# Selection of RNA Aptamers to Distinguish the V600E Mutation Status of BRAF Protein: A Potential *in silico* Approach

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***Abstract:*** *The valine to glutamate substitution at the 600th residue of B-type rapidly accelerated fibrosarcoma protein (BRAF V600E) is the most common mutation in the BRAF gene. Due to its high prevalence in a number of cancers, development of efficient diagnostic and prognostic assays and therapeutics is essential for their management. Aptamers have become promising candidates in a variety of biomedical applications due to many favourable properties. However, no aptamers have been experimentally determined that can distinguish the V600E mutation status of the BRAF protein. Therefore, this study was conducted to create an initial knowledge-base for in silico design of aptamers for wild-type and mutant (V600E) BRAF (mutant BRAF) proteins. It was achieved using molecular docking employing HADDOCK 2.4 web server. In the absence of aptamers for BRAF, five RNA aptamers targeted to the activation loop of ERK 1&2 proteins were selected for docking, considering the similarity of the 3D structure of the kinase domains of the above proteins to BRAF. Docking was done for ten protein-aptamer combinations (five aptamers with wild-type BRAF and mutant BRAF). Three complexes were selected based on the HADDOCK score and their intermolecular hydrogen bonds and salt bridges were determined. Three aptamers obtained negative HADDOCK scores signifying they presumably target the activation loop of wild-type and mutant BRAF. Considering the total of intermolecular hydrogen bonds and salt bridges, Aptamers_1 and 3 (Apta-Index IDs: 481 and 263) would preferably bind with wild-type and mutant BRAF, respectively. They have a potential to be used as starting structures in the in-silico aptamer modeling workflow for wild-type and mutant BRAF proteins.*

***Keywords:*** *Aptamers, BRAF V600E, Hydrogen bonds, Molecular docking, Salt bridges*

## 1. Introduction

B-type rapidly accelerated fibrosarcoma (BRAF) protein is a Ser/Thr kinase involved in the extracellular signal-regulated kinase (ERK)/ mitogen activated protein kinase (MAPK) signaling pathway, which plays a crucial role in cell proliferation and differentiation under both normal and pathological conditions (Guo et al., 2020). BRAF protein consists of three conserved regions. Two of them are regulatory domains and the other is a catalytic protein kinase domain (Roskoski, 2012). BRAF is activated at the protein level by amino acid (AA) variations in the kinase domain, as a result of various mutations leading to a variety of cancers (Hussain et al., 2015). The BRAF V600E mutation, which results in substitution of glutamic acid for valine, located at the activation loop is the most common among all BRAF mutations (Cohen et al., 2003). This mutation accounts for 90% of BRAF mutations with a high prevalence in metastatic melanoma, papillary thyroid carcinoma, colorectal cancer and serous ovarian cancer (Hussain et al., 2015). Development of efficient diagnostic and prognostic assays, imaging technologies and therapeutics is crucial for precise management of these malignancies.

Aptamers are potential candidates in a variety of biomedical applications such as, bio sensing probes, diagnostic and therapeutic agents, drug discovery and as targeting molecules in drug delivery systems (Chandola et al., 2016; Emami et al., 2020). They are short, single stranded, artificial nucleic acid (DNA/RNA) or peptide sequences, which can bind to their specific targets with high affinity and specificity due to their 3D structures. Hence, they are considered analogous to antibodies (Emami et al., 2020; Buglak et al., 2020). Aptamers interact with their targets *via* various intermolecular interactions such as, electrostatic interactions, van der Waal's forces, hydrogen bonds, 3D shape and stacking. Moreover, they can fold in an array of secondary and tertiary structural elements including stem loops, kinks, pseudoknots and buldges, which aid in the formation of multiple target binding sites (Chandola et al., 2016).

