	1	GTP	145	N	O1PA	278	44.06	16	LYSH	1	GTP	139	NZ	O1PG	55	8.18
16	LYSH	1	GTP	139	NZ	O2PB	1	0.27	17	SER	1	GTP	145	N	O2PA	30	4.75	16	LYSH	1	GTP	139	NZ	O2PG	73	10.86
16	LYSH	1	GTP	139	NZ	O3PB	4	1.08	17	SER	1	GTP	145	N	O3PA	1	0.16	16	LYSH	1	GTP	139	NZ	O3PB	19	2.83
16	LYSH	1	GTP	139	NZ	O2PG	1	0.27	17	SER	1	GTP	145	N	O2PB	234	37.08	16	LYSH	1	GTP	139	NZ	O1PG	76	11.31
16	LYSH	1	GTP	139	NZ	O1PB	47	12.67	17	SER	1	GTP	149	OC	O2PA	629	99.68	16	LYSH	1	GTP	139	NZ	O2PG	74	11.01
16	LYSH	1	GTP	139	NZ	O3PB	4	1.08	18	ALA	1	GTP	153	N	O1PA	418	66.24	16	LYSH	1	GTP	139	NZ	O3PB	17	2.53
16	LYSH	1	GTP	139	NZ	O1PG	1	0.27	18	ALA	1	GTP	153	N	O2PA	20	3.17	16	LYSH	1	GTP	139	NZ	O1PG	74	11.01
16	LYSH	1	GTP	139	NZ	O1PB	60	16.17	28	PHE	1	GTP	262	CE	O2*	2	0.32	16	LYSH	1	GTP	139	NZ	O2PG	77	11.46
16	LYSH	1	GTP	139	NZ	O2PB	1	0.27	28	PHE	1	GTP	266	CZ	N7	1	0.16	17	SER	1	GTP	145	N	O1PB	666	99.11
16	LYSH	1	GTP	139	NZ	O3PB	4	1.08	28	PHE	1	GTP	266	CZ	O2*	3	0.48	17	SER	1	GTP	145	N	O2PB	1	0.15
16	LYSH	1	GTP	139	NZ	O2PG	1	0.27	117	LYSH	1	GTP	1184	NZ	N3	1	0.16	17	SER	1	GTP	149	OC	O1PA	655	97.47
17	SER	1	GTP	145	N	O1PA	12	3.23	117	LYSH	1	GTP	1184	NZ	O4*	3	0.48	17	SER	1	GTP	149	OC	O3PA	1	0.15
17	SER	1	GTP	145	N	O2PB	360	97.04	117	LYSH	1	GTP	1184	NZ	O4*	2	0.32	17	SER	1	GTP	149	OC	O1PB	1	0.15
17	SER	1	GTP	149	OC	O1PA	1	0.27	117	LYSH	1	GTP	1184	NZ	N3	2	0.32	28	PHE	1	GTP	266	CZ	N9	2	0.3
17	SER	1	GTP	149	OC	O2PA	113	30.46	145	SER	1	GTP	1463	OC	O6	2	0.32	28	PHE	1	GTP	266	CZ	N7	1	0.15
17	SER	1	GTP	149	OC	O2PB	221	59.57	146	ALA	1	GTP	1467	N	O6	21	3.33	28	PHE	1	GTP	266	CZ	O2*	20	2.98
18	ALA	1	GTP	153	N	O1PA	371	100	146	ALA	1	GTP	1467	N	O7	1	0.16	32	TYR	1	GTP	297	N	O2*	1	0.15
28	PHE	1	GTP	264	CE	N7	1	0.27	147	LYSH	1	GTP	1473	N	O6	577	91.44	32	TYR	1	GTP	297	N	O3*	2	0.3
28	PHE	1	GTP	266	CZ	O2*	15	4.04	147	LYSH	1	GTP	1473	N	N7	45	7.13	32	TYR	1	GTP	304	CD	O1PA	13	1.93
60	GLY	1	GTP	571	N	O1PG	306	82.48	147	LYSH	1	GTP	1480	NZ	N3	1	0.16	32	TYR	1	GTP	304	CD	O2PA	50	7.44
