Peptide inhibition of the mTORC1 signaling: A trend in Cancer Therapeutics

Ashok Tiwari
Jaypee Institute of Information Technology
Anuj Kumar
ICMR-National Institute of Cancer Prevention and Research
Rachana R (rachana.dr@iitbombay.org)
Jaypee Institute of Information Technology

Research Article

Keywords: mTOR signaling, RHEB, HADDOCK, Docking, mTORC1 complex.

Posted Date: April 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1548859/v1

Abstract

Cancer is the second most leading cause of deaths in the world and has been manifested by various hallmarks including uncontrolled cell proliferation, increased cell survival, abnormal angiogenesis, eluding of antitumor immunity and acquisition of metabolic processes unique to cancers. Interestingly, mTOR signaling is found to be associated with all of these hallmarks which makes it the most appropriate choice for target therapies against cancer. Inhibition of the target protein by peptides designed by using the interfacial amino acids of its interacting partner is one of the approaches of target therapies. The same approach has been engaged here to inhibit association of the mTOR kinase and RHEB. The sequential amino acids of the mTOR kinase were utilized to design three different native peptides which were further modified by incorporating evolutionary changes as the mutations. All the peptides were tested for their affinity with the RHEB by performing protein-peptide docking on the HADDOCK 2.4 docking interface and the binding energy calculation on the FoldX5.0 interface. Also, the interactions were analyzed using the ligplot^{+} tool. The peptide H2P1 and H3P1 showed good binding energies (-14.28 kcal/mol and − 14.53 kcal/mol, respectively) in comparison to the entire mTOR-RHEB complex (-15.83 kcal/mol). This was also supported by a good number of Hydrogens bonds and other positive interactions. All these evidences support their potency to interrupt the mTORC1 signaling which can be further established based on these shreds of evidences.

Introduction

Cancer is amongst the topmost causes of deaths in the entire world as estimated by the World Health Organization (WHO). Both of its morbidity and mortality incidences are increasing at an alarming pace [Sung et al. 2021]. Prostate, Breast and Lung cancer are at the main focus point among all types of cancers [Hassanpour and Dehghani 2017]. Cancer generally refers to the hyperproliferation and uncontrolled growth of the cells which leads to tumor formation and ultimately leading to metastasis. The stimulus from the carcinogenesis associated risk factors work as the driving force for the progression of cancerous conditions [Sun et al. 2017]. Although there have been various mechanisms of cancer progression and pathogenesis, hyperactivation of some cell signaling pathways like: the Ras/Raf pathway, PI3K/AKT pathway and mammalian Target Of Rapamycin (mTOR) pathway are amongst the most discussed ones.

The mTOR pathway is a central pathway that interconnects several signaling pathways (**Fig-1**). The mTOR, a serine/threonine-protein kinase, is the central component of this pathway and forms two distinct catalytic complexes i.e. mTOR Complex1 (mTORC1) and mTORC2 along with other components.The mTORC1 constitutes mTOR kinase, RAPTOR (regulatory associated protein of mTOR) - a scaffolding protein that assembles the mTORC1 complex, a negative regulator, PRAS40, mLST8(mammalian lethal with sec-13) and the negative regulator DEPTOR where as the mTORC2 complex consists of mTOR kinase, RICTOR (rapamycin-insensitive companion of mTOR) - scaffolding protein, PROTOR (protein observed with rictor 1 and 2), mSIN1(mammalian stress-activated map kinase-interacting protein 1), mLST8 and DEPTOR. The pathway is functionally involved in regulating cell survival, cell growth, cell

metabolism, protein synthesis, cell proliferation and autophagy, along with homeostasis. Both these complexes drive these biological functions distinctly [Ghosh et al. 2015]. Signaling factors including amino acids, oxygen levels, glucose, growth factors & hormones, cellular energy status (ATP) and stress regulates the mTOR pathway through activation and deactivation of transcription factors and other proteins via their receptors (see **Fig-1**) [Saxton and Sabatini 2017]. Primarily, three signaling cascades converge to activate the mTOR pathway. First, the growth factor regulated PI3K/AKT pathway inhibits the TSC1/2 complex which in turn inhibits the RHEB. Inhibition of RHEB leads to the activation of the mTOR pathway. Secondly, the low energy status stimulates the serine/threonine protein kinase STK11 which phosphorylates AMPK. The Phosphorylated AMPK leads to the TSC2 mediated inhibition of the mTOR. And the third way of the mTOR regulation is amino acid dependent. Here, GAP activity towards rags (GATOR1) complex (DEP domain-containing protein 5, DEPDC5; nitrogen permease regulator proteins NPRL2 and NPRL3) regulates mTOR in response to changes in levels of amino acids, especially leucine and arginine: when amino acids are low, GATOR1 inhibits mTOR signaling; when levels are normal, GATOR1 inhibition of mTOR is released [Crino 2016].

But, various studies have revealed the correlation between dysregulation of mTOR and cancer progression. The deregulation of the upstream regulators of the mTOR kinase results in hyperactivation of the mTOR pathway, ultimately leading to cancer. Majorly, the hyperactivation of mTORC1 has been observed in various cancer types. This hyperactivation commonly occurs due the Loss-of-Function mutations of the tumor suppressors like phosphatase and tensin homolog (*PTEN*), tuberous sclerosis 1/2 (*TSC1/2*), neurofibromin 1/2 (*NF1/2*). However some of the mutations like S2215Y and R2505P are characterized in the mTOR kinase itself [Grabiner et al. 2014; Kim et al. 2017].

The mTOR inhibitors like Rapamycin and rapalogs have been studied on various cancers. Rapamycin used as monotherapy is reported ineffective due to drug resistance. The combinatorial therapies of Rapamycin are the ones which are still under clinical and preclinical trials for various types of tumors. Hence, there is a strong need for a novel mTORC1 inhibitor which can solely inhibit this pathway and might be useful for the treatment of many types of cancers [Tian et al. 2019]. In the mTOR pathway when Rheb protein binds to mTOR protein, it gets activated and can lead to aggravation of carcinogenesis. Various small molecules have been tried to inhibit mTOR but they are found to be unable to disrupt the protein-protein interactions (PPIs) between mTOR and Rheb as they are unable to cover the large interfacial regions in PPIs. The other way of inhibiting mTOR could be that derivatives of Rheb proteins can interact and does not allow the real Rheb to interact and so inhibit the action of mTOR. In this way various peptides can be designed very easily which can have the capability to target the protein and so, peptide inhibitors have now become the novel choice for PPI inhibition. Peptides with 20–60 residues are the better choice as small peptides of this size can not maintain their secondary structures [Chowdhury et al. 2020; Pirogova et al. 2011].