Aptamers have some key advantages over antibodies. Unlike antibodies, aptamers can withstand extremely high or low temperatures and pH ranges. This makes possible to select aptamers under non-physiological conditions and makes them suitable for applications performed under harsh conditions. They can be selected using an *in vitro* process by screening against an artificial oligonucleotide library, while antibodies need cell lines or animals for selection (Gonzalez et al., 2016). Large amounts of highly pure nucleic acid aptamers can be produced using the polymerase chain reaction, which is relatively inexpensive than the production of antibodies (Li et al., 2014). Further, they can be synthesized with minimum batch-to-batch variations. They are about tenfold smaller than antibodies, which makes them easier to be synthesized in large quantities and modified with a wide range of chemical groups. Despite their smaller size, aptamers can form complex, folded tertiary structures, with recognition surface areas even greater than antibodies. All these properties make aptamers prospective and complementary to antibodies for biomedical applications.

The conventional *in vitro* process for selection of aptamers against targets is termed Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Tuerk and Gold, 1990). This process requires repetitive rounds of selection and amplification thus, is time and labour consuming, have a low cost efficiency rate and often fails to generate aptamers with high affinity (Emami et al., 2020; Buglak et al., 2020). To overcome the problems associated with SELEX, several computational methods in aptamer sciences have been developed in the recent years (Emami *et al*., 2020). These techniques, combined with different branches of technologies have drawn significant attention in aptamer scientists as they are simple, time and cost effective and do not require sophisticated instrumentation (Ahirwar et al., 2016).

Computational methods, namely, docking and molecular dynamics (MD), have been introduced as an alternate to SELEX to design aptamers against targets ranging from small molecules to complex biopolymers like proteins. This *in silico* approach can be accompanied with SELEX and high throughput sequencing to improve efficacy of aptamer research. The main advantage of using molecular modeling methods over SELEX is that it is possible to find new aptamers with better affinity and specificity to the target and also to identify structural patterns responsible for aptamer-target interactions (Emami et al., 2020; Buglak et al., 2020). The typical modeling workflow for *in silico* design and optimization of an aptamer for a target is described in the review by Buglak et al. (2020).

Experimentally determined aptamers for a particular target are required as the starting structures for the *in silico* aptamer design workflow. To the best of our knowledge, no aptamers have been experimentally determined for wild-type and mutant BRAF proteins using an *in vitro* process so far. Yet, aptamers for other Ser/Thr kinases such as, ERK 1 &2, have been experimentally determined and available in

aptamer databases. The kinase domains of BRAF and ERK 1&2 have structurally similar subdomains (conserved fold) despite the dissimilarity in their AA sequences and catalyze the same reaction (Kobe and Kemp, 2003).

As an initial approach, an attempt was made in this study to find out whether the aptamers targeted to the activation loop of ERK 1&2 and the dual phosphorylated form of ERK2 (ppERK2) have an ability to target the activation loops of wild-type and mutant BRAF proteins, using molecular docking. To the best of our knowledge, this is a pioneering study, which aims to deduce the binding ability of RNA aptamers to the BRAF protein, which were designed for another protein kinase, assuming the similarity in their structures. This paper introduces an approach to select possible starting structures of aptamers for the *in silico* aptamer modeling workflow to obtain aptamers with high affinity and specificity to wild-type and mutant BRAF proteins, where experimentally determined aptamers are not available. Further, important concerns on designing computational studies and subsequent analysis of their results are highlighted.

## 2. Methodology

*A. Retrieval and preparation of protein structures for docking*

The crystal structures of the kinase domain of WT BRAF and mutant BRAF proteins were retrieved from the RCSB PDB database; <https://www.rcsb.org> (RCSB PDB, 2021). The following criteria were adopted to select protein structures; structures with a resolution better than 3 Å, more than 50% ligand structure quality (goodness of fit), minimum missing residues at the activation loop and the presence of the V600E point mutation site at the activation loop. One crystal structure, which best satisfied the selection criteria was chosen for each protein and the respective PDB files were downloaded (PDB IDs: 5VAM, 5JRQ). The protein structures were cleaned for docking by removing undesired chains and ligands using UCSF Chimera version 1.15 (Pettersen et al., 2004).

