Evaluation of Pathogenicity and Structural Alterations for the Mutations Identified in the Conserved Region of C-Terminal Kinase Domain of human- Ribosomal S6 Kinase 1

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Keywords: RSK1; missense mutation; cancer; molecular dynamics simulation; PCA.

Legend:

The conservation scale:

1 2 3 4 5 6 7 8 9

Variable **Average** **Conserved**

e - An exposed residue according to the neural-network algorithm.
b - A buried residue according to the neural-network algorithm.
f - A predicted functional residue (highly conserved and exposed).
s - A predicted structural residue (highly conserved and buried).
x - Insufficient data - the calculation for this site was performed on less than 10% of the sequences.

Figure S1. Evolutionary conservancy of RSK1-CTKD generated by ConSurf. Based on multiple sequence alignment ConSurf generates a colour-coded output. The higher the score more is the residue conserved. Residue can be predicted to be exposed (e), buried (b), functional highly conserved, and exposed residue (f), structural highly conserved, buried (s), and x insufficient data

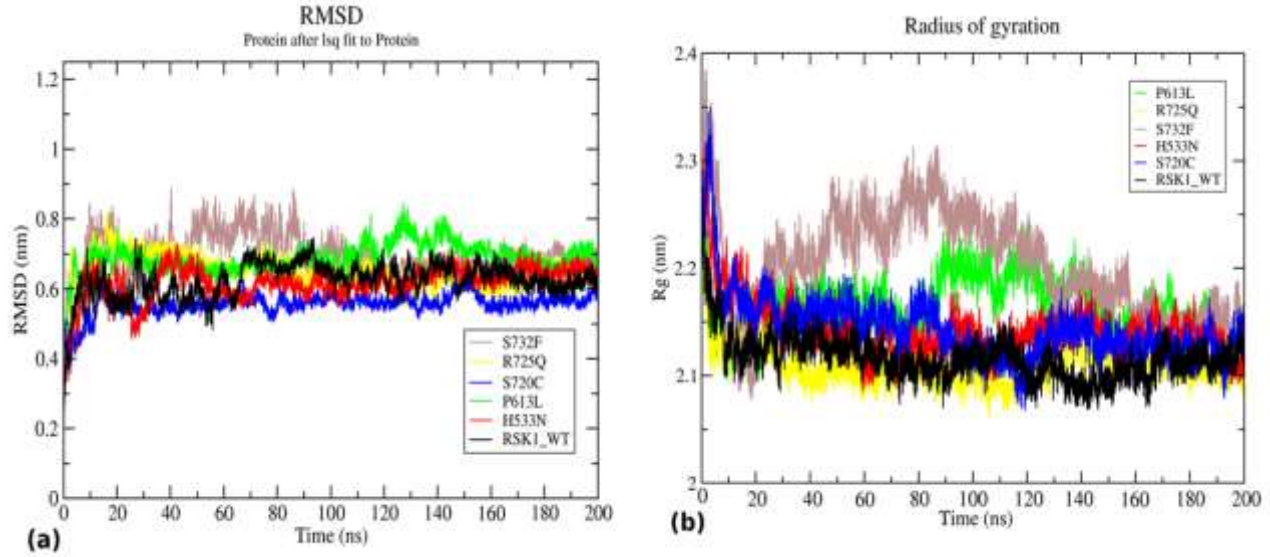


Figure S2. Structural stability and compactness analysis of RSK1-CTKD WT (black) and mutants H533N (red), P613L (green), S720C (blue), R725Q (yellow), and S732F (brown), **(a)** RMSD of WT and mutants showing similar pattern, **(b)** Rg of WT and mutant indicating alteration to some extent in compactness of a structure

