CHAPTER 8

STUDY OF THE EFFECT OF PEPTIDES ON α-SYNUCLEIN AGGREGATION

Study of the effect of peptides on α-Synuclein aggregation

8.1. Abstract:

The αS aggregation is a promising therapeutic target for Synucleinopathies, a range of neurodegenerative disorders. Recently, two novel peptides K84s (FLVWGCLRGSAIGECVVHGGPPSRH) and K102s (FLKRWARSTRWGTASCGGS) have been reported to significantly inhibit αS oligomerization and aggregation. However, a little is known about the interaction sites between these peptides and αS. The objective of this work is to probe the interaction sites of K84s and K102s with αS using computational tools. In this study, the 3-D structure of the K84s and K102s peptides were constructed using PEPFOLD3 server. The two peptides were then docked to αS molecule using Patchdock online server. The two resultant docked complexes were then subjected to 50 ns of Molecular Dynamics (MD) simulations using the Amberff99SBildn force field. From the analysis of the corresponding MD simulation trajectories, the structure, dynamics, energy profiles and binding modes of the two complexes were determined. The interface residues in the two complexes were obtained by submitting their lowest energy structure to the PDBsum server. The contribution of each residue present in αS of (α-Synuclein-K84s/K102s) complexes to the total binding free energy were determined from the per residue energy decomposition (PRED) analysis with the MM-GBSA method. The binding free energies between the αS protein and the two peptides were found to be indeed high. The (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes have binding free energies of -33.61 kcal mol^{-1} and -40.88 kcal mol^{-1} respectively. Using PDBsum server analysis, in the (α-Synuclein-K84s) complex, the residues GLY 25, ALA 29, VAL 49, LEU 38, VAL 40, GLU 28, GLY 47, LYS 32, GLU 35, GLY 36, TYR 39, VAL 48 and VAL 26 (from α-Synuclein) and SER 23, LEU 7, ILE 12, HIS 25, PHE 1, HIS 18, CYS 6, ARG 24, PRO 21 and ARG 8 (from K84s peptide) were identified to be present at the interface. In the (α-Synuclein-K102s) complex, the residues VAL40, GLY36, GLU35, TYR39, LYS45, LEU38, LYS43, VAL37, THR44, VAL49, VAL48 and GLU46 (from α-Synuclein) and ARG 10, GLY 12, GLY 18, SER 15, THR 13, SER 19, TRP 11, ALA 14, CYS 16, ARG 7, ARG 4 and GLY 17 (from K102s peptide) were identified to be present at the interface. The PRED analysis using MM-GBSA algorithm showed that their intermolecular interaction can be credited to the residues PHE 1, LEU 7, ILE 12, LEU 2, VAL 3, GLY 5 and PRO 21 of

K84s peptide and residues VAL 48, ALA 29, VAL 40, TYR 39, VAL 49, VAL 26 and GLY 36 present in the αS of (α-Synuclein-K84s) complex. In case of the (α-Synuclein-K102s) complex, the inter-molecular interaction can be credited to the residues ARG 4, ARG 10, TRP 11, ALA 14, SER 15, CYS 16 and SER 19 of K102s peptide and residues GLU 46, LYS 45, VAL 49, GLU 35, VAL 48, TYR 39 and VAL 40 of αS. It has also been noticed that the αS has retained the helical content to significant extent when it is in complex form with the K84s and K102s peptides. Taken together the data suggest that the two novel peptides studied here could be suitable candidates for future therapeutic development against αS aggregation**.**

8.2. Introduction:

Protein aggregation and self-assembly have been linked to neurological diseases such as Parkinson's disease (PD). They are one of the most important problems addressing modern medicine; they are widespread, and there are currently no treatments available to delay or stop the progression of neurodegeneration [651-653]. A number of strategies are being investigated to reduce the toxicity caused by protein aggregation. One of them is the use of passive or active immunization. Another technique is to reduce protein expression or improve clearance processes, as well as methods to prevent misfolded protein transfer from cell to cell. The use of chaperones or modulator and inhibitor drugs to target the aggregation process directly is an appealing therapeutic alternative. The bulk of medications now on the market are small molecules; nonetheless, peptides are becoming increasingly popular as pharmaceuticals [654-656].

Peptides are more specific to a single target than tiny compounds, making them less likely to cause unwanted side effects. Many of the difficulties that have previously hampered peptide clinical development (for example, proteolytic degradation and localization) are now better known and can be researched further [657, 658]. Peptides are particularly appealing in neurodegenerative research involving protein aggregation because of their ability to block broad and shallow protein-protein interactions, and thus fibril formation. Some peptides have been found to produce β-hairpin structures that can "cap" fibrils, preventing the addition of more monomeric units and so limiting their elongation, such as for amylin and αS [659-662].Another option is to use N-methylated peptides to limit hydrogen bond formation or short peptides with residues that impede subsequent protein attachment to prohibit assembly on one side of the protein. The

development of novel peptide-based inhibitors to counteract the toxicity of aggregation-prone proteins such αS is consequently of great interest [663].

Recently two novel peptides (K84s and K102s, of 25 and 19 amino acids respectively) have been identified that inhibit αS toxicity and aggregation [664]. These peptides have been proposed as potential αS aggregation antagonists for future therapeutic approaches. Understanding the anti-aggregation process will require identifying the peptides' contact locations with αS. In this study, computational techniques have been used to investigate the interaction locations of these two new peptides with αS [665].

8.3. Materials and Methods:

8.3.1. Preparation of the system

8.3.1.1. Preparation of the ligands:

By submitting their respective amino acid sequences to the PEP-FOLD3 server, the initial 3-D structures of K84s (FLVWGCLRGSAIGECVVHGGPPSRH) and K102s (FLKRWARSTRWGTASCGGS) peptides were obtained [666].

8.3.1.2. Preparation of the receptor:

The initial 3-D structure of the receptor molecule (α-Synuclein: PDB ID 1XQ8) [275] was obtained from the RCSB PDB [502, 503].

8.3.1.3. Preparation of the complexes:

Using the Patchdock online docking server [511], the two complexes (α-Synuclein-K84s) and (α-Synuclein-K102s) were created by docking the corresponding peptides to the αS. The 3-D structures of the two complexes have been shown in **Figures 8.1(A) and 8.1(B).**

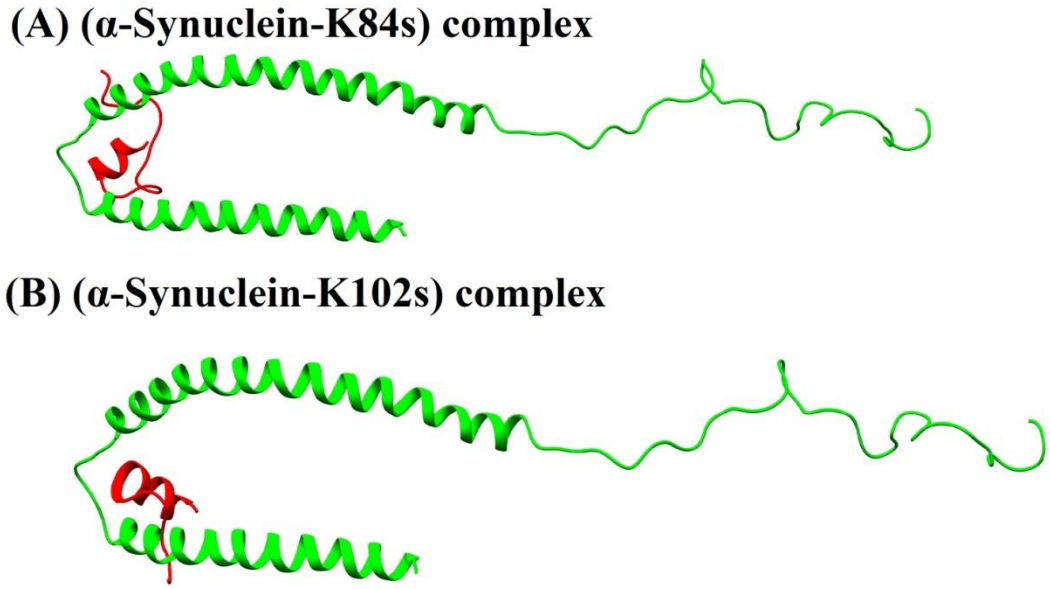

Figure 8.1. *Schematic representation of the interaction of K84s and K102s peptides with α-Synuclein as shown in (A) and (B) respectively.*