In the present study, peptide inhibitors, based on amino acids present surface of Rheb protein and their evolutionary mutations, are designed against the mTOR-RHEB interface to potentially interrupt the interaction of the Rheb with the mTOR kinase for mTORC1 inactivation during cancerous conditions.

Methodology

The mTOR-RHEB interface and Binding affinity prediction:

The Cryo-EM structure of the RHEB activated mTORC1 (PDB Id: 6BCU) was retrieved from the Protein Data Bank (PDB) and used for mTOR kinase-RHEB interface prediction and Protein-Protein Docking. As stated above, the mTORC1 complex has mTOR kinase, RAPTOR, PRAS40, mLST8 and DEPTOR as its constituent proteins, of which mTOR kinase is a homodimer (Chain A&B in 6BCU). The mTOR kinase has three domains i.e N-Heat, M-Heat and FAT domain all of which interact with the RHEB (Chain R&S in 6BCU, It is also a homodimer) for activation of mTORC1 cascade. The interacting amino acids of the interfacial region of the mTOR kinase (Chain A:6BCU) and the RHEB (Chain S: 6BCU) were identified using the Ligplot + suit. The Ligplot + suit uses an updated version of the original DIMPLOT for protein-protein interface (PPI) prediction [Laskowski and Swindells 2011].

Similarly, the chain A&S of 6BCU were also used for the prediction of the free energy change (ΔG) of the mTOR-RHEB complex using FoldX5.0 and thus, the dissociation constant (K_d). The Foldx uses various linear combinations of empirical terms for free energy change (ΔG) calculation. It relies on the terms as depicted in the equation given below.

ΔG = a·ΔGvdw + b·ΔGsolvH + c·ΔGsolvP + d·ΔGwb + e·ΔGhbond + f·ΔGel + g·ΔGkno + h·TΔSmc + k·TΔSsc + l·TΔSclash

In this equation, the initial alphabetical characters before each energy term signifies its weight in free energy change (ΔG) calculation.

Based on the energy predicted by the equation given above, the dissociation constant of the reaction can be evaluated using-

$\Delta G = RT \ln K_d$

where R is the ideal gas constant (in kcal $K^{-1}mol^{-1}$), T is the temperature (in K) and ΔG is the predicted free energy. The temperature was considered to be 310.15 K, as the normal body temperature is nearly 310.15K.

Mutations retrieval and the Evolutionary changes identification in the mTOR kinase

Mutation retrieval in mTOR kinase

The natural and the experimental mutations in the mTOR and the RHEB were listed from the literature survey and Uniprot database but none of these mutations were found to be present on the interfacial

regions [**Fig. 5 (A**)] of either of the proteins. Hence, they were not of much significance for the peptide designing process hence were not incorporated into “designed peptides”.

Identification of evolutionary changes in the mTOR kinase

The multiple sequence alignment was performed using MEGA-X (Molecular Evolutionary Genetics Analysis X) [Kumar et al. 2018]. The FASTA sequences of the mTOR kinases from 50 vertebrates (selected randomly from each class) were retrieved from the Uniprot database and were used for multiple sequence alignment on MEGA-X. Two algorithms Clustal-W or MUSCLE are available on MEGA-X for the evolutionary analyses but this work was performed on the Clustal-W platform. The evolutionary changes, as observed in the residues from the interacting regions of the N-Heat domain of the mTOR kinase in different vertebrates, are summarized in **Table-1.** Also, the closeness of the vertebrates with the human was analyzed by drawing a phylogeny tree on the MEGA-X platform.

Peptide designing:

H1P0, H2P0 and H3P0 were designed by using the residues from interacting regions of the N-Heat domain of mTOR kinase. The residues 90I to 119D and the residues 130I to 160R from interacting regions of the N-Heat domain were used as the peptide H1P0 and H2P0, respectively while H3P0 was designed by using the residues 101A-154E which included some residues of H1P0 and H2P0. These peptides were further enhanced to design six modified peptides i.e. H1P1, H1P2, H2P1, H2P2, H3P1 and H3P2. Firstly, the peptides H1P1, H2P1 and H3P1 were designed by introducing all the evolutionary changes (found in the sequence regions of H1P0, H2P0 and H3P0) as mutations. Thereafter, the destructive mutations based on the change in their ΔG i.e. ddG/dΔG/ΔΔG were again replaced by their native amino acid residues from the peptides H1P1, H2P1 and H3P1 which resulted in the designing of the H1P2, H2P2 and the H3P2 peptides. The dΔG of each mutation was calculated with Position-Specific Scoring Matrix (PSSM) approach using the FoldX5.0 plugin and YASARA View tools. The mutations in the peptides H1P0, H2P0 and H3P0 for designing the rest of all peptides were produced using the tool UCSF Chimera 1.15rc [Pettersen et al. 2004] and also the mutated structures were locally minimized using the same tool.

Protein-Peptide Docking:

The peptide-protein docking was performed on HADDOCK 2.4 (*H*igh *A*mbiguity *D*riven biomolecular *DOCK*ing) webportal [Charlier et al. 2017] and the results of docking interface were further water refined on refinement interface of the HADDOCK 2.4. The free energy changes (ΔG) of the refined RHEB-peptide complexes were analyzed with the FoldX5.0 interface after minimizing the refined complexes [Vanhee, et al. 2011; Petukhov et al. 1999; Munoz and Serrano 1994; Abagyan and Totrov 1994; Vijayakumar et al. 1998; Schymkowitz et al. 2005; Baeten et al. 2008].

The HADDOCK server produced docking results in ten Clusters constituting almost 100 models of the RHEB-peptide complex each, Out of these, only four to five clusters with their best four models were depicted as results on the result panel. Also, out of these clusters, only the top two clusters with their best

four models were selected for further water refinement and energy calculation. So, eight models (four models of each of the top two clusters) of each peptide were water refined which resulted in four new refined models for each model. Only the top most model of these four refined models was selected for energy minimization and ΔG calculation. The ΔGs of all eight refined models were calculated and compared among themselves.

Results

The mTOR-RHEB Interface:

It was necessary to find out the interacting residues between the mTOR-kinase and the RHEB for peptide designing process and hence, the interactions between the mTOR and the RHEB were predicted by LigPlot + as recorded in **Fig-2.** The mTOR-RHEB interface is big enough, the interactions between the mTOR kinase and the RHEB are predominantly formed between the N-Heat domain of the mTOR kinase and the switch II (residue 63–79) of the RHEB. The RHEB shows eight hydrogen bond interactions with the mTOR kinase along with so many other interactions. Residues 69H, 101A, 106R, 109N, 112R and 154Q (all of them are from the N-Heat domain of the mTOR kinase) are involved in hydrogen bonding with residues 109K, 72G, 105D, 75S, 76I and 7R of the RHEB GTPase, respectively. Rest all residues form other kinds of interactions including salt bridges, hydrophobic interactions, pi-pi bonds etc. (Fig. 2).