*B. Modeling the missing segments, refining and external validation of protein models*

The missing segments at the activation loop of 5VAM and 5JRQ structures were modeled using MODELLER ver. 10.1 (Sali and Blundell, 1993). They were further refined to obtain more reliable structures for docking using ISOLDE ver. 1.2.0 (Croll, 2018) and directed to external validation

Table 1. The selected aptamers for docking from Apta-Index database

| Aptamer | Name | Target | Reference |
|---|---|---|---|
| Aptamer_1 | ERK1/ ERK2 (Family II - Truncated) (ID# 481) | ERK 1 and ERK2 | Seiwert et al. (2000) |
| Aptamer_2 | Unphosphorylated ERK2 (ID# 264) | Unphosphorylated ERK2 | Vaish et al. (2002) |
| Aptamer_3 | Phosphorylated ERK2 (ID# 263) | Phosphorylated ERK2 | Vaish et al. (2002) |
| Aptamer_4 | ERK 1/ ERK2 (Family II) (ID# 143) | ERK 1 and ERK2 | Seiwert et al. (2000) |
| Aptamer_5 | ppERK2/ERK2 (ID# 73) | ppERK2/ERK2 | Seiwert, et al. (2000) |

ERK1- extracellular signal- regulated kinase 1, ERK2- extracellular signal-regulated kinase 2, ppERK2 - dual phosphorylated extracellular signal- regulated kinase 2

using PROCHECK, ERRAT and Verify3D; <https://saves.mbi.ucla.edu> (SAVESv6.0, 2021).

*C. Retrieval and modification of aptamer sequences*

The typical workflow for *in silico* modeling of aptamers requires aptamer sequences selected *in vitro* for the particular target. The relevant sequences can be retrieved from the Apta-Index database by Aptagen; <https://www.aptagen.com> (Aptagen, 2021), which is the only database of aptamer-target interactions currently available, with related publications (Emami et al., 2020). Aptamers for BRAF were searched in Apta-Index database using the appropriate search parameters. In the absence of aptamers for BRAF, the database containing 347 entries was manually searched for aptamers targeted to the kinase domain of protein kinases other than BRAF. Aptamers targeted to the kinase domain of Ser/Thr kinases were selected as BRAF belongs to the same category. Accordingly, five entries of RNA aptamers targeted to the kinase domain of ERK 1& 2 were selected and their sequences were retrieved. The targeted fragment of the protein by the aptamers was obtained

*D. Secondary (2D) and tertiary (3D) structure prediction and optimization of RNA aptamers*

Since crystal structures of the aptamer-target complexes were not available in the RCSB PDB or any other database, the 2D and 3D structures of the aptamers were predicted using web servers. The 2D structures were predicted using the

RNAfold web server; http://rna.tbi.univie.ac.at/RNAWebSuite/help (RNAfold, 2021) and minimum free energy, optimal secondary structures were obtained. The 3D structures of the aptamers were predicted using the 3dRNA v2.0 web server; <http://biophy.hust.edu.cn/new/3dRNA> (Wang, 2021). Predicted, energy minimized 3D model with the lowest 3dRNA score for each aptamer was selected for docking and the relevant PDB files were downloaded.

*E. Molecular docking and analysis of intermolecular interactions*

Docking was performed for 10 combinations of protein- aptamer complexes comprised of the five aptamers selected and WT BRAF and mutant BRAF proteins using the EASY interface of the HADDOCK 2.4 web server; https://wenmr.science.uu.nl/haddock2.4/ (HADDOCK, 2021). HADDOCK is an information-driven flexible docking approach for the modeling of biomolecular complexes. It uses experimentally or bioinformatically from the relevant publication for the particular entry. Details of these aptamers are listed in Table 1.

Two of the five entries are allosteric ribozymes activated either by the unphosphorylated or phosphorylated forms of ERK2, which contain an ERK2 binding domain, attenuated stem structure and a hammerhead catalytic motif (Vaish et al., 2002). The hammerhead catalytic motif sequences were removed as they are not responsible for binding to the target protein and its sequence complementarity to the attenuated stem structure, which can cause unnecessary structure formation.

available interaction information to predict minimal energy docked conformations (Ahirwar et al., 2016). Docking was performed using default parameters of the EASY interface of HADDOCK, since this is an elementary experiment. Some of the default parameters in the software were automatically changed to optimum values when the server identified nucleic acids in theinput https://wenmr.science.uu.nl/haddock2.4/ (HADDOCK, 2021).