8.3.2. Molecular Dynamics (MD) simulation of (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes:

The requisite coordinate and topology files of the (α-Synuclein-K84s) and (α-Synuclein-K102s) complex structures to perform MD simulation were constructed using the Leap module of the AMBER 14 [478] software package and the AMBERff99SBildn force field [474, 667-669]. Both the complex systems were solvated in explicit TIP3P (transferable intermolecular potential with 3 points) water model [480] with the buffer distance of 10 Å in a cubic periodic box. The two systems were neutralized by adding appropriate numbers of counter ions and then subjected to energy minimization to remove the strong van der Waals. The MD analysis followed a standard approach as discussed in *Section 4.3.2.*

8.3.3. MD analyses:

In order to analyse the conformational changes of the (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes during simulations, its backbone and all heavy atoms was superimposed with the initial structure obtained from Patchdock server. Root mean-square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bonding and secondary structure information were analysed by using CPPTRAJ module in AMBER 14. To account for changes in secondary structure properties, the Dictionary of

Secondary Structure of Protein plot for both the complexes were investigated at various time intervals throughout the simulation.

8.3.4. Binding free energy:

The BFE calculations were performed for the two complexes as per the methods discussed elaborately in *Section 3.1.3.* By adding all of a residue's interactions with the other residues in the system, the per-residue energy decomposition (PRED) approach [497] determines the energy contribution of a single residue. By using this method, one can ascertain how these residues interact with the overall ligand binding free energy. The MM-GBSA module of the AMBER 14 software was used to estimate the PRED values.

8.4. Result and Discussion:

8.4.1. Structural properties of the (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes from the MD simulation

8.4.1.1. Root Mean Square Deviation (RMSD) analysis:

The RMSD values of all the C_α atoms of the peptide and αS components were calculated for the (α-Synuclein-K84s) complex referenced to their starting structures to assess the stability. **Figures 8.2(A) and 8.2(B)** depicts the RMSD calculated with respect to time period for the peptide and αS components of the (α-Synuclein-K84s) complex respectively. From these two figures, it is observed that the conformation of the peptide and the αS is stable in the complex throughout the simulation period. The RMSD value of the peptide component of (α-Synuclein-K84s) complex reached plateau around 4 Å after approximately 10 ns of the simulation and the RMSD value of the αS component of the (α-Synuclein-K84s) complex reached plateau around 12 Å after 30 ns of simulation period.

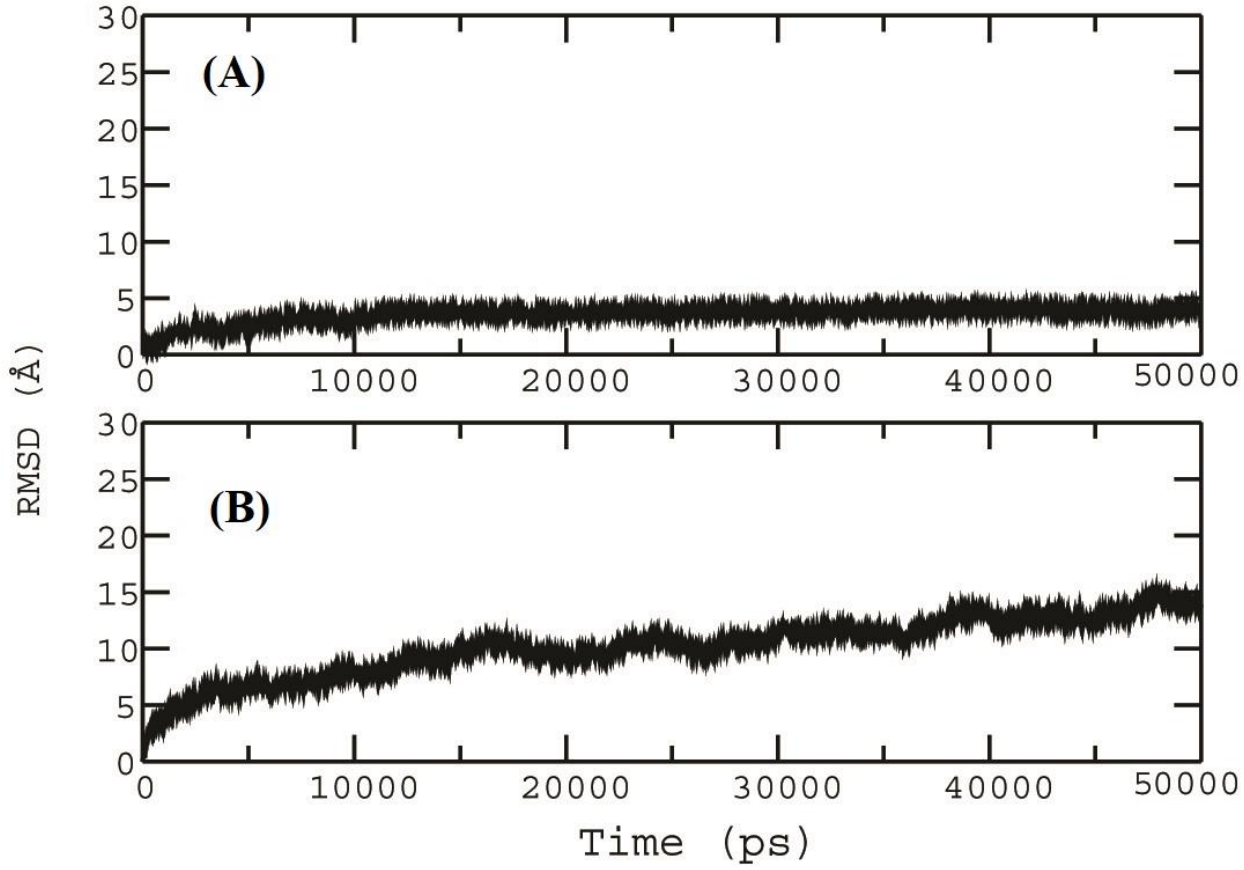

Figure 8.2. *Molecular Dynamics analysis of Root mean square deviation (RMSD) with respect to time period for the (α-Synuclein-K84s) complex for (A) peptide (K84s) and (B) α-Synuclein.*

The RMSD values of C_α atoms of the peptide and αS components were calculated for the (α-Synuclein-K102s) complex referenced to their starting structures to assess the stability. **Figures 8.3(A) and 8.3(B)** depicts the RMSD calculated with respect to time period for the peptide and αS component of the (α-Synuclein-K102s) complex respectively. From these two figures, it is observed that the conformation of the peptide and the αS is stable in the complex throughout the simulation period. The RMSD value of the peptide component of (α-Synuclein-K102s) complex reached plateau around 3 Å after approximately 20 ns of the simulation while the RMSD value of the αS component of the (α-Synuclein-K102s) complex reached plateau around 20 Å after 30 ns of simulation period. Thus, it can be inferred from the RMSD analysis that both the systems have attained stability.