The Evolutionary changes

Here, the term “evolutionary changes" signifies the change in the nature of residue at a specific position occurring due to evolution. This concept hypothesizes that as all the vertebrates evolved from a common ancestor, during the course of evolution, the changes in the amino acid sequence of the mTOR kinase of all these vertebrates might have occurred.

Hence, the evolutionary changes occurring in the Vertebrate mTOR kinase were identified. As mentioned earlier, the peptide enhancement process required knowledge about the evolutionary changes that occurred during the course of evolution. So, in total, 50 sequences of the mTOR kinase from vertebrates were aligned with the Human mTOR kinase. The evolutionary changes observed in the process were recorded and are briefly summarized in “**Table-1**” given below. Here, mutations with positive *dΔG* indicate the destructive mutations while the negative *dΔG* indicates the constructiveness of the mutations.

Table-1 The mutations of the mTOR Kinase in the interfacial region of mTOR and RHEB.

Residue	Changes	Description	dΔG kcal/mol
91A	**V**	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	0.0
98G	**A**	In *Phascolarctos cinereus*, *Vombatus ursinus*, Involved in interface with the RHEB.	0.0
100N	**Y**	In *Phascolarctos cinereus*, *Vombatus ursinus*, Involved in interface formation with RHEB	0.21
101A	**S**	Serine replaces the alanine at position 101 in *Mesocricetus auratus* (Golden Hamster). (Involved in interface with Rheb from N-Heat and also involved in direct interaction with switch II of RHEB)	0.25
101A	**V**	*Podarcis muralis* (Common Wall Lizard)	*-0.35*
105G	**S**	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	*-0.36*
118N	**S**	Serine replaces the arginine at position 118 in *Mesocricetus auratus* (Involved in interface with the RHEB from N-Heat)	0.0
130I	**M**	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	*0.0*
132R	**H**	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	*0.0*
134A	**S**	*Myripristis murdjan, Anabas testudineus, Lepisosteus oculatus, Sphaeramia orbicularis*	*0.05*
135M	**L**	*Gopherus agassizii*	*0.0*
136A	**T**	*Alligator sinensis*	*0.02*
140F	**C**	In *Vombatus ursinus* (Common Wombat), Involved in interface formation	0.30

Designed Peptides:

There are various types of peptide inhibitors that are being worked upon or are under the process such as: peptide inhibitors for Ras/NF-κB/c-Myc activation, MAP kinases and p53 functions for treating cancerous conditions. One of the 'rational' methods for designing peptides is using a portion of interacting domains from the protein-protein interface [Han and Král 2020].

In the present case the interface analysis of the mTOR-RHEB complex showed that in total eight hydrogen bonds are formed between the RHEB and the mTOR kinase which are solely formed by the N-Heat domain of the mTOR kinase. Also, most of the interfacial contacts are shown by the N-Heat domain of

the mTOR kinase with the RHEB (Fig. 2). Hence, the portions with residues 90I to 119D, residues 130I to 160R and the residues 101A to 154E of the N-Heat domain were selected as native peptides namely H1P0, H2P0 and H3P0, respectively (**Fig-3**). The modifications in these three native peptides resulted in the rest of the peptides and were named H1P1, H1P2, H2P1, H2P2, H3P1 and H3P2 (Refer to **Table-2**).

Table-2 The sequences of the designed peptides and their binding affinity.

S. No.	Peptide Sequence	Peptide length
1	IASLIGVEGGNATRIGRFANYLRNLLPSND	30
2	IVSLIGVEAGYSTRIGRFANYLRNLLPSSD	30
3	IVSLIGVEAGNVTRISRFANYLRNLLPSSD	30
4	IGRLAMAGDTFTAEYVEFEVKRALEWLGADR	31
5	MGHLSLTGDTFTAEYVEFEVKRALEWLGADR	31
6	MGHLALAGDTFTAEYVEFEVKRALEWLGADR	31
7	ATRIGRFANYLRNLLPSNDPVVMEMASKAIGRLAMAGDTFTAEYVEFEVKRALE	54
8	VTRISRFANYLRNLLPSSDPVVMEMASKAMGHLSLTGDTCTAEYVEFEVKRALE	54
9	VTRISRFANYLRNLLPSSDPVVMEMASKAMGHLALAGDTFTAEYVEFEVKRALE	54

RHEB-peptide Docking

When these newly prepared peptides i.e. H1P0, H1P1, H1P2, H2P0, H2P1, H2P2, H3P0, H3P1 and H3P2 were introduced for protein-peptide docking with the RHEB as receptor protein on the HADDOCK docking interface. The selective possible models of each RHEB-peptide complex were refined and then introduced to binding affinity analysis. After comparing all the models of each RHEB-peptide complex amongst themselves in terms of ΔG, the best RHEB-peptide model for each peptide was compared with the best models of other RHEB-peptide complexes. For example, amongst all models of RHEB-H1P0 complex, the model CL1.2 (model 2 of cluster 1) was the best, which was then compared with the best models of other RHEB-Peptide complexes i.e H1P1, H1P2 and so on. The results are summarized as below in table-3.

Table-3 The comparison of RHEB-Peptide complexes in terms of ΔG and interactions.

Set No.	Complex/Peptide	Lowest ΔG (kcal/mol)	H - Bonds	Total Interactions
	mTOR-RHEB	**-15.83**	**8**	**37**
1	**H1P0**	-9.34	8	18
	H1P1	-9.18	8	23
	H1P2	-10.21	7	22
2	**H2P0**	-9.50	4	21
	H2P1	-14.28	7	24
	H2P2	-9.18	5	21
3	**H3P0**	-12.17	8	22
	H3P1	-14.53	8	20
	H3P3	-13.25	11	23

The Peptide H2P1 and H3P1 show a better ΔG and a good number of bonding interactions between the peptides and the mTOR kinase. The ΔG for H3P1-mTOR and H2P1-mTOR complexes is almost near to the ΔG of the mTOR-RHEB complex. This is indicative of the good potential of these peptides to be used for further analysis and experiments. The favorable results can prove them to be good anticancer peptides.

The graphical binding energy comparison of all the peptides shows the peptide H2P1 and H3P1 as the best candidates for mTORC1 inactivation. The peptide H2P1 has a binding energy of -14.28 kcal/mol which proves its candidacy to be an anticancer peptide. Also, its amino acid length is comparatively shorter than H3P1. The peptide H3P1 has even better figures in terms of the free energy change but its larger size may be a footfall.