According to literature, these aptamers are targeted to the activation loop of ERK 1 &2 and ppERK2 protein kinases (Seiwert et al., 2000; Vaish et al., 2002). Therefore, the AAs at the activation loop were given as active residues in HADDOCK for proteins. Regarding aptamers, all nucleotides were defined as active residues. The

PDB files of the best clusters ranked first by HADDOCK were downloaded for further analyses.

The H-Bonds tool of UCSF ChimeraX 1.2.5 was used for further analysis of the binding of three selected aptamers to proteins in each model, by determining the number of intermolecular hydrogen bonds (H bonds) and salt bridges. The analysis was performed using default parameters and thresholds given in the software.

## 3. Results and Discussion

### *A. Details of the best clusters of docked protein-aptamer complexes*

The average values of the HADDOCK score (H_score) and its contributing energy components and structural features of the best cluster for each docking are listed in Table 2. The predicted binding ability of the selected aptamers with WT and mutant BRAF was deduced. The HADDOCK scoring function has been successful in selecting near-native docking poses in a variety of cases (Kastritis et al., 2014). A negative H_score of a complex can be considered as an indication of favourable binding of two molecules according to defined restraints, while a positive H_score indicates the binding is not favourable (Rodrigues et al., 2020). In this study, negative H_scores were resulted for the docked complexes of both WT and mutant BRAF with Aptamers, 1, 2 and 3 and for WT BRAF with Aptamer 5 (Table 2). According to this result, these aptamers presumably target the activation loop of the WT and mutant BRAF proteins. This was a positive finding to proceed with more thorough analysis of binding. Accuracy of the docking results can be guaranteed since both protein structures met all external validation quality criteria given in Table 3 after modeling and further refinement.

### *B. Intermolecular interactions of the selected docked complexes*

It is not recommended to use the H_score to compare binding affinities of docked complexes (Vries et al., 2010). Intermolecular electrostatic energy (Eelec) is recognized as the most discriminatory energy term, which contributes to the H_score among others. In addition, buried surface area (BSA) also correlates strongly with the H_score (Rodrigues et al., 2020). Hydrogen bonds and salt bridges are important electrostatic interactions, which contribute substantially to the free energy of a bound complex.

In this study, the number of H bonds and salt bridges formed between Aptamers, 1, 2 and 3 and WT and mutant BRAF proteins were determined. Aptamer_1 formed 16 H bonds and 3 salt bridges with WT BRAF where, only 8 H bonds were formed with mutant BRAF. Therefore, Aptamer_1 can be considered to exhibit more stable binding with WT BRAF compared to mutant. Aptamer_2 formed 14 H bonds with WT BRAF and 10 H bonds and 5 salt bridges with the mutant. Therefore, the two proteins do not show a marked advantage over each other when binding with Aptamer_2. Considering the fact that salt bridges are stronger interactions than regular H bonds, the presence of 5 salt bridges in the complex of Aptamer_2 and mutant BRAF, may cause better binding of the two molecules. Aptamer_3 formed 9 H bonds and 1 salt bridge with WT BRAF and 7 H bonds and 7 salt bridges with the mutant. This result suggests that Aptamer_3 could bind more stably with mutant BRAF compared to WT. Accordingly,

Aptamer_1 and Aptamer_3 can be considered as possible starting structures for the *in silico* protocol for modeling aptamers specific to WT and mutant BRAF proteins, respectively.

Table 2. Average HADDOCK score, individual energy terms (Evdw- van der Waals energy, Eelec- electrostatics energy, Edesol- desolvation energy, Eair- restraints violation energy) and BSA (buried surface area) of the docked complexes