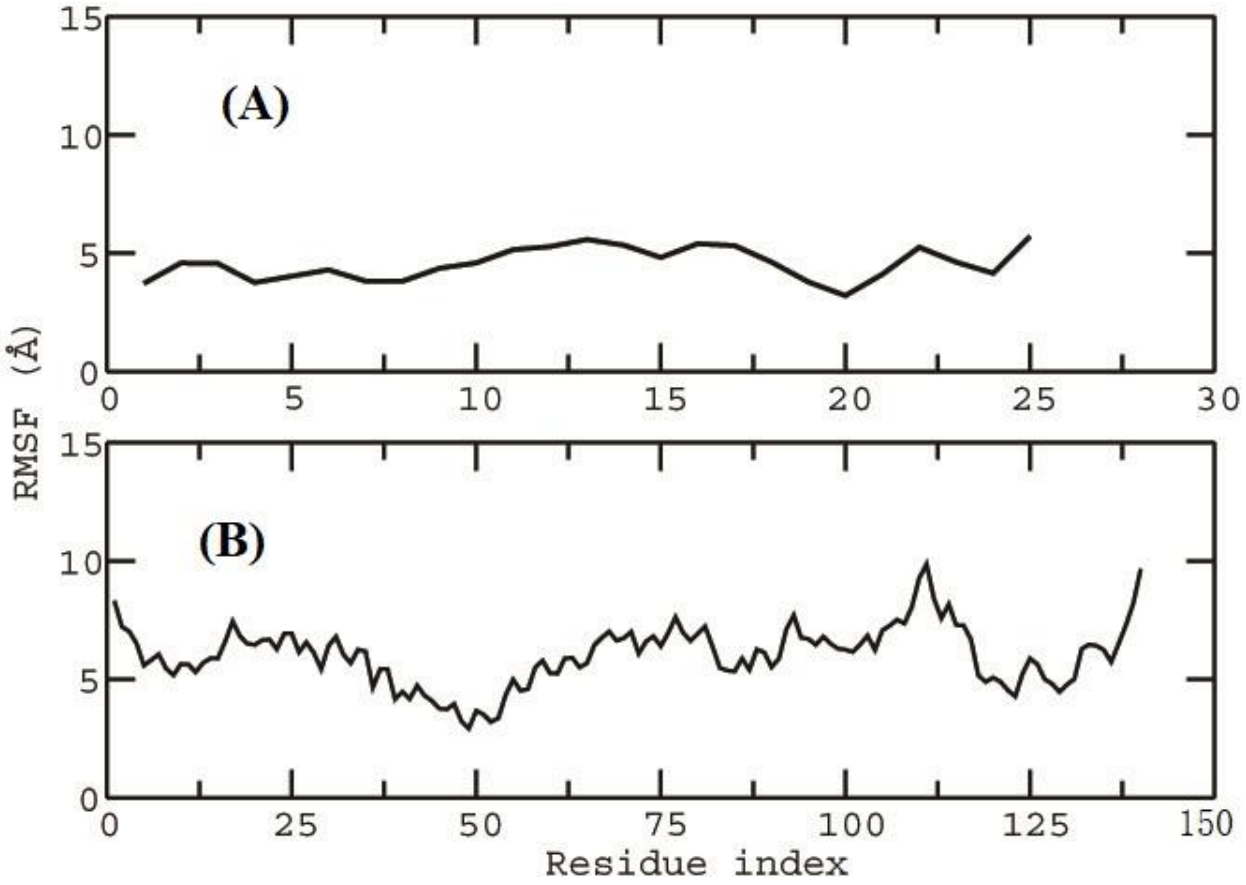

Figure 8.3. *Molecular Dynamics analysis of Root mean square deviation (RMSD) with respect to time period for the (α-Synuclein-K102s) complex for (A) peptide (K102s) and (B) α-Synuclein.*

8.4.1.2. Root Mean Square Fluctuation (RMSF) analysis:

The RMSF values of all the C_α-atoms referenced to their starting structures of the peptide and αS components for the two systems: (a) (α-Synuclein-K84s) complex and (b) (α-Synuclein-K102s) complex were determined to assess individual residue flexibility during the simulation time. RMSF per residue is typically plotted vs. residue number, and can indicate structurally which amino acids in a protein/peptide contribute the most to a molecular motion. **Figures 8.4(A) and 8.4(B)** shows RMSF values for the peptide and αS components of the (α-Synuclein-K84s) complex respectively. From **Figure 8.4(A),** it can be seen that no residues in the peptide exhibited distinct atom positional fluctuation amplitudes during 50 ns MD simulation. From the **Figure 8.4(B),** it can be seen that in the region where the peptide has been bound to αS exhibit relatively lower atom positional fluctuation amplitudes throughout the simulation time. The results indicated that the K84s peptide bound to αS can make it less flexible and maintain an ordered structure of αS thereby allowing the tighter fitting of K84s peptide into the αS

active site. **Figures 8.5(A) and 8.5(B)** shows RMSF values for the peptide and αS components of the (α-Synuclein-K102s) complex respectively. From **Figure 8.5(A),** it is observed that no residues in the peptide exhibited distinct atom positional fluctuation amplitudes during the entire MD simulation. From the **Figure 8.5(B),** it can be seen that in the region where the peptide has been bound to αS exhibit relatively lower atom positional fluctuation amplitudes throughout the simulation time. The results indicated that the K84s peptide bound to αS can make it less flexible and maintain an ordered structure of αS thereby allowing the tighter fitting of K84s peptide into the αS active site. From **Figures 8.4 and 8.5**, it is observed that (α-Synuclein-K102s) complex is relatively more ordered than the (α-Synuclein-K84s) complex.

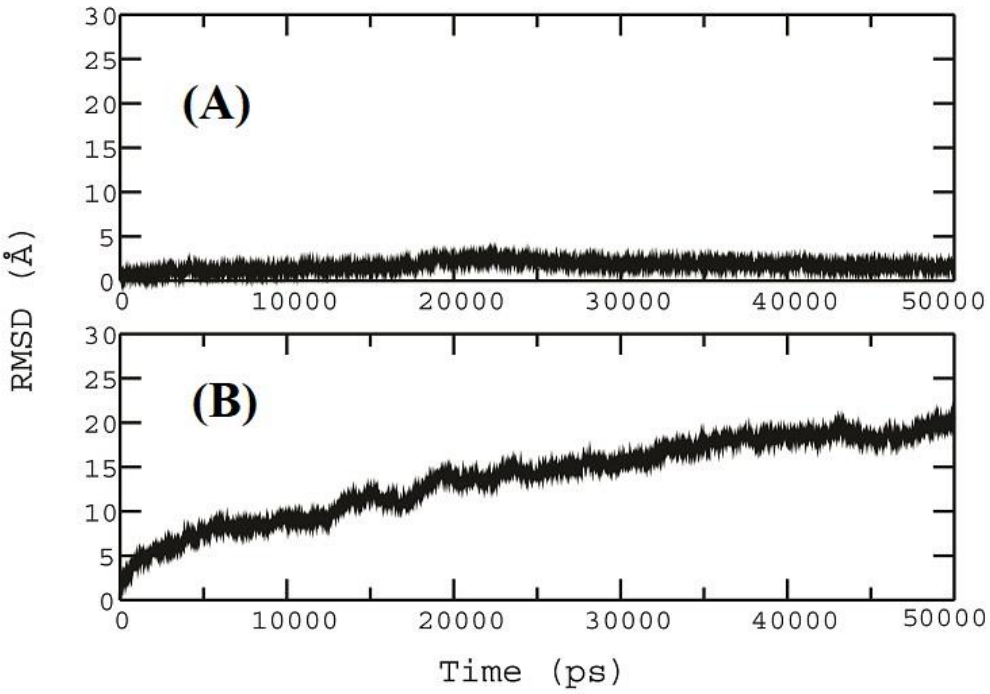

Figure 8.4. *Molecular Dynamics analysis of Root mean square fluctuation (RMSF) with respect to time period for the (α-Synuclein-K84s) complex for (A) peptide (K84s) and (B) α-Synuclein.*