Discussion

The mTOR pathway has been widely studied and many of its protein members are acknowledged as the therapeutic target. The mTORC1 is one of the most extensively studied one and is referred to as the most potential therapeutic target for cancer as it has been found to be deregulated in almost all of the cancer hallmarks [Mahoney et al. 2018]. The mTORC1 inactivation by mTOR kinase inhibition using small inhibitors is one of the basic approaches to prevent these hallmarks.

Various mTOR inhibitors of mTOR have been designed so far, including Rapamycin and its derivatives (rapalogs). All of these have been found to have very serious side effects of the and also inhibit the activities of the mTORC2 complex which may lead to improper cellular functions of healthy cells [Mahoney et al. 2018]. Hence, the mTOR-RHEB binding interruption using peptides could be a good approach to inactivate the mTORC1. Also, anticancer peptide therapy has gained momentum in the last

two decades as the peptides can be designed to target any protein. The main rationale behind it is that the peptides can be designed by utilizing the amino acid chains or the helices from the interacting domain. Also, these peptides can be synthesized very easily and modified accordingly using some biological and chemical techniques.

The most important thing required to proceed with this work was the knowledge about the mTOR-RHEB interface which was priorly also determined by Yang et al in 2017. According to Yang and colleagues, the RHEB binds to the mTOR kinase at HEAT domains and FAT domain. The Switch I (residues 33D-41N) and Switch II (residues 63G-79N) along with residues 5K-7R and 106M-111Q of the RHEB GTPase binds at the residues 60S-157G of N-Heat domain, residues 966H-1020V of M-Heat domain and residues 1277K-1307A of FAT domain of the mTOR kinase. But this information was not residue-specific and thus insufficient to proceed with. Hence, the Ligplot predicted residue-specific interactions between the mTOR and the RHEB were preceded with. The results from the Ligplot matched the findings from Yang's work. According to Yang et al, the major interactions between the mTOR and the RHEB are formed by the N-Heat of the mTOR and the Switch II of the RHEB [Yang et al. 2017].

Further peptides were to be designed to dock with the RHEB GTPase and ultimately use them for therapeutic uses. The two rationals which were used for this peptide designing were: one based on the merging of structural and combinatorial chemistry technology and the second one is based on mutations and evolutionary changes.

The latter has a problem with its originality and precision as it uses the Genetic Algorithms for predicting the evolutionary changes in the given protein sequence [Pirogova et al. 2011]. In this study, the mutations and the evolutionary changes in the mTOR kinase within vertebrates were recorded. Surprisingly, most of the evolutionary mutations in the mTOR kinase lay outside the mTOR-RHEB interface and nearly 40 evolutionary changes in the residues were noted at the interface as well (**Fig. 5**).

As shown in figure A) All of the natural and the experimental mutations of the mTOR kinase laid away from the mTOR-RHEB interface. So, they can’t participate in the peptide designing process. B) Most of the residues which show evolutionary changes in different vertebrates lay in the interfacial region of the mTOR and the RHEB. So, changes related to these residues can be helpful during peptide designing for the peptide enhancement process. The identified evolutionary changes in residues of the mTOR kinase were induced as mutations in the native form of peptides which resulted in new peptides. As expected, the newly designed peptides were even more effective and showed a better binding with the RHEB.

The targeted therapies have been a new generation in cancer-preventing drugs and this sentence fits in. This approach aims at interfering with a specific molecule (mainly protein) that plays a significant role in tumorigenesis or cancer progression. Detailed knowledge about the molecular changes involved in cancer initiation and progression helps in selecting these targets [Sawyers 2004]. The prior knowledge about mTORC1 signaling and the role of RHEB in mTORC1 signaling has been utilized very significantly. Mahoney and colleagues in 2018, selected the RHEB GTPase as their target for interrupting the mTORC1

signaling using a small molecule (NR1) which successfully interrupted the mTORC1 signaling by binding to the Switch II residues of the RHEB. [Mahoney et al. 2018].

Similarly, this work also presents the RHEB as a possible target for halting the cascade process of the mTORC1 but in a very different yet effective manner i.e. using anticancer peptides. The use of anticancer peptides has been increasing day by day, one such example of exploring peptides as anticancer therapy is the work performed by Matthew Pincus. His group worked on the Ras, a member of the GTPase family, to inhibit its interactions with various partners. In another study on Ras, Chung et al, tried to inhibit oocyte maturation using peptides from the interface of its interacting partners [Bidwell III and Raucher 2009]. This shows that the use of peptides to inhibit some signaling pathway or the process holds a strong position and hence provides a strong base to the current work.

Although, the utility of the peptides to treat cancerous conditions is limited due to their poor pharmacokinetics so far. During *in-vivo* applications of the peptides, these are degraded by the action of various proteases in the serum. Also, their relatively bigger size and charged nature make them impermeable to the cell membrane. Yet, the recent advances in drug delivery e.g. use of macromolecules to deliver peptides to the target site and the *retro-D-inverso* approach of peptide designing have made it easy to overcome such problems [Bidwell III and Raucher 2009].

Also, selecting the RHEB as a target molecule for mTORC1 inactivation is not a bad idea, as previously successful works have been done on the same.

Conclusion

The main aim of this project was to design inhibitory peptides of the mTOR-RHEB interface and then to prove their potency to interrupt the mTORC1 signaling for cancer therapeutics. The project work includes some short objectives as mentioned in chapter-3 of this thesis. All the objectives and the aim were achieved successfully and can be concluded as:

1. The interactions and binding affinity of the mTOR-RHEB complex were determined which showed a total 37 bonds including 8 H-bonds and the **ΔG** was predicted to be -15.83 kcal/mol.
2. The mTOR-RHEB interface prediction was completed by Ligplot which showed that most of the interaction between the mTOR and RHEB complex are confined to the N-Heat domain of the mTOR kinase and the Switch I & II of the RHEB GTPase. The knowledge was further utilized for peptide designing which was the ultimate reason for interface prediction.
3. The mutations in the evolutionary changes in mTOR kinase were identified and were used in peptide designing. The mutations induced in the native forms of the peptide for peptide enhancement purpose proved to be favorable for expectations.
4. Total 6 new peptides were designed and were docked to the RHEB along with the 3 native peptides (peptides from interface) and further refining of the docking results was conducted.
5. The binding affinities and interactions were analyzed and compared for all peptides and were found to be best for the peptide H3P1 and H2P1 with the energies − 14.53 kcal/mol and − 14.28 kcal/mol

(very much near to the mTOR-RHEB complex) and 7 and 8 H-bonds, respectively.
6. Hence, these peptides prove to be the best inhibitors among the peptides chosen of mTORC1 in initial studies.
7. These peptides can be further explored by in-vitro and in-vivo tests as potential lead peptides against the cancers.