116	ASN	1	GTP	1172	NC	N7	2	0.54	147	LYSH	1	GTP	1480	NZ	O2*	13	2.06	32	TYR	1	GTP	308	CE	O1PA	11	1.64
117	LYSH	1	GTP	1184	NZ	O4*	3	0.81	147	LYSH	1	GTP	1480	NZ	N3	1	0.16	32	TYR	1	GTP	308	CE	O2PA	7	1.04
117	LYSH	1	GTP	1184	NZ	O4*	5	1.35	147	LYSH	1	GTP	1480	NZ	O2*	11	1.74	32	TYR	1	GTP	311	OF	O1PA	10	1.49
117	LYSH	1	GTP	1184	NZ	O3*	1	0.27	147	LYSH	1	GTP	1480	NZ	N3	3	0.48	32	TYR	1	GTP	311	OF	O2PA	4	0.6
145	SER	1	GTP	1463	OC	O6	1	0.27	147	LYSH	1	GTP	1480	NZ	O2*	8	1.27	58	THR	1	GTP	560	OG	O2PB	5	0.74
146	ALA	1	GTP	1467	N	O6	19	5.12	148	THR	1	GTP	1486	N	O6	37	5.86	60	GLY	1	GTP	571	N	O3PA	3	0.45
147	LYSH	1	GTP	1473	N	O6	348	93.8										60	GLY	1	GTP	571	N	O3PB	8	1.19
147	LYSH	1	GTP	1473	N	N7	8	2.16										116	ASN	1	GTP	1172	NC	O6	3	0.45
147	LYSH	1	GTP	1480	NZ	N3	1	0.27										116	ASN	1	GTP	1172	NC	N7	597	88.84
147	LYSH	1	GTP	1480	NZ	O2*	3	0.81										117	LYSH	1	GTP	1177	N	O6	7	1.04
147	LYSH	1	GTP	1480	NZ	N3	2	0.54										146	ALA	1	GTP	1467	N	O6	471	70.09
147	LYSH	1	GTP	1480	NZ	O2*	4	1.08										147	LYSH	1	GTP	1473	N	O6	208	30.95
147	LYSH	1	GTP	1480	NZ	N3	1	0.27										147	LYSH	1	GTP	1480	NZ	N3	3	0.45
147	LYSH	1	GTP	1480	NZ	N2	1	0.27										147	LYSH	1	GTP	1480	NZ	N2	3	0.45
147	LYSH	1	GTP	1480	NZ	O2*	6	1.62										147	LYSH	1	GTP	1480	NZ	O2*	5	0.74
																		147	LYSH	1	GTP	1480	NZ	N3	4	0.6
																		147	LYSH	1	GTP	1480	NZ	N2	3	0.45
																		147	LYSH	1	GTP	1480	NZ	O2*	3	0.45
																		147	LYSH	1	GTP	1480	NZ	N3	2	0.3
																		147	LYSH	1	GTP	1480	NZ	N2	5	0.74
																		147	LYSH	1	GTP	1480	NZ	O2*	5	0.74

Figure 31: Occurrence of h-bonds in G12C-GTP→WT-GTP perturbation. Colored in yellow is P-loop, in red Switch-I loop, in blue Switch-II loop, and in orange the h-bond between sulfur in cysteine and P γ /P β oxygen. Three colours represent three different replicas, numerated from left to right.

Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%	Res	DONO	Res	ACC	Atom	D	A	OCCUR	%			
12	CYSH	-	1	GTP	110	SC	O3PB	1	0.09	12	CYSH	-	1	GTP	110	SC	O1PB	36	6.81	12	CYSH	-	1	GTP	110	SC	O1PG	1	0.19
12	CYSH	-	1	GTP	110	SC	O1PG	220	20.39	12	CYSH	-	1	GTP	110	SC	O1PB	14	2.65	12	CYSH	-	1	GTP	110	SC	O2PG	6	1.13
12	CYSH	-	1	GTP	110	SC	O3PG	2	0.19	12	CYSH	-	1	GTP	110	SC	O2PG	3	0.57	12	CYSH	-	1	GTP	110	SC	O3PG	1	0.19
13	GLY	-	1	GTP	114	N	O3PA	10	0.93	13	GLY	-	1	GTP	110	SC	O3PG	4	0.76	13	GLY	-	1	GTP	114	N	O3PB	41	7.72
13	GLY	-	1	GTP	114	N	O1PB	34	3.15	13	GLY	-	1	GTP	114	N	O1PA	3	0.57	13	GLY	-	1	GTP	114	N	O1PG	26	4.9
13	GLY	-	1	GTP	114	N	O3PB	459	42.54	13	GLY	-	1	GTP	114	N	O3PA	20	3.78	13	GLY	-	1	GTP	114	N	O2PG	152	28.63
13	GLY	-	1	GTP	114	N	O1PG	146	13.53	13	GLY	-	1	GTP	114	N	O1PB	179	33.84	13	GLY	-	1	GTP	114	N	O3RG	168	31.64
13	GLY	-	1	GTP	114	N	O2PG	74	6.86	13	GLY	-	1	GTP	114	N	O3PB	14	2.65	14	VAL	-	1	GTP	119	N	O1PB	12	2.26
13	GLY	-	1	GTP	114	N	O3PG	172	15.94	13	GLY	-	1	GTP	114	N	O1PG	23	4.35	14	VAL	-	1	GTP	119	N	O1PG	141	26.55
14	VAL	-	1	GTP	119	N	O1PB	112	10.38	13	GLY	-	1	GTP	114	N	O2PG	21	3.97	14	VAL	-	1	GTP	119	N	O2PG	6	1.13
14	VAL	-	1	GTP	119	N	O1PG	20	1.85	13	GLY	-	1	GTP	114	N	O3PG	11	2.08	14	VAL	-	1	GTP	119	N	O3RG	2	0.38
14	VAL	-	1	GTP	119	N	O2PG	9	0.83	14	VAL	-	1	GTP	119	N	O1PA	15	2.84	15	GLY	-	1	GTP	127	N	O3PA	15	2.82
14	VAL	-	1	GTP	119	N	O3PG	12	1.11	14	VAL	-	1	GTP	119	N	O2PA	1	0.19	15	GLY	-	1	GTP	127	N	O1PB	240	45.2
15	GLY	-	1	GTP	127	N	O1PA	3	0.28	14	VAL	-	1	GTP	119	N	O1PB	4	0.76	15	GLY	-	1	GTP	127	N	O1PG	136	25.61
15	GLY	-	1	GTP	127	N	O3PA	78	7.23	14	VAL	-	1	GTP	119	N	O3PG	2	0.38	15	GLY	-	1	GTP	127	N	O3RG	7	1.32
15	GLY	-	1	GTP	127	N	O1PB	810	75.07	15	GLY	-	1	GTP	127	N	O5*	29	5.48	16	LYSH	-	1	GTP	132	N	O3PA	4	0.75
16	LYSH	-	1	GTP	132	N	O1PA	2	0.19	15	GLY	-	1	GTP	127	N	O1PA	17	3.21	16	LYSH	-	1	GTP	132	N	O1PB	444	83.62
16	LYSH	-	1	GTP	132	N	O3PA	10	0.93	15	GLY	-	1	GTP	127	N	O2PA	17	3.21	16	LYSH	-	1	GTP	132	N	O3PB	2	0.38
16	LYSH	-	1	GTP	132	N	O1PB	1049	97.22	15	GLY	-	1	GTP	127	N	O3PA	4	0.76	16	LYSH	-	1	GTP	132	N	O1RG	76	14.31