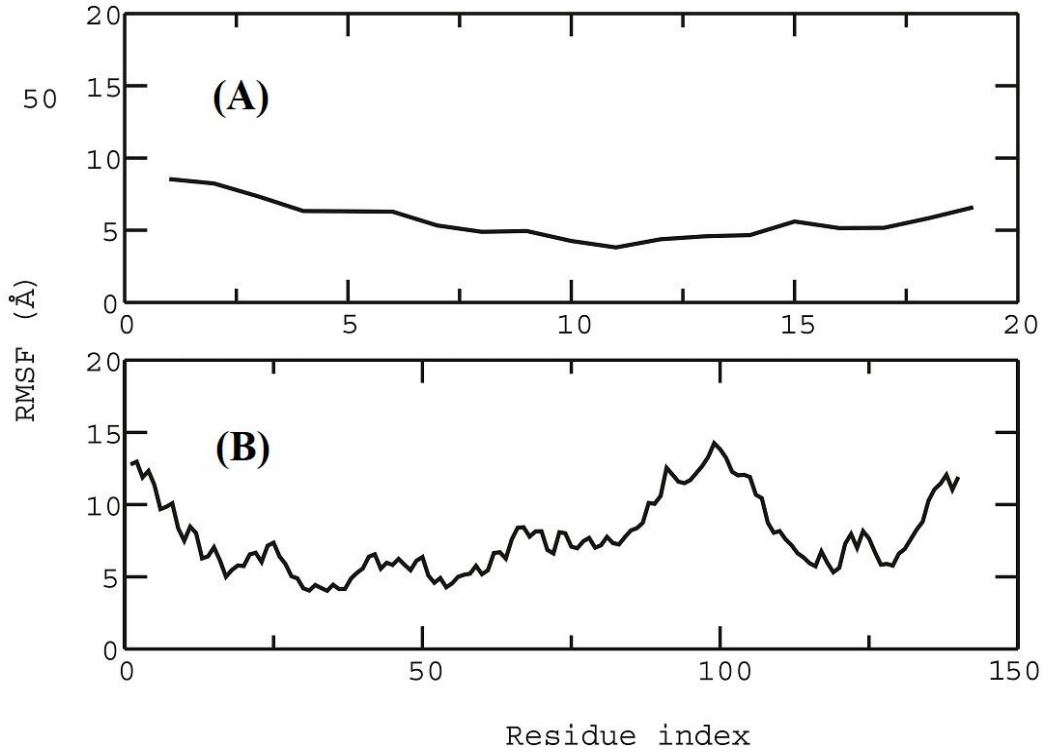

Figure 8.5. *Molecular Dynamics analysis of Root mean square fluctuation (RMSF) with respect to time period for the (α-Synuclein-K102s) complex for (A) peptide (K102s) and (B) α-Synuclein.*

8.4.2. Hydrogen bond Analysis:

The inter-molecular hydrogen bond analysis for the ligand and receptor components of (α-Synuclein-K84s) and (α-Synuclein-K102s) complex are shown in **Tables 8.1(A), 8.1(B)** and **Tables 8.2(A), 8.2(B)** respectively. To calculate the hydrogen bond, the cut-off for angle and distance was set to 120° and 3.5 Å respectively. The results were stipulated according to the occupancy, bond length, and the bond angle formed (HA–H–HD) between the hydrogen bond donor (HD) and acceptor (HA) atoms. The summary of the hydrogen bonds that are responsible for the stability of the two complexes are listed in the **Tables 8.1 and 8.2.**

Table 8.1(A). *Inter-molecular Hydrogen bond occupancy between ligand and receptor components of (α-Synuclein-K84s) complex considering K84s peptide as hydrogen donor and α-Synuclein as hydrogen acceptor*

Acceptor	DonorH	Donor	Average Distance (Å)	Average Angle(°)
LEU_7@O	VAL_90@H	VAL_90@N	2.8690	159.44
LEU_7@O	GLY_91@H	GLY_91@N	2.8773	147.4444
ARG_8@HA	TYR_89@HA	TYR_89@CA	2.9316	148.0497
LEU_2@O	LYS_82@HZ3	LYS_82@NZ	2.8606	157.2629
LEU_2@O	LYS_82@HZ1	LYS_82@NZ	2.8232	157.4491
ARG_8@HA	VAL_90@H	VAL_90@N	2.8505	147.8811
HIE_25@OXT	LYS_95@HZ2	LYS_95@NZ	2.769	158.1853
LEU_2@HD22	VAL_76@HA	VAL_76@CA	2.9394	145.1037
ARG_8@HA	TYR_89@HD1	TYR_89@CD1	2.9192	143.0079
LEU_2@HD23	VAL_76@HA	VAL_76@CA	2.9573	145.0862

Table 8.1(B). *Inter-molecular Hydrogen bond occupancy between ligand and receptor components of (α-Synuclein-K84s) complex considering α-Synuclein as hydrogen donor and K84s peptide as hydrogen acceptor*

Acceptor	DonorH	Donor	Average Distance (Å)	Average Angle(°)
VAL_98@O	ARG_24@HH11	ARG_24@NH1	2.8182	159.6087
GLU_78@OE1	LEU_2@H	LEU_2@N	2.8322	156.0124
LYS_95@O	SER_23@HG	SER_23@OG	2.7478	160.7089
GLU_78@OE1	PHE_1@H1	PHE_1@N	2.811	147.0814
GLU_78@OE2	LEU_2@H	LEU_2@N	2.8216	157.3895
GLU_78@OE1	PHE_1@H3	PHE_1@N	2.7953	147.9754
GLU_78@OE1	PHE_1@H2	PHE_1@N	2.7995	148.8844
TYR_89@HA	ARG_8@HA	ARG_8@CA	2.9313	146.6175
TYR_89@HD1	ARG_8@HA	ARG_8@CA	2.927	141.7441
GLY_75@O	HIE_18@HE2	HIE_18@NE2	2.8568	155.2709
GLU_85@OE2	ARG_24@HH22	ARG_24@NH2	2.8091	159.9239
VAL_90@H	ARG_8@HA	ARG_8@CA	2.8971	157.0215

Table 8.2(A). *Inter-molecular Hydrogen bond occupancy between ligand and receptor components of (α-Synuclein-K102s) complex considering K102s as hydrogen donor and α-Synuclein as hydrogen acceptor.*

Acceptor	DonorH	Donor	Average Distance (Å)	Average Angle (º)
VAL_86@O	ARG_7@HH11	ARG_7@NH1	2.8358	160.4316
LYS_81@O	SER_15@HG	SER_15@OG	2.691	161.3233
VAL_75@O	THR_13@HG1	THR_13@OG1	2.7978	174.6761
VAL_78@O	SER_15@H	SER_15@N	2.8918	178.4776
GLU_84@OE1	ARG_4@HH11	ARG_4@NH1	2.7666	158.3978
GLU_73@OE2	ARG_10@HH21	ARG_10@NH2	2.8153	174.7345
GLU_73@OE2	ARG_10@HH11	ARG_10@NH1	2.7972	156.7178
GLU_73@OE2	ARG_10@HE	ARG_10@NE	2.8338	156.9113
GLU_84@OE2	ARG_4@HH11	ARG_4@NH1	2.7783	178.1949
GLU_73@OE1	ARG_10@HH21	ARG_10@NH2	2.8138	157.2388

Table 8.2(B). *Inter-molecular Hydrogen bond occupancy between ligand and receptor components of (α-Synuclein-K102s) complex considering α-Synuclein as hydrogen donor and K102s as hydrogen acceptor.*

Acceptor	DonorH	Donor	Average Distance (Å)	Average Angle (º)
ALA_14@HA	VAL_78@H	VAL_78@N	2.8666	158.0145
SER_19@O	LYS_83@HZ2	LYS_83@NZ	2.7631	178.0613
SER_19@O	LYS_83@HZ1	LYS_83@NZ	2.7706	158.7538
SER_19@O	LYS_83@HZ3	LYS_83@NZ	2.7665	158.6272
SER_15@O	LYS_83@HZ2	LYS_83@NZ	2.8224	154.7149
SER_15@OG	TYR_77@HH	TYR_77@OH	2.7998	178.0073
GLY_17@O	LYS_83@HZ3	LYS_83@NZ	2.8106	150.4178
GLY_17@O	LYS_83@HZ1	LYS_83@NZ	2.8113	149.1762
GLY_17@O	LYS_83@HZ2	LYS_83@NZ	2.8125	149.3336
SER_15@O	LYS_83@HZ3	LYS_83@NZ	2.8243	156.1353