Declarations

- **Competing Interests:** No financial or non financial interests reside among authors.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.CA: a cancer journal for clinicians,71(3),209–249
2. Hassanpour SH, Dehghani M (2017) Review of cancer from perspective of molecular.Journal of Cancer Research and Practice,4(4),127–129
3. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, Zhu HP (2017) Risk factors and preventions of breast cancer.International journal of biological sciences,13(11),1387
4. Ghosh AP, Marshall CB, Coric T, Shim EH, Kirkman R, Ballestas ME, Sudarshan S (2015) Point mutations of the mTOR-RHEB pathway in renal cell carcinoma.Oncotarget,6(20),17895
5. Saxton RA, Sabatini DM (2017) mTOR signaling in growth, metabolism, and disease.Cell,168(6),960–976
6. Crino PB (2016) The mTOR signalling cascade: paving new roads to cure neurological disease.Nature Reviews Neurology,12(7),379–392.https://doi.org/10.1038/nrneurol.2016.81
7. Grabiner BC, Nardi V, Birsoy K, Possemato R, Shen K, Sinha S, Sabatini DM (2014) A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity.Cancer discovery,4(5),554–563
8. Kim LC, Cook RS, Chen J (2017) mTORC1 and mTORC2 in cancer and the tumor microenvironment.Oncogene,36(16),2191–2201
9. Tian T, Li X, Zhang J (2019) mTOR signaling in cancer and mTOR inhibitors in solid tumor targeting therapy.International journal of molecular sciences,20(3),755
10. Chowdhury SM, Talukder SA, Khan AM, Afrin N, Ali MA, Islam R, Halim MA (2020) Antiviral peptides as promising therapeutics against SARS-CoV-2. J Phys Chem B 124(44):9785–9792
11. Pirogova E, Istivan T, Gan E, Cosic I (2011) Advances in methods for therapeutic peptide discovery, design and development.Current Pharmaceutical Biotechnology,12(8),1117–1127
12. Laskowski RA, Swindells MB (2011) LigPlot+:multiple ligand–protein interaction diagrams for drug discovery

13. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms.Molecular biology and evolution,35(6),1547
14. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization system for exploratory research and analysis.Journal of computational chemistry,25(13),1605–1612
15. Vanhee P, Verschueren E, Baeten L, Stricher F, Serrano L, Rousseau F, Schymkowitz J (2011) BriX: a database of protein building blocks for structural analysis, modeling and design.Nucleic acids research,39(suppl_1),D435-D442
16. Petukhov M, Cregut D, Soares CM, Serrano L (1999) Local water bridges and protein conformational stability.Protein Science,8(10),1982–1989
17. Munoz V, Serrano L (1994) Intrinsic secondary structure propensities of the amino acids, using statistical ϕ−ψ matrices: comparison with experimental scales.Proteins: Structure, Function, and Bioinformatics,20(4),301–311
18. Abagyan R, Totrov M (1994) Biased probability Monte Carlo conformational searches and electrostatic calculations for peptides and proteins.Journal of molecular biology,235(3),983–1002
19. Vijayakumar M, Wong KY, Schreiber G, Fersht AR, Szabo A, Zhou HX (1998) Electrostatic enhancement of diffusion-controlled protein-protein association: comparison of theory and experiment on barnase and barstar.Journal of molecular biology,278(5),1015–1024
20. Schymkowitz JW, Rousseau F, Martins IC, Ferkinghoff-Borg J, Stricher F, Serrano L (2005) PredictionofwaterandmetalbindingsitesandtheiraffinitiesbyusingtheFold-Xforcefield.*Proceedings of the National Academy of Sciences*,*102*(29),10147–10152
21. Baeten L, Reumers J, Tur V, Stricher F, Lenaerts T, Serrano L, Schymkowitz J (2008) Reconstruction of protein backbones from the BriX collection of canonical protein fragments.PLoS computational biology,4(5),e1000083
22. Han Y, Král P (2020) Computational design of ACE2-based peptide inhibitors of SARS-CoV-2.ACS nano,14(4),5143–5147
23. Mahoney SJ, Narayan S, Molz L, Berstler LA, Kang SA, Vlasuk GP, Saiah E (2018) A small molecule inhibitor of Rheb selectively targets mTORC1 signaling.Nature communications,9(1),1–12
24. Yang H, Jiang X, Li B, Yang HJ, Miller M, Yang A, Pavletich NP (2017) Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40.Nature,552(7685),368–373
25. Sawyers C (2004) Targeted cancer therapy.Nature,432(7015),294–297
26. Bidwell III, Raucher D (2009) Therapeutic peptides for cancer therapy. Part I–peptide inhibitors of signal transduction cascades.Expert opinion on drug delivery,6(10),1033–1047