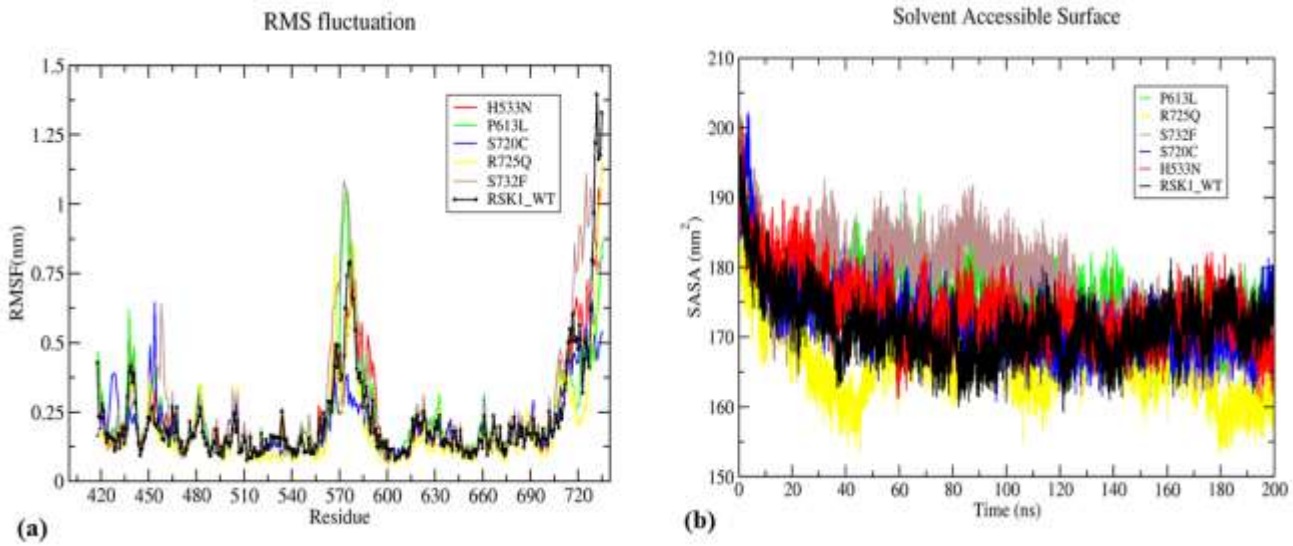


Figure S3. Structural flexibility and Solvent accessible surface area (SASA) analysis of RSK1-CTKD WT (black) mutants H533N (red), P613L (green), S720C (blue), R725Q (yellow), and S732F (brown). **(a)** RMSF of WT and mutants showing minor changes in the flexibility of a protein, (b) SASA of wild-type and mutant indicating not much alteration in an area accessible to the solvent