8.4.3. Contribution of interface residues:

From the PDBsum server analysis the interface residues in the (α-Synuclein-K84s) complex and the (α-Synuclein-K102s) complexes were indentified. In the (α-Synuclein-K84s) complex, the residues GLY 25, ALA 29, VAL 49, LEU 38, VAL 40, GLU 28, GLY 47, LYS 32, GLU 35, GLY 36, TYR 39, VAL 48 and VAL 26 (from αS) and SER 23, LEU 7, ILE 12, HIS 25, PHE 1, HIS 18, CYS 6, ARG 24, PRO 21 and ARG 8 (from K84s peptide) were identified to be present at the interface. The summary of hydrogen bond and non-bonded interactions observed in the (α-Synuclein-K84s) complex has been tabulated in **Tables 8.3(A) and 8.3(B)** respectively. The interface area shared by the αS and the peptide in forming complex along with the number of hydrogen bond and non-bonded interactions is shown in **Table 8.4.**

Table 8.3(A). *List of atom-atom interactions (4 Hydrogen bonds) across the protein-protein interface in (α-Synuclein–K84s peptide) complex from PDBsum server.*

K84s peptide (Chain A)				**Non-bonded contacts**	α-Synuclein (Chain B)				
Atom number	**Atom name**	**Residue name**	**Residue number**		**Atom number**	**Atom name**	**Residue name**	**Residue number**	**Distance (Å)**
1	N	PHE	1	<-->	784	OE2	GLU	28	3.06
23	N	LEU	2	<-->	784	OE2	GLU	28	3.21
118	O	LEU	7	<-->	950	N	VAL	40	2.71
341	NH1	ARG	24	<-->	1079	O	VAL	48	3.18

Table 8.3.(B) *List of atom-atom interactions (41 Non-bonded contacts) across the protein-protein interface in (α-Synuclein-K84s peptide) complex from PDBsum server.*

K84s peptide (Chain A)				**Non-bonded contacts**	α-Synuclein (Chain B)				
Atom number	**Atom name**	**Residue name**	**Residue number**		**Atom number**	**Atom name**	**Residue name**	**Residue number**	**Distance (Å)**
1	N	PHE	1	<-->	784	OE2	GLU	28	3.06
5	CA	PHE	1	<-->	784	OE2	GLU	28	3.36
21	C	PHE	1	<-->	784	OE2	GLU	28	3.79
23	N	LEU	2	<-->	784	OE2	GLU	28	3.21
36	CD2	LEU	2	<-->	745	O	GLY	25	3.15
36	CD2	LEU	2	<-->	748	CA	VAL	26	3.77
99	O	CYS	6	<-->	889	CA	GLY	36	3.77
96	SG	CYS	6	<-->	886	O	GLU	35	3.87
102	CA	LEU	7	<-->	928	O	LEU	38	3.72
117	C	LEU	7	<-->	950	N	VAL	40	3.67
118	O	LEU	7	<-->	931	CA	TYR	39	3.34
118	O	LEU	7	<-->	948	C	TYR	39	3.35
118	O	LEU	7	<-->	936	CG	TYR	39	3.81
118	O	LEU	7	<-->	937	CD1	TYR	39	3.13
118	O	LEU	7	<-->	939	CE1	TYR	39	3.55
118	O	LEU	7	<-->	950	N	VAL	40	2.71
118	O	LEU	7	<-->	952	CA	VAL	40	3.77
118	O	LEU	7	<-->	965	O	VAL	40	3.54
118	O	LEU	7	<-->	960	CG2	VAL	40	3.83
104	CB	LEU	7	<-->	960	CG2	VAL	40	3.85
113	CD2	LEU	7	<-->	928	O	LEU	38	3.72
113	CD2	LEU	7	<-->	914	CB	LEU	38	3.52
113	CD2	LEU	7	<-->	1063	O	GLY	47	3.57
126	CG	ARG	8	<-->	965	O	VAL	40	3.88
126	CG	ARG	8	<-->	960	CG2	VAL	40	3.63
175	CB	ILE	12	<-->	796	O	ALA	29	3.53
177	CG2	ILE	12	<-->	835	O	LYS	32	3.17

184	CD1	ILE	12	<-->	796	O	ALA	29	3.52
184	CD1	ILE	12	<-->	834	C	LYS	32	3.8
184	CD1	ILE	12	<-->	835	O	LYS	32	3.65
264	CE1	HIE	18	<-->	782	CD	GLU	28	3.82
264	CE1	HIE	18	<-->	783	OE1	GLU	28	3.68
290	CG	PRO	21	<-->	1068	CB	VAL	48	3.85
290	CG	PRO	21	<-->	1074	CG2	VAL	48	3.88
287	CD	PRO	21	<-->	1068	CB	VAL	48	3.67
321	OG	SER	23	<-->	1086	CG1	VAL	49	3.77
332	CG	ARG	24	<-->	1079	O	VAL	48	3.16
335	CD	ARG	24	<-->	1079	O	VAL	48	3.28
341	NH1	ARG	24	<-->	1078	C	VAL	48	3.81
341	NH1	ARG	24	<-->	1079	O	VAL	48	3.18
366	OXT	HIE	25	<-->	784	OE2	GLU	28	3.71

Table 8.4. *Interface statistics of (α-Synuclein-K84s) complex.*

System	No. of interface residues	Interface area (Å^2)	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
K84s	11	616	-	-	4	41
α-Synuclein	13	591				

In the(α-Synuclein-K102s) complex, the residues VAL40, GLY36, GLU35, TYR39, LYS45, LEU38, LYS43, VAL37, THR44, VAL49, VAL48 and GLU46 (from αS) and ARG 10, GLY 12, GLY 18, SER 15, THR 13, SER 19, TRP 11, ALA 14, CYS 16, ARG 7, ARG 4 and GLY 17 (from K102s peptide) were identified to be present at the interface. The summary of hydrogen bond, non-bonded, salt-bridge interactions observed in the (α-Synuclein-K102s) complex have been tabulated in **Tables 8.5(A), 8.5(B) and 8.5(C)** respectively. The interface area shared by the αS and the peptide in forming complex along with the number of hydrogen bond and non-bonded interactions is shown in **Table 8.6.** The contribution of the interface residues in the (α-Synuclein-K102s) complex and the (α-Synuclein-K102s) complex using PDBsum server is depicted in **Figures 8.6(A) and 8.6(B)** respectively. **Figures 8.7 and 8.8** shows the schematic representation of (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes respectively showing the residues of αS interacting with the novel peptides.

Table 8.5(A). *List of atom-atom interactions (9 Hydrogen bonds) across the protein-protein interface in (α-Synuclein - K102s peptide) complex from PDBsum server*

K102s peptide (Chain A)				**Hydrogen bonds**	α-Synuclein (Chain B)				
Atom number	**Atom name**	**Residue name**	**Residue number**		**Atom no.**	**Atom name**	**Residue name**	**Residue number**	**Distance (Å)**
80	NH1	ARG	4	<-->	985	OE2	GLU	46	3.05
83	NH2	ARG	4	<-->	984	OE1	GLU	46	2.75
184	NE	ARG	10	<-->	814	OE1	GLU	35	2.85
219	N	GLY	12	<-->	824	O	GLY	36	3.25
236	OG1	THR	13	<-->	824	O	GLY	36	2.84
257	OG	SER	15	<-->	873	OH	TYR	39	3.35
278	O	GLY	17	<-->	967	NZ	LYS	45	2.83
285	O	GLY	18	<-->	967	NZ	LYS	45	3.24
296	O	SER	19	<-->	967	NZ	LYS	45	2.68

Table 8.5(B). *List of atom-atom interactions (78 Non-bonded contacts) across the protein-protein interface in (α-Synuclein-K102s peptide) complex from PDBsum server*