Figures

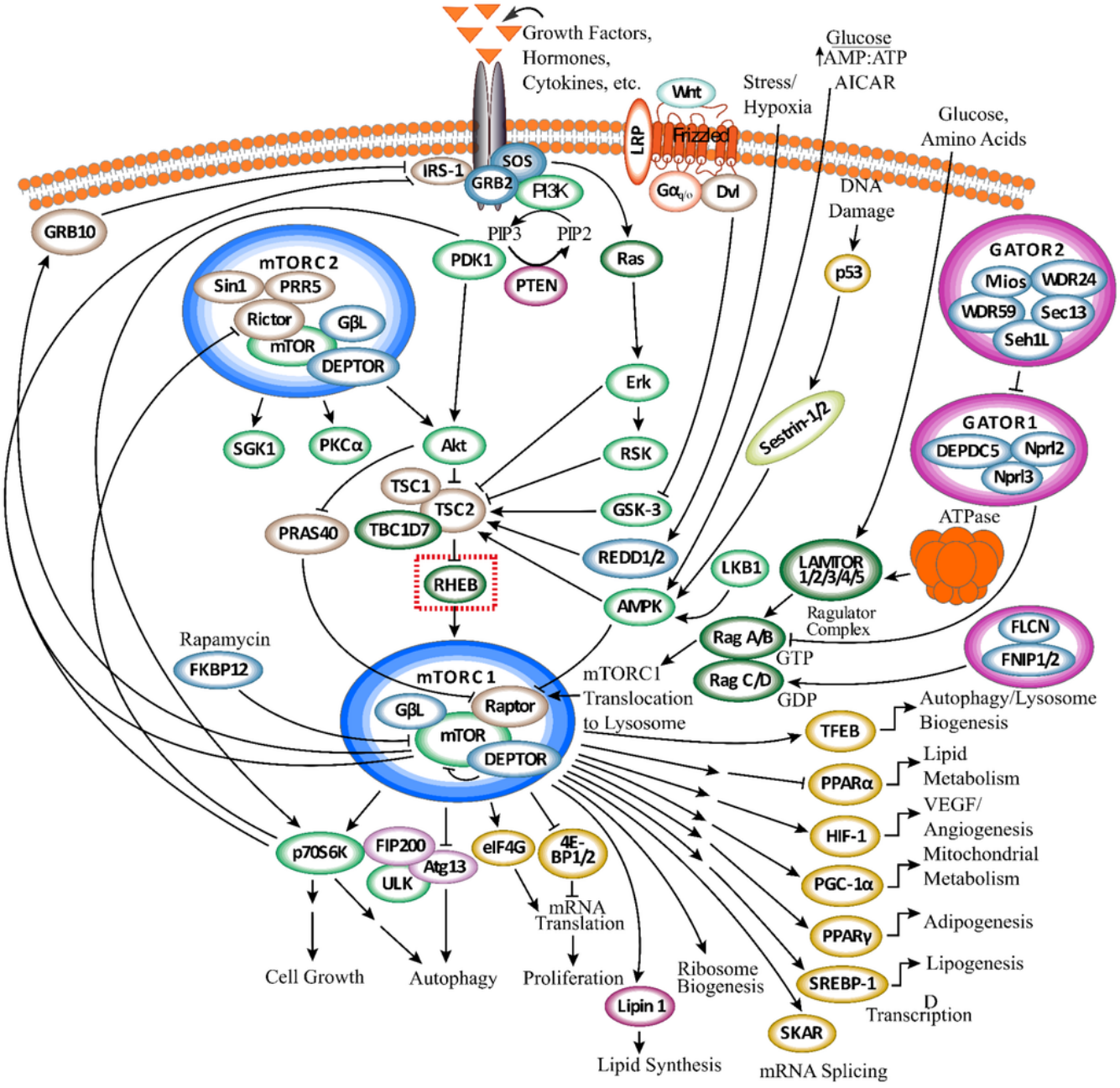

Figure 1

S6K1 (S6 Kinase 1) and 4E-BP1 (eIF-4E binding protein 1) are two well-characterized mTORC1 substrates that associate with mRNAs and regulate both mRNA translation initiation and progression, thus enhancing protein synthesis. As such, mTOR is normally subject to stringent regulation by nutrient conditions and growth factors. The presence of the growth factors and the good nutrient conditions lead to the dimerization of the Receptor Tyrosine kinases (RTKs). The activated RTKs result in the activation of

the PI3K/AKT pathway which ultimately inhibits the heterodimer consisting of TSC1 (tuberous sclerosis 1; also known as hamartin) and TSC2 (tuberous sclerosis 2; also known as tuberin). It is a key upstream regulator of mTORC1 and functions as a GTPase-activating protein (GAP) for RHEB. The GTP-bound form of RHEB directly interacts with mTORC1 and strongly stimulates its kinase activity. As a RHEB GAP, TSC1/2 negatively regulates mTORC1 by converting RHEB into its inactive GDP-bound state. mTORC2 substrates include members of the AGC (protein kinase A/protein kinase G/protein kinase C) family that regulate cell survival and cell cycle progression. One of the most well characterized downstream targets of mTORC2 is AKT. mTORC2 directly activates AKT by phosphorylating its hydrophobic motif (Ser473), a site required for its maximal activation [Ghosh et al. 2015]**.** The pathway diagram was designed by using “Inkscape 0.92.4”.