16	LYSH	-	1	GTP	132	N	O2PB	23	2.13	15	GLY	-	1	GTP	127	N	O1PB	34	6.43	16	LYSH	-	1	GTP	139	NZ	O1PB	7	1.32
16	LYSH	-	1	GTP	139	NZ	O1PB	17	1.58	15	GLY	-	1	GTP	127	N	O1PG	1	0.19	16	LYSH	-	1	GTP	139	NZ	O3PB	3	0.56
16	LYSH	-	1	GTP	139	NZ	O3PB	1	0.09	16	LYSH	-	1	GTP	132	N	O5*	3	0.57	16	LYSH	-	1	GTP	139	NZ	O1PG	27	5.08
16	LYSH	-	1	GTP	139	NZ	O1PG	6	0.56	16	LYSH	-	1	GTP	132	N	O1PA	129	24.39	16	LYSH	-	1	GTP	139	NZ	O2PG	23	4.33
16	LYSH	-	1	GTP	139	NZ	O2PG	12	1.11	16	LYSH	-	1	GTP	132	N	O2PA	19	3.59	16	LYSH	-	1	GTP	139	NZ	O3RG	1	0.19
16	LYSH	-	1	GTP	139	NZ	O1PB	11	1.02	16	LYSH	-	1	GTP	132	N	O3PA	131	24.76	16	LYSH	-	1	GTP	139	NZ	O1PB	5	0.94
16	LYSH	-	1	GTP	139	NZ	O3PB	1	0.09	16	LYSH	-	1	GTP	132	N	O1PB	124	23.44	16	LYSH	-	1	GTP	139	NZ	O3PB	4	0.75
16	LYSH	-	1	GTP	139	NZ	O1PG	7	0.65	16	LYSH	-	1	GTP	132	N	O2PB	1	0.19	16	LYSH	-	1	GTP	139	NZ	O1PG	20	3.77
16	LYSH	-	1	GTP	139	NZ	O2PG	4	0.37	16	LYSH	-	1	GTP	139	NZ	O1PB	10	1.89	16	LYSH	-	1	GTP	139	NZ	O2PG	18	3.39
16	LYSH	-	1	GTP	139	NZ	O3PG	1	0.09	16	LYSH	-	1	GTP	139	NZ	O3PB	1	0.19	16	LYSH	-	1	GTP	139	NZ	O3RG	2	0.38
16	LYSH	-	1	GTP	139	NZ	O1PB	24	2.22	16	LYSH	-	1	GTP	139	NZ	O1PG	6	1.13	16	LYSH	-	1	GTP	139	NZ	O3PB	6	1.13
16	LYSH	-	1	GTP	139	NZ	O3PB	5	0.46	16	LYSH	-	1	GTP	139	NZ	O2PG	3	0.57	16	LYSH	-	1	GTP	139	NZ	O1PG	17	3.2
16	LYSH	-	1	GTP	139	NZ	O1PG	4	0.37	16	LYSH	-	1	GTP	139	NZ	O1PA	3	0.57	16	LYSH	-	1	GTP	139	NZ	O2PG	30	5.65
16	LYSH	-	1	GTP	139	NZ	O3PG	3	0.28	16	LYSH	-	1	GTP	139	NZ	O1PB	10	1.89	16	LYSH	-	1	GTP	139	NZ	O3PG	1	0.19
17	SER	-	1	GTP	145	N	O1PA	730	67.66	16	LYSH	-	1	GTP	139	NZ	O1PG	4	0.76	17	SER	-	1	GTP	145	N	O1PA	351	66.1
17	SER	-	1	GTP	145	N	O3PA	44	4.08	16	LYSH	-	1	GTP	139	NZ	O2PG	2	0.38	17	SER	-	1	GTP	145	N	O1PB	148	27.87
17	SER	-	1	GTP	145	N	O2PB	205	19	16	LYSH	-	1	GTP	139	NZ	O3PG	2	0.38	17	SER	-	1	GTP	145	N	O2PB	3	0.56
17	SER	-	1	GTP	149	OC	O1PA	158	14.64	16	LYSH	-	1	GTP	139	NZ	O1PB	4	0.76	17	SER	-	1	GTP	149	OC	O1PA	253	47.65
17	SER	-	1	GTP	149	OC	O2PA	870	80.63	16	LYSH	-	1	GTP	139	NZ	O1PG	3	0.57	17	SER	-	1	GTP	149	OC	O2PA	283	53.3
17	SER	-	1	GTP	149	OC	O3PA	1	0.09	16	LYSH	-	1	GTP	139	NZ	O2PG	1	0.19	17	SER	-	1	GTP	149	OC	O1PB	1	0.19