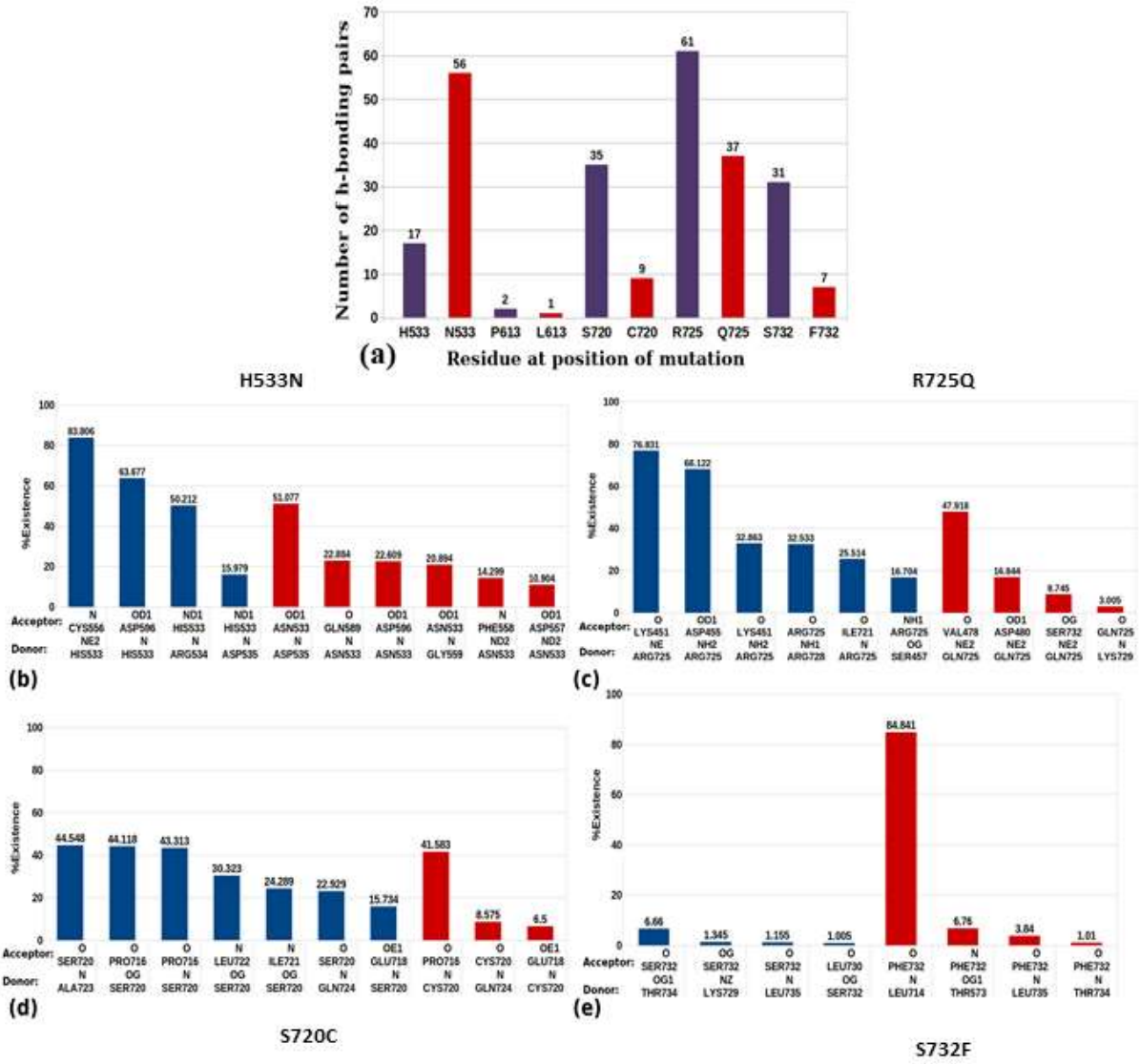


Figure S4. Several intramolecular hydrogen bonding pairs and their % existence throughout the simulation of WT and mutated residue **(a)** number of H-bonding pair formed by WT residue and mutated residue, **(b-e)** high scored H-bond existence formed by WT residue and mutated residue in **(b)** H533N **(c)** R725Q **(d)** S720C, and **(e)** S732F