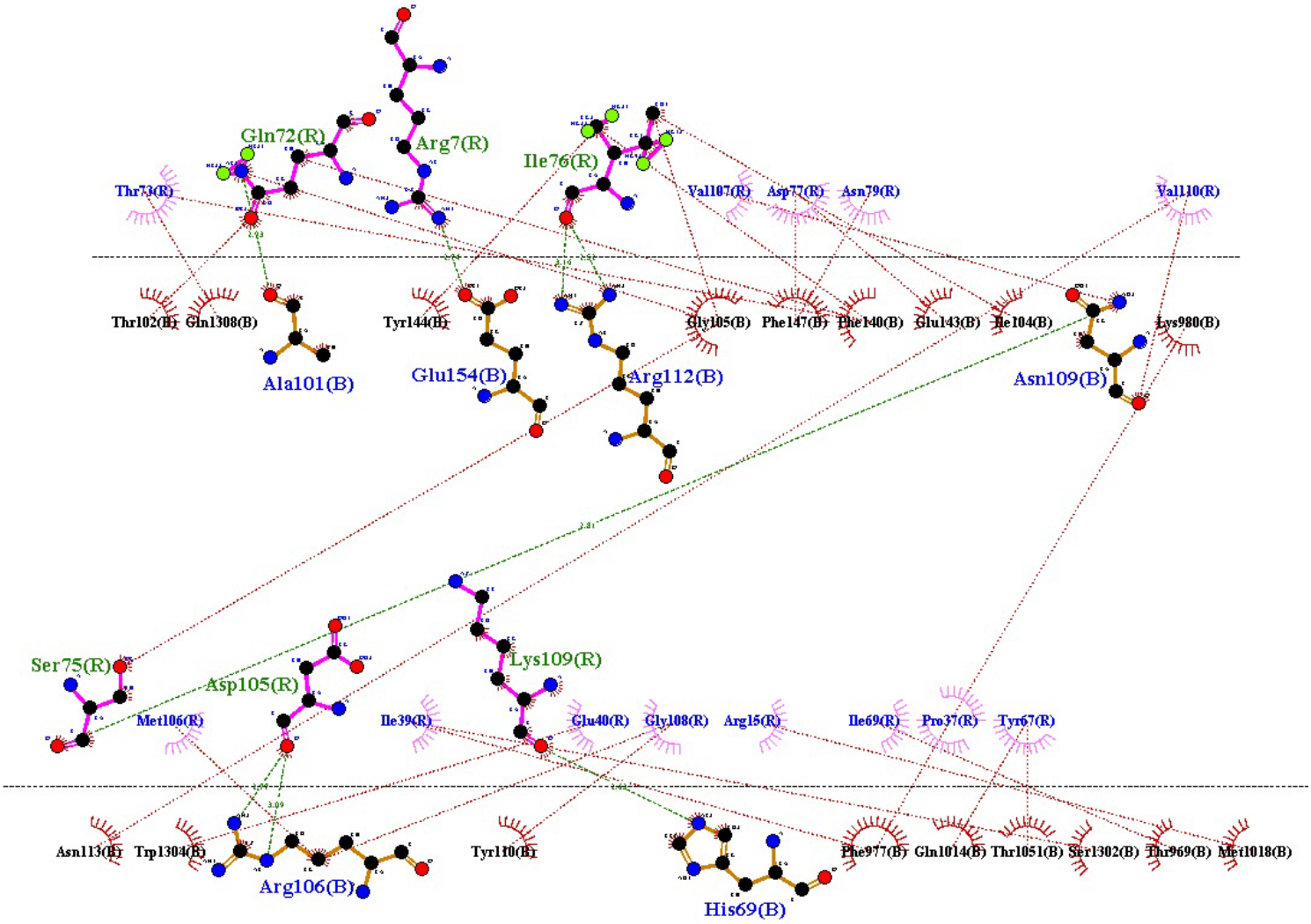

Figure 2

The mTOR kinase and RHEB GTPase interface: The ligplot diagram shows residues His69, Ala101, Arg106, Asn109, Arg112 and Glu 154 from the N-Heat domain of the mTOR kinase are involved in the Hydrogen bond (Green) formation while the residues Arg7, Gln72, Ser75, Ile76, Asp105 and Lys109 are

from the RHEB. The rest all interacting residues of the mTOR are in brown color while that of RHEB are in Pink.

Labellings: Red: Hydrophobic residues from RHEB, Pink: Hydrophobic residues from N-Heat, Green Bonds: Hydrogen bonds, Red Bonds: Interaction other than H-Bonds.

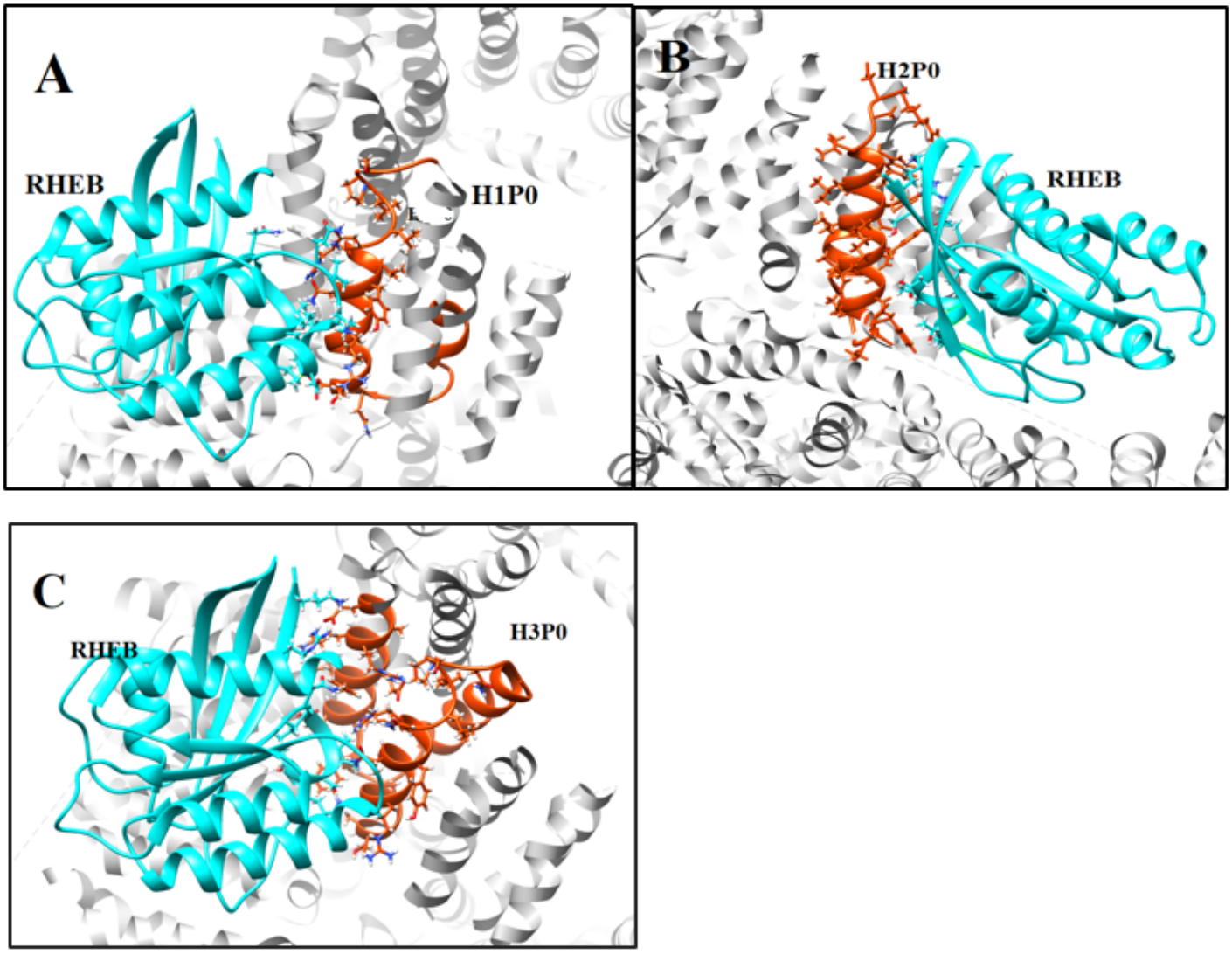

Figure 3

The Native peptides: The peptides H1P0, H2P0 and H3P0 designed from the interacting regions of the N-Heat domain of the mTOR kinase. A) The peptide prepared by utilizing the sequence 90I-119D named H1P0. B) The peptide is prepared by utilizing the sequence 130I-160R named ad H2P0. C) The peptide designed by utilizing the sequence 101A-154E named H3P0.