K102s peptide (Chain A)				**Hydrogen bonds**	α-Synuclein (Chain B)				
Atom number	**Atom name**	**Residue name**	**Residue number**		**Atom no.**	**Atom name**	**Residue name**	**Residue number**	**Distance (Å)**
66	CA	ARG	4	<-->	1017	CG1	VAL	49	3.77
87	O	ARG	4	<-->	1017	CG1	VAL	49	3.66
79	CZ	ARG	4	<-->	983	CD	GLU	46	3.89
79	CZ	ARG	4	<-->	984	OE1	GLU	46	3.35
79	CZ	ARG	4	<-->	985	OE2	GLU	46	3.84
80	NH1	ARG	4	<-->	983	CD	GLU	46	3.42
80	NH1	ARG	4	<-->	984	OE1	GLU	46	3.06
80	NH1	ARG	4	<-->	985	OE2	GLU	46	3.05
83	NH2	ARG	4	<-->	983	CD	GLU	46	3.49
83	NH2	ARG	4	<-->	984	OE1	GLU	46	2.75
83	NH2	ARG	4	<-->	985	OE2	GLU	46	3.86
126	CB	ARG	7	<-->	1010	O	VAL	48	3.61
132	CD	ARG	7	<-->	1010	O	VAL	48	3.67
135	NE	ARG	7	<-->	1017	CG1	VAL	49	3.65
178	CG	ARG	10	<-->	814	OE1	GLU	35	3.81
184	NE	ARG	10	<-->	813	CD	GLU	35	3.6
184	NE	ARG	10	<-->	814	OE1	GLU	35	2.85
186	CZ	ARG	10	<-->	814	OE1	GLU	35	3.36
186	CZ	ARG	10	<-->	815	OE2	GLU	35	3.8
190	NH2	ARG	10	<-->	813	CD	GLU	35	3.33
190	NH2	ARG	10	<-->	814	OE1	GLU	35	3

190	NH2	ARG	10	<-->	815	OE2	GLU	35	2.89
212	CZ3	TRP	11	<-->	999	CB	VAL	48	3.67
212	CZ3	TRP	11	<-->	1001	CG1	VAL	48	3.42
212	CZ3	TRP	11	<-->	1005	CG2	VAL	48	3.82
210	CH2	TRP	11	<-->	999	CB	VAL	48	3.79
210	CH2	TRP	11	<-->	1001	CG1	VAL	48	3.27
219	N	GLY	12	<-->	820	CA	GLY	36	3.64
219	N	GLY	12	<-->	824	O	GLY	36	3.25
221	CA	GLY	12	<-->	824	O	GLY	36	3.24
224	C	GLY	12	<-->	824	O	GLY	36	3.36
226	N	THR	13	<-->	824	O	GLY	36	3.18
238	C	THR	13	<-->	859	O	LEU	38	3.86
239	O	THR	13	<-->	868	CD1	TYR	39	3.56
230	CB	THR	13	<-->	824	O	GLY	36	3.61
230	CB	THR	13	<-->	859	O	LEU	38	3.82
236	OG1	THR	13	<-->	823	C	GLY	36	3.88
236	OG1	THR	13	<-->	824	O	GLY	36	2.84
236	OG1	THR	13	<-->	827	CA	VAL	37	3.46
236	OG1	THR	13	<-->	839	C	VAL	37	3.86
240	N	ALA	14	<-->	859	O	LEU	38	3.21
242	CA	ALA	14	<-->	859	O	LEU	38	3.65
242	CA	ALA	14	<-->	868	CD1	TYR	39	3.87
244	CB	ALA	14	<-->	859	O	LEU	38	3.42
244	CB	ALA	14	<-->	881	N	VAL	40	3.84
250	N	SER	15	<-->	896	O	VAL	40	3.58
252	CA	SER	15	<-->	936	O	LYS	43	3.73
259	C	SER	15	<-->	936	O	LYS	43	3.4
260	O	SER	15	<-->	936	O	LYS	43	3.79
260	O	SER	15	<-->	951	N	LYS	45	3.73
260	O	SER	15	<-->	958	CG	LYS	45	3.73
260	O	SER	15	<-->	964	CE	LYS	45	3.85
254	CB	SER	15	<-->	936	O	LYS	43	3.19
257	OG	SER	15	<-->	870	CE1	TYR	39	3.2
257	OG	SER	15	<-->	872	CZ	TYR	39	3.6
257	OG	SER	15	<-->	873	OH	TYR	39	3.35
257	OG	SER	15	<-->	896	O	VAL	40	3.8
261	N	CYS	16	<-->	936	O	LYS	43	3.57
263	CA	CYS	16	<-->	949	C	THR	44	3.49
263	CA	CYS	16	<-->	950	O	THR	44	3.35
263	CA	CYS	16	<-->	951	N	LYS	45	3.65
263	CA	CYS	16	<-->	953	CA	LYS	45	3.82
271	O	CYS	16	<-->	953	CA	LYS	45	3.72
271	O	CYS	16	<-->	1005	CG2	VAL	48	3.81
265	CB	CYS	16	<-->	949	C	THR	44	3.74
265	CB	CYS	16	<-->	950	O	THR	44	3.09
265	CB	CYS	16	<-->	1001	CG1	VAL	48	3.45

265	CB	CYS	16	<-->	1005	CG2	VAL	48	3.88
277	C	GLY	17	<-->	967	NZ	LYS	45	3.88
278	O	GLY	17	<-->	964	CE	LYS	45	3.82
278	O	GLY	17	<-->	967	NZ	LYS	45	2.83
284	C	GLY	18	<-->	967	NZ	LYS	45	3.65
285	O	GLY	18	<-->	958	CG	LYS	45	3.67
285	O	GLY	18	<-->	967	NZ	LYS	45	3.24
295	C	SER	19	<-->	967	NZ	LYS	45	3.7
296	O	SER	19	<-->	961	CD	LYS	45	3.82
296	O	SER	19	<-->	964	CE	LYS	45	3.57
296	O	SER	19	<-->	967	NZ	LYS	45	2.68

Table 8.5(C). *List of atom-atom interactions (2 salt-bridges) across the protein-protein interface in (α-Synuclein-K102s peptide) complex from PDBsum server*

K102s peptide (Chain A)				**Hydrogen bonds**	α-Synuclein (Chain B)				
Atom number	**Atom name**	**Residue name**	**Residue number**		**Atom no.**	**Atom name**	**Residue name**	**Residue number**	**Distance (Å)**
83	NH2	ARG	4	<-->	985	OE2	GLU	46	2.75
184	NE	ARG	10	<-->	814	OE1	GLU	35	2.85

Table 8.6. *Interface statistics of (α-Synuclein-K102s)complex.*

System	**No. of interface residues**	**Interface area (Å^2)**	**No. of salt bridges**	**No. of disulphide bonds**	**No. of hydrogen bonds**	**No. of non-bonded contacts**
K102s	12	628	2	-	9	78
α-Synuclein	12	639				