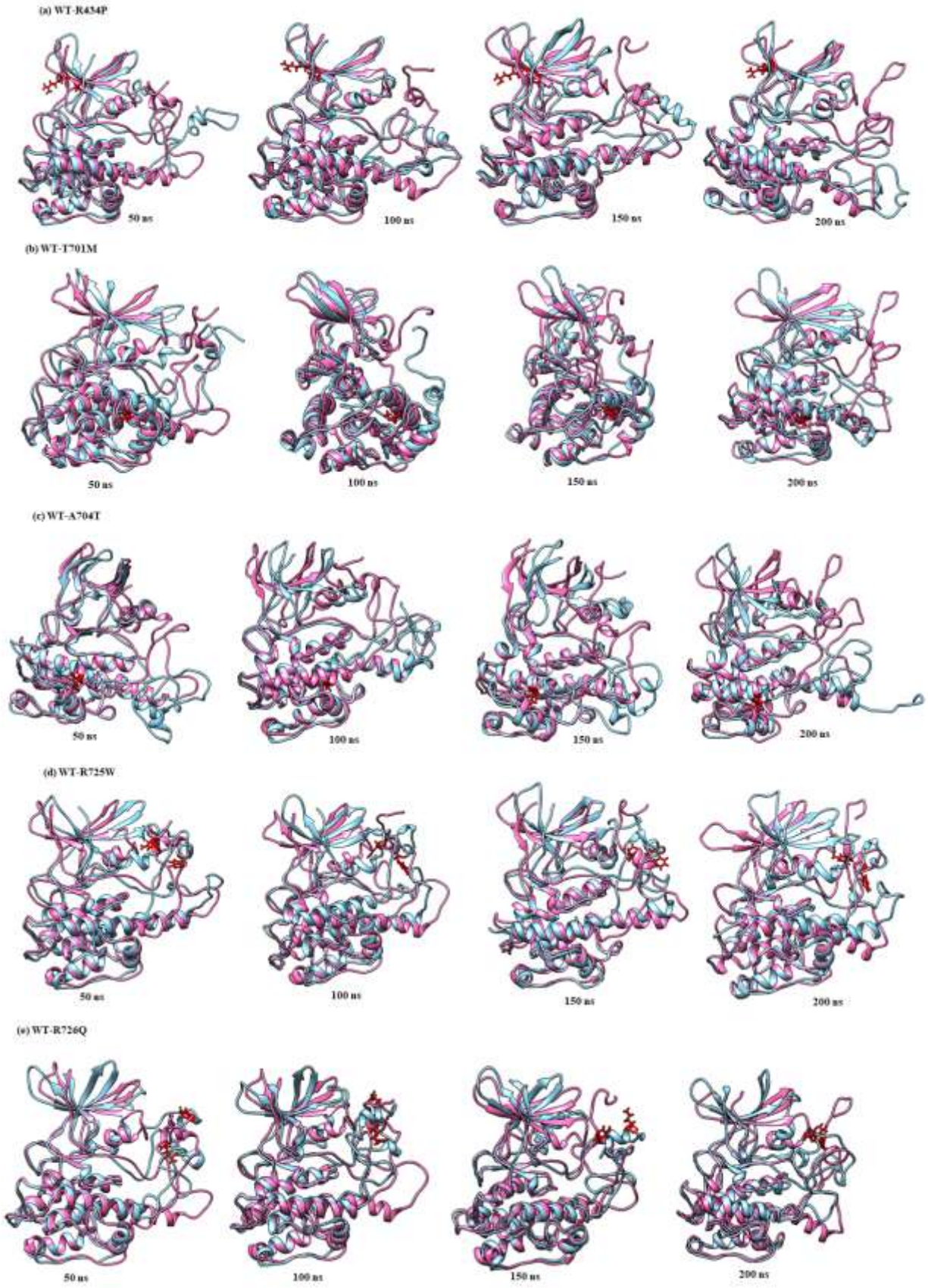


Figure S5. Snapshot of WT and mutant (a) R434P, (b) T701M, (c) A704T, (d), R725W, and (e) R726Q RSK1-CTKD confirmation at different simulation time steps

Table S1: Functional consequences of missense mutations predicted by SIFT, PolyPhen2, PhD-SNP, PMut, and PROVEAN