17	SER	-	1	GTP	149	OC	O2PB	61	5.65	16	LYSH	-	1	GTP	139	NZ	O3PG	2	0.38	18	ALA	-	1	GTP	153	N	O1PA	7	1.32
18	ALA	-	1	GTP	153	N	O1PA	451	41.8	17	SER	-	1	GTP	145	N	O1PA	294	55.58	28	PHE	-	1	GTP	262	CE	O6	1	0.19
28	PHE	-	1	GTP	262	CE	N7	3	0.28	17	SER	-	1	GTP	145	N	O2PA	70	13.23	28	PHE	-	1	GTP	262	CE	N7	2	0.38
28	PHE	-	1	GTP	264	CE	O2*	7	0.65	17	SER	-	1	GTP	145	N	O3PA	43	8.13	28	PHE	-	1	GTP	264	CE	O2*	1	0.19
28	PHE	-	1	GTP	266	CZ	N3	8	0.74	17	SER	-	1	GTP	145	N	O2PB	7	1.32	28	PHE	-	1	GTP	266	CZ	N9	1	0.19
28	PHE	-	1	GTP	266	CZ	N7	6	0.56	17	SER	-	1	GTP	149	OC	O1PA	176	33.27	28	PHE	-	1	GTP	266	CZ	O2*	18	3.39
28	PHE	-	1	GTP	266	CZ	O2*	49	4.54	17	SER	-	1	GTP	149	OC	O2PA	301	56.9	32	TYR	-	1	GTP	297	N	O2*	10	1.88
28	PHE	-	1	GTP	266	CZ	O3*	1	0.09	17	SER	-	1	GTP	149	OC	O1PG	2	0.38	32	TYR	-	1	GTP	297	N	O3*	4	0.75
32	TYR	-	1	GTP	297	N	O2*	5	0.46	17	SER	-	1	GTP	149	OC	O3PG	2	0.38	32	TYR	-	1	GTP	304	CD	O1PA	4	0.75
32	TYR	-	1	GTP	304	CD	O5*	3	0.28	18	ALA	-	1	GTP	153	N	O1PA	38	7.18	32	TYR	-	1	GTP	304	CD	O2PA	72	13.56
32	TYR	-	1	GTP	304	CD	O2PA	18	1.67	18	ALA	-	1	GTP	153	N	O2PA	1	0.19	58	THR	-	1	GTP	560	OG	O2PB	10	1.88
32	TYR	-	1	GTP	304	CD	O3PG	1	0.09	28	PHE	-	1	GTP	262	CE	O6	1	0.19	60	GLY	-	1	GTP	571	N	O2PA	5	0.94
32	TYR	-	1	GTP	308	CE	O2PA	4	0.37	28	PHE	-	1	GTP	262	CE	N7	5	0.95	60	GLY	-	1	GTP	571	N	O3PB	5	0.94
60	GLY	-	1	GTP	571	N	O1PG	167	15.48	28	PHE	-	1	GTP	264	CE	N7	1	0.19	60	GLY	-	1	GTP	571	N	O1PG	7	1.32
60	GLY	-	1	GTP	571	N	O2PG	22	2.04	28	PHE	-	1	GTP	266	CZ	O2*	6	1.13	60	GLY	-	1	GTP	571	N	O2PG	1	0.19
60	GLY	-	1	GTP	571	N	O3PG	11	1.02	32	TYR	-	1	GTP	297	N	O3*	1	0.19	60	GLY	-	1	GTP	571	N	O3PG	2	0.38
116	ASN	-	1	GTP	1172	NC	O6	29	2.69	32	TYR	-	1	GTP	304	CD	O1PA	5	0.95	116	ASN	-	1	GTP	1172	NC	O6	1	0.19
116	ASN	-	1	GTP	1172	NC	N7	280	25.95	32	TYR	-	1	GTP	304	CD	O2PA	1	0.19	116	ASN	-	1	GTP	1172	NC	N7	333	62.71
117	LYSH	-	1	GTP	1177	N	O6	12	1.11	32	TYR	-	1	GTP	304	CD	O3PG	1	0.19	116	ASN	-	1	GTP	1172	NC	O4*	10	1.88
117	LYSH	-	1	GTP	1184	NZ	O4*	8	0.74	58	THR	-	1	GTP	560	OG	O1PB	15	2.84	117	LYSH	-	1	GTP	1177	N</			