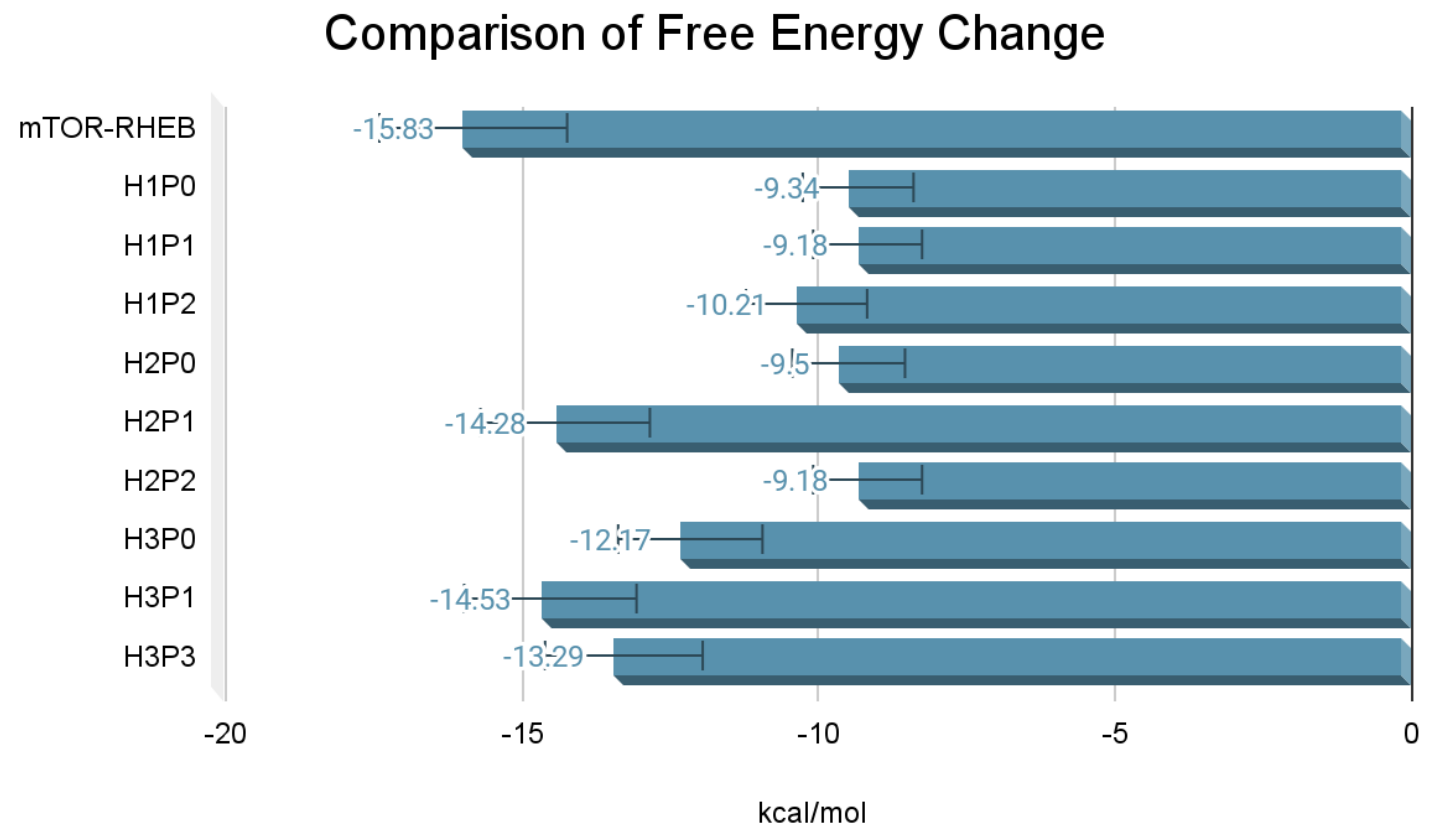

Figure 4

Legend not included with this version

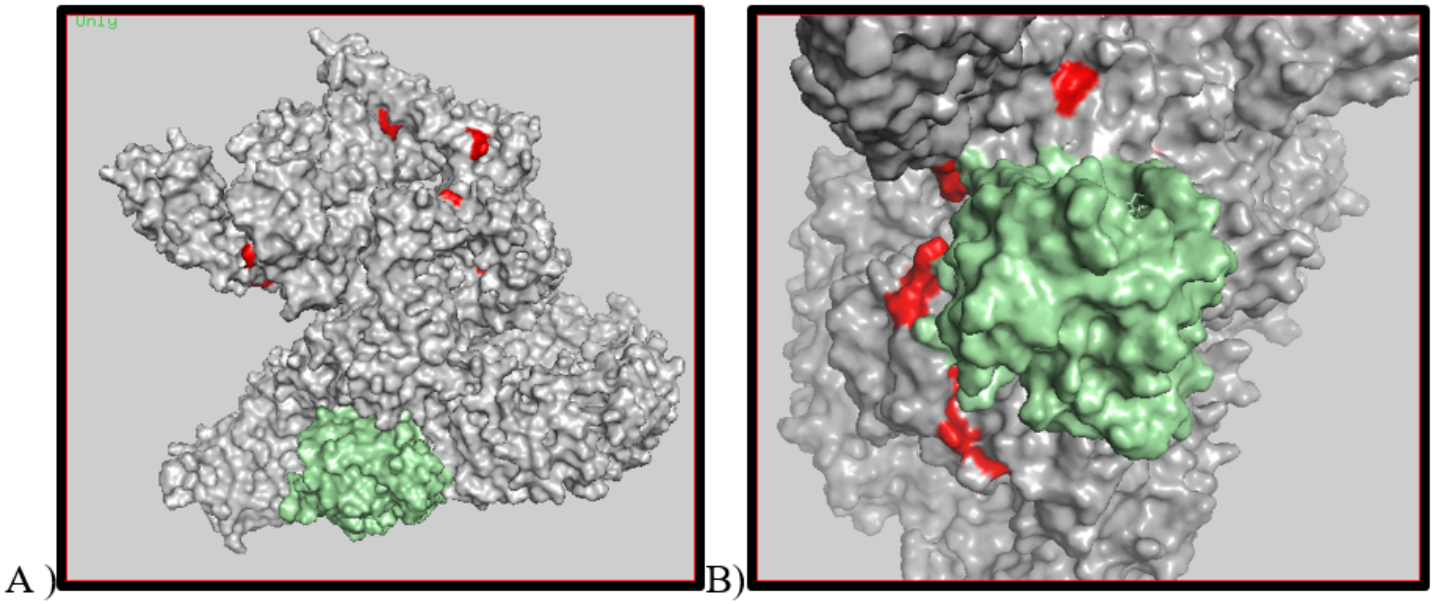

Figure 5

The positions of the retrieved mutations and the “evolutionary changes identified” in the mTOR kinase sequence. Color code - Red: Mutations, Gray: mTOR kinase and Green: RHEB.

As shown in figure A) All of the natural and the experimental mutations of the mTOR kinase laid away from the mTOR-RHEB interface. So, they can’t participate in the peptide designing process. B) Most of the residues which show evolutionary changes in different vertebrates lay in the interfacial region of the mTOR and the RHEB. So, changes related to these residues can be helpful during peptide designing for the peptide enhancement process. The identified evolutionary changes in residues of the mTOR kinase were induced as mutations in the native form of peptides which resulted in new peptides. As expected, the newly designed peptides were even more effective and showed a better binding with the RHEB.