Protein Change	phD-SNP	Pmut	PROVEAN	SIFT	PolyPhen2
S457L	neutral	disease	Deleterious	Deleterious	Benign
R588L	Disease	disease	Deleterious	Deleterious	Probably damaging
R434H	neutral	disease	Deleterious	Deleterious	Probably damaging
S719P	neutral	neutral	Neutral	Tolerated	Benign
G530R	Disease	disease	Deleterious	Deleterious	Probably damaging
R503Q	Disease	neutral	Tolerated	Tolerated	Benign
G520D	Disease	neutral	Neutral	Deleterious	Possibly damaging
R493W	Disease	disease	Deleterious	Deleterious	Probably damaging
E623K	neutral	disease	Tolerated	Tolerated	Benign
E490V	Disease	disease	Deleterious	Deleterious	Probably damaging
L463F	Disease	disease	Deleterious	Deleterious	Probably damaging
G617S	neutral	neutral	Tolerated	Tolerated	Probably damaging
G495E	Disease	disease	Deleterious	Deleterious	Probably damaging
E524G	neutral	disease	Deleterious	Tolerated	Probably damaging
G639V	Disease	disease	Deleterious	Deleterious	Benign
S539C	Disease	disease	Deleterious	Deleterious	Probably damaging
R565Q	neutral	disease	Deleterious	Deleterious	Probably damaging
G630S	neutral	neutral	Deleterious	Tolerated	Benign
R554H	neutral	disease	Deleterious	Deleterious	Probably damaging
Y605H	Disease	disease	Deleterious	Deleterious	Possibly damaging
A704T	Disease	disease	Deleterious	Deleterious	Probably damaging
E524Q	neutral	neutral	Neutral	Tolerated	Probably damaging
P682S	neutral	neutral	Tolerated	Tolerated	Probably damaging
T701M	Disease	disease	Deleterious	Deleterious	Probably damaging
K451M	Disease	disease	Deleterious	Deleterious	Probably damaging
S720C	neutral	disease	Deleterious	Deleterious	Probably damaging
T577A	Disease	disease	Deleterious	Deleterious	Possibly damaging
S708F	neutral	disease	Deleterious	Tolerated	Benign
A582V	Disease	disease	Deleterious	Deleterious	Probably damaging
R726Q	Disease	disease	Deleterious	Deleterious	Benign
V515D	Disease	disease	Deleterious	Deleterious	Probably damaging
P613L	Disease	disease	Deleterious	Deleterious	Probably damaging
P712S	neutral	disease	Deleterious	Tolerated	Benign
F614L	Disease	disease	Deleterious	Deleterious	Probably damaging
V531F	Disease	neutral	Deleterious	Deleterious	Probably damaging
K421N	neutral	disease	Deleterious	Deleterious	Benign
V419M	neutral	neutral	Neutral	Tolerated	Benign
R725W	Disease	disease	Deleterious	Deleterious	Probably damaging
R588H	neutral	neutral	Deleterious	Tolerated	Probably damaging
A439T	neutral	neutral	Neutral	Tolerated	Benign

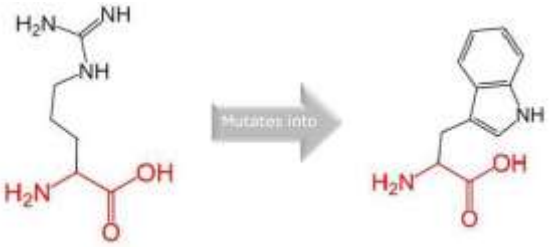
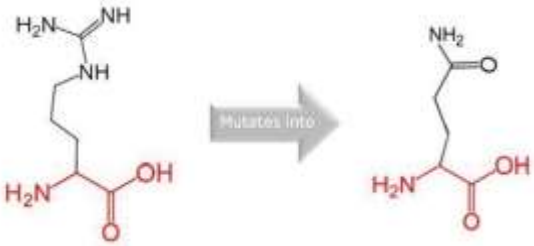
S732F	neutral	neutral	Deleterious	Deleterious	Benign
N411S	neutral	neutral	Neutral	Tolerated	Benign
V652M	neutral	disease	Neutral	Deleterious	Possibly damaging
V485A	Disease	neutral	Deleterious	Tolerated	Possibly damaging
H533N	Disease	disease	Deleterious	Deleterious	Benign
L463P	Disease	disease	Deleterious	Deleterious	Probably damaging
V446I	neutral	neutral	Neutral	Deleterious	Probably damaging
L536M	Disease	disease	Neutral	Deleterious	Probably damaging
G610D	Disease	disease	Deleterious	Deleterious	Possibly damaging
T606I	Disease	neutral	Deleterious	Tolerated	Benign
G630D	Disease	disease	Deleterious	Tolerated	Probably damaging
P673L	Disease	disease	Deleterious	Deleterious	Benign
R663C	Disease	disease	Deleterious	Deleterious	Probably damaging
R725Q	Disease	disease	Deleterious	Deleterious	Possibly damaging
K521R	neutral	neutral	Neutral	Tolerated	Benign
L463I	neutral	neutral	Neutral	Deleterious	Probably damaging
R434P	Disease	disease	Deleterious	Deleterious	Probably damaging
Q468L	Disease	disease	Deleterious	Deleterious	Probably damaging
R510Q	neutral	neutral	Neutral	Tolerated	Benign
L636P	Disease	disease	Deleterious	Deleterious	Possibly damaging
L502M	neutral	neutral	Neutral	Tolerated	Probably damaging