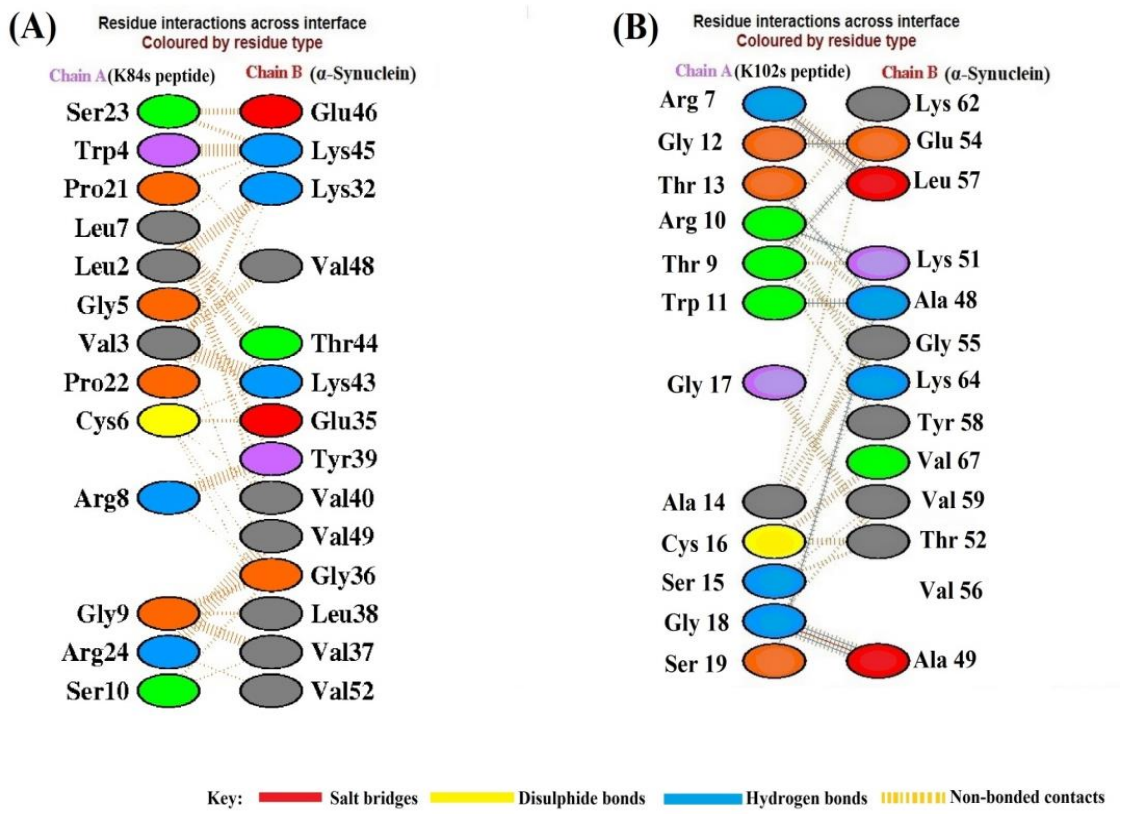

Figure 8.6. *Schematic representation of interacting residues of (A) K84s with residues of α-Synuclein and (B) K102s with residues of α-Synuclein*

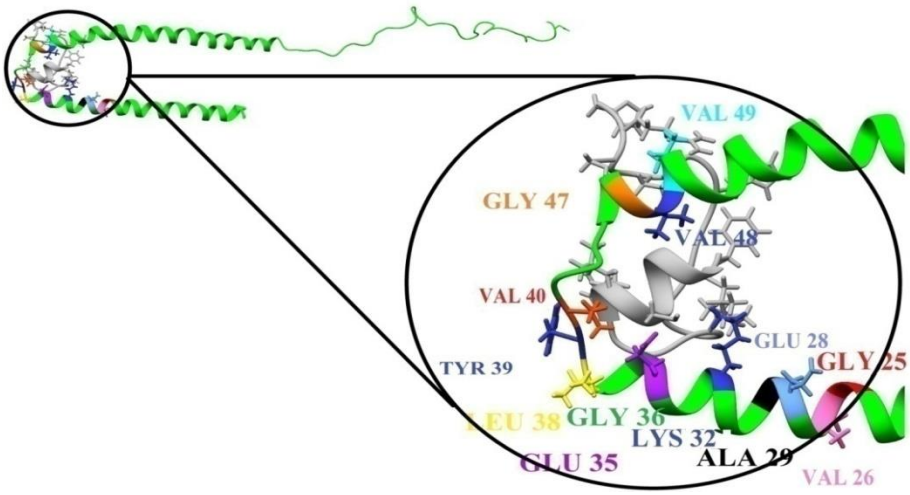

Figure 8.7. *Schematic representation of (α-Synuclein-K84s) complex showing the residues of α-Synuclein interacting with K84s peptide*

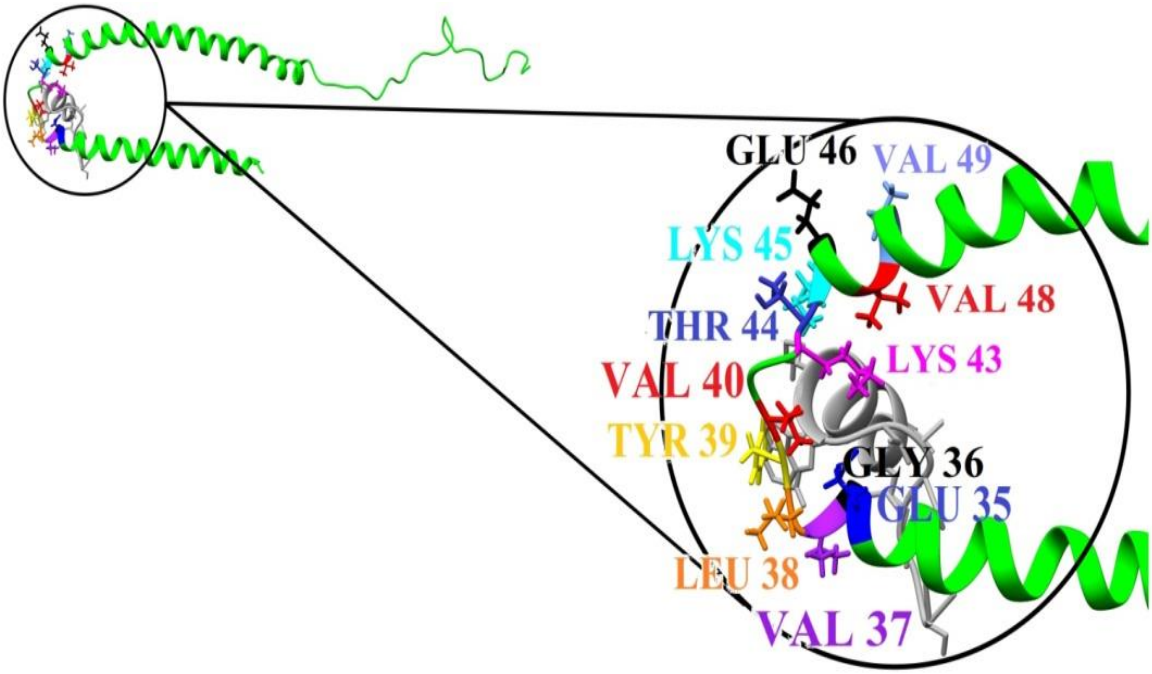

Figure 8.8. *Schematic representation of (α-Synuclein-K102s) complex showing the residues of α-Synuclein interacting with K102s peptide.*

8.4.4. Secondary structure analysis:

The secondary structure analysis for the two complexes was carried out using the Kabsch and Sander algorithm incorporated in their DSSP (Dictionary of Secondary Structure for Protein) program. The probable secondary structure that can be adopted in the case of the apo and complex form of αS as a function of residue index have been quantified as shown in **Figures 8.9 and 8.10** respectively. It has been observed that the helical content in the secondary structure of both the complexes is holding. The presence of anti-parallel β-sheets is lower in the (α-Synuclein-K102s) complex than in the (α-Synuclein-K84s) complex. These observations are compared with the secondary structure analysis performed for apo of αS [540], where rapid changes are seen in the secondary structure in the N-terminal and NAC domain of αS. The presence of anti-parallel β-sheets is more in the apo structure of αS than in the (α-Synuclein-K102s) complex. This means that the binding of K102s peptide has helped in preventing the aggregation of αS. This inference can be correlated to the experimental analysis performed by B.Popova et. al [665], where it has been reported that the peptides K84s, K102s significantly decreased the accumulation of soluble oligomeric species *in vitro*. The authors of this work suggested that K84s and K102s peptides as potent suppressors

of toxicity by decreasing the load of oligomeric species and inhibiting the aggregation process of αS.