| Docked complex | HADDOCK score (a.u.) | Evdw (kcal/mol) | Eelec (kcal/mol) | Edesol (kcal/mol) | BSA (Å²) | Eair (kcal/mol) |
|---|---|---|---|---|---|---|
| P1_Apt1 | -98.7 | -99.8 | -521.4 | 34.0 | 3084.2 | 714.6 |
| P2_Apt1 | -52.8 | -91.5 | -230.6 | 7.3 | 2369.6 | 774.7 |
| P1_Apt2 | -64.7 | -83.8 | -315.5 | 22.5 | 2445.8 | 597.1 |
| P2_Apt2 | -70.5 | -68.7 | -404.7 | 6.6 | 2003.2 | 725.1 |
| P1_Apt3 | -16.0 | -69.6 | -377.7 | 23.7 | 2170.2 | 1054.3 |
| P2_Apt3 | -10.1 | -70.8 | -359.5 | 11.9 | 2164.3 | 1206.8 |
| P1_Apt4 | 127.0 | -68.8 | -420.8 | 30.6 | 2499.9 | 2493.2 |
| P2_Apt4 | 120.4 | -85.8 | -336.6 | 33.3 | 2712.7 | 2401.9 |
| P1_Apt5 | -7.9 | -128.8 | -665.9 | 42.6 | 3925.7 | 2115.5 |
| P2_Apt5 | 39.8 | -95.2 | -475.2 | 21.4 | 3213.4 | 2087.5 |

P1- wild-type BRAF, P2- mutant BRAF

Table 3. External validation results of refined protein models

| Refined protein model | PROCHECK-Ramachandran analysis | | | | | ERRAT | Verify3D |
|---|---|---|---|---|---|---|---|
| | Core % | Allow % | General % | Disallow % | Overall G factor | Overall quality factor | Score (%) |
| Wild-type BRAF | 90 | 9.5 | 0.4 | 0 | -0.02 | 99.2 | 100, Pass |
| Mutant BRAF | 90 | 9.5 | 0.4 | 0 | -0.12 | 98.3 | 99, Pass |

The term buried surface area (BSA) defines the surface area buried upon binding of aptamers with proteins; higher the BSA, the greater the biding ability. A higher BSA would increase the number of interactions at the interface, including electrostatic, van der Waals and hydrophobic interactions, leading to a well-established, stable binding. The docked complexes between WT BRAF and Aptamer_1 and mutant BRAF and Aptamer_1, resulted in BSAs of 3084.2 $Å^2$ and 2369.6 $Å^2$, respectively (Table 2). This suggests Aptamer_1 has an ability to form a more stable binding with WT BRAF than with the mutant. Hence, it agrees with the former result obtained for Aptamer_1 through analysis of H bonds and salt bridges. The docked complexes between WT BRAF and Aptamer_3 and mutant BRAF and Aptamer_3, resulted in BSAs of 2170.2 $Å^2$ and 2164.3$Å^2$, respectively (Table 2). As the values for BSA are very much similar, BSA cannot be used as a feature to distinguish binding of Aptamer_3 with the two proteins.

*C. Limitations and future perspectives*

X-ray crystallography structures of the aptamers used in the study were not available in the RCSB PDB or any other database, and therefore their 2D and 3D structures were predicted. These structure prediction software for aptamers are relatively new bioinformatics tools and the field of *de novo* aptamer designing is still in its infancy. Hence, external validation of predicted models was not possible as validation methods are not available.

This study used only the HADDOCK 2.4 web server for docking. The reliability and reproducibility of the results need to be confirmed by performing docking with other software that uses different docking algorithms. In the present study, the H bonds, salt bridges and BSA analyzed, only correspond to just one best pose of the docked complexes given by HADDOCK. Although, molecular docking provides valuable information regarding the interactions between the proteins and aptamers, the stability and

consistence of these bonds cannot be guaranteed. In order to obtain that information, the dynamic properties of the interacting complexes need to be studied real time. Therefore, it is suggested to perform MD simulations for more accurate analysis of bond formation and binding free energy calculation. Further, careful interpretation and validation of results in computational predictions are essential when designing aptamers that specifically bind to a particular protein.

**4. Conclusion**

Aptamers, 1, 2 and 3 revealed negative H_scores when docked with both WT and mutant BRAF proteins. This suggests these aptamers presumably target the activation loop of the WT and mutant BRAF, too. Consequently, Aptamers 1 and 3 can be regarded as starting structures for the *in silico* aptamer modeling workflow for WT BRAF and mutant BRAF, respectively. This study provides a basic plan for modeling interactions between WT and mutant BRAF with aptamers designed for another protein kinase (ERK2), which exhibit similar structural domains. The knowledge gathered will be of immense importance for future studies on *in silico* design of aptamers with high affinity, specificity and stability for WT and mutant BRAF proteins.

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