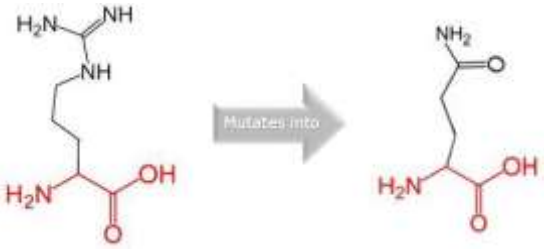
Table S2: Effect of 10 mutations over RSK1-CTKD predicted by HOPE server

Protein Change	Structure	Properties
R434P		1. Mutant residue is smaller and hydrophobic than wild type. 2. Positive wild type residue mutated into neutral charge residue. 3. Disruption in Salt bridge interaction between Glutamic acid 422 and 443. 4. The mutated residue is located in a domain that is important for the binding of other molecules. The mutated residue is in contact with residues in another domain. The mutation may disturb these contacts.
H533N		1. Mutant residue is smaller and hydrophobic than wild type. 2. Disruption in hydrogen bonding between aspartic acid 535 and cysteine 556. 3. The mutated residue is located in a domain that is important for the activity of the protein and in contact with residues in another domain. This interaction may be important for the correct function of the protein. The mutation can affect this interaction and as such affect protein function.
P613L		1. The mutant residue is bigger than the wild-type residue. 2. wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. 3. The mutated residue is located in a domain that is important for the

		activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.
T701M		1. Mutant residue bigger and more hydrophobic than wild type. 2. Disruption of a hydrogen bond at alanine 697 3. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. The mutation will cause loss of hydrogen bonds in the core of the protein and as a result, disturb correct folding. 4. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.

A704T		1. Mutant residue bigger and more hydrophobic than wild type. 2. The wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit and will probably leads to loss of hydrophobic interactions in the core of the protein. 3. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.
S720C		1. Mutated residue is more hydrophobic than wild type and will cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding. 2. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.

R725W		1. Mutant residue is smaller and hydrophobic than wild type. 2. Positive wild type residue mutated into neutral charge residue. 3. The residue is located on the surface of the protein, mutation of this residue can disturb external interactions with other molecules or other parts of the protein. 4. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.
R725Q		1. Mutant residue is smaller and hydrophobic than wild type. 2. Positive wild type residue mutated into neutral charge residue. 3. The residue is located on the surface of the protein, mutation of this residue can disturb external interactions with other molecules or other parts of the protein. 4. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.

R726Q		1. Mutant residue is smaller and hydrophobic than wild type. 2. Positive wild type residue mutated into neutral charge residue. 3. The residue is located on the surface of the protein, mutation of this residue can disturb external interactions with other molecules or other parts of the protein. 4. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.
S732F		1. Mutant residue is bigger and hydrophobic than wild type. 2. The mutated residue is located in a domain that is important for the activity of the protein and in contact with another domain that is known to be involved in binding. The interaction between these domains could be disturbed by the mutation, which might affect the signal transduction between the domains.

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