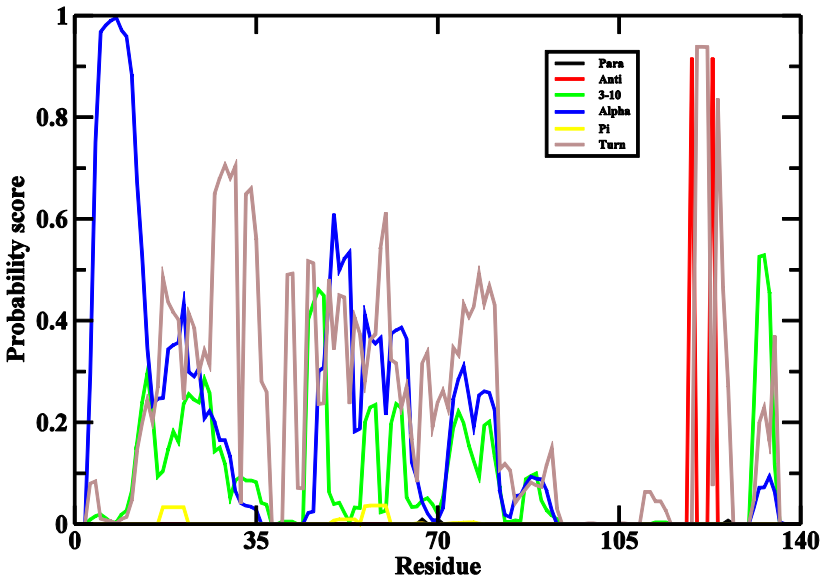

Figure 8.9. *Probability score of the residues of α-Synuclein of (α-Synuclein-K84s) complex*

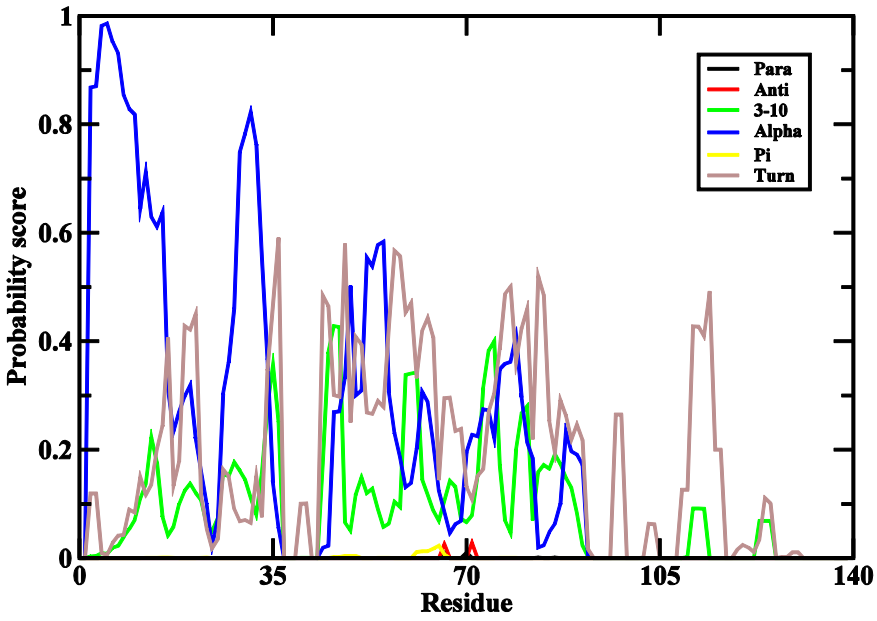

Figure 8.10. *Probability score of the residues of α-Synuclein of (α-Synuclein-K102s) complex*

8.4.5. Binding free energy calculation:

The MM-GBSA technique is used to calculate the binding free energy of (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes. This approach yields accurate results at a cheaper cost and provide information about the various contributions to free energies such as van der Waals, electrostatic, and solvation energy. **Tables 8.7 and 8.8** describe the features of the BFE profile using MM-GBSA calculations for the (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes.The ΔGB_{TOTAL} value for the (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes are -33.61 kcal mol^{-1} and -40.88 kcal mol^{-1} respectively. From these values, it can be inferred that peptides K84s and K102s are strongly bound to the protein αS exclusively.

Table 8.7. *The various components of the Binding Free Energy (kcal mol^{-1}) calculated by Molecular Mechanics-Generalized Borne Surface Area (MM-GBSA) method for (α-Synuclein-K84s) complex.*

Energy components	**COMPLEX (α-Synuclein-K84s)**	**LIGAND (K84s)**	**RECEPTOR (α-Synuclein)**	**DELTA** $\Delta G_{\alpha\text{-Synuclein-K84s}}$ - (ΔG_{K84s}+ $\Delta G_{\alpha\text{-Synuclein}}$)
	Energy (kcal mol^{-1})	**Energy (kcal mol^{-1})**	**Energy (kcal mol^{-1})**	**Energy (kcal mol^{-1})**
E_{vdw}	-771.24	-127.31	-585.34	-58.59
E_{ele}	-10186.02	-1573.56	-8535.51	-76.95
E_{PB}	-4750.45	-321.87	-4538.56	109.98
E_{SURF}	107.41	15.41	100.03	-8.03
G_{gas}	-10957.26	-1700.87	-9120.84	-135.55
G_{solv}	-4643.04	-306.46	-4438.53	101.94
GB_{TOTAL}	**-15600.31**	**-2007.33**	**-13559.37**	**-33.61**

*Abbreviations expanded under **Table 5.4.**

Table 8.8. *The various components of the Binding Free Energy (kcal mol^{-1}) calculated by Molecular Mechanics-Generalized Borne Surface Area (MM-GBSA) method for (α-Synuclein-K102s) complex.*

Energy components	**COMPLEX (α-Synuclein-K102s)**	**LIGAND (K102s)**	**RECEPTOR (α-Synuclein)**	**DELTA** $\Delta G_{\alpha\text{-Synuclein-K102s}}$ - $(\Delta G_{K102s} + \Delta G_{\alpha\text{-Synuclein}})$
	Energy (kcal mol^{-1})	**Energy (kcal mol^{-1})**	**Energy (kcal mol^{-1})**	**Energy (kcal mol^{-1})**
E_{vdw}	-699.79	-92.60	-549.73	-57.46
E_{ele}	-9774.78	-687.78	-8930.59	-156.40
E_{PB}	-4691.74	-657.11	-4216.42	181.79
E_{SURF}	109.61	13.64	104.78	-8.81
G_{gas}	-10474.57	-780.39	-9480.32	-213.86
G_{solv}	-4582.13	-643.47	-4111.64	172.98
GB_{TOTAL}	**-15056.70**	**-1423.86**	**-13591.96**	**-40.88**

*Abbreviations expanded under **Table 5.4.**

The PRED analysis graphs for the peptide K84s and αS components present in the (α-Synuclein-K84s) complex are depicted in **Figures 8.11** and **8.12**, respectively. The PRED analysis plots for the peptide K102s and αS components present in the (α-Synuclein-K102s) complex are shown in **Figures 8.13 and 8.14**, respectively. The PRED analysis utilising the MM-GBSA algorithm revealed that the peptide to αS binding affinity is indeed high, and their intermolecular interaction can be attributed to the residues PHE 1, LEU 7, ILE 12, LEU 2, VAL 3, GLY 5 and PRO 21 of K84s peptide and residues VAL 48, ALA 29, VAL 40, TYR 39, VAL 49, VAL 26 and GLY 36 present in the αS of the (α-Synuclein-K84s) complex. In case of the (α-Synuclein-K102s) complex, the inter-molecular interaction can be credited to the residues ARG 4, ARG 10, TRP 11, ALA 14, SER 15, CYS 16 and SER 19 of K102s peptide and residues GLU 46, LYS 45, VAL 49, GLU 35, VAL 48, TYR 39 and VAL 40 of αS. In the experimental study by B. Popova et. al [665], they concluded that peptides obtained from the screenings (K84s and K102s notably) may help to identify the amino acids involved in

interaction and rescue function. Henceforth, the PRED analysis alongwith the PDBsum server analysis has helped us in the identification of the interacting residues of the two novel peptides K84s and K102s with the αS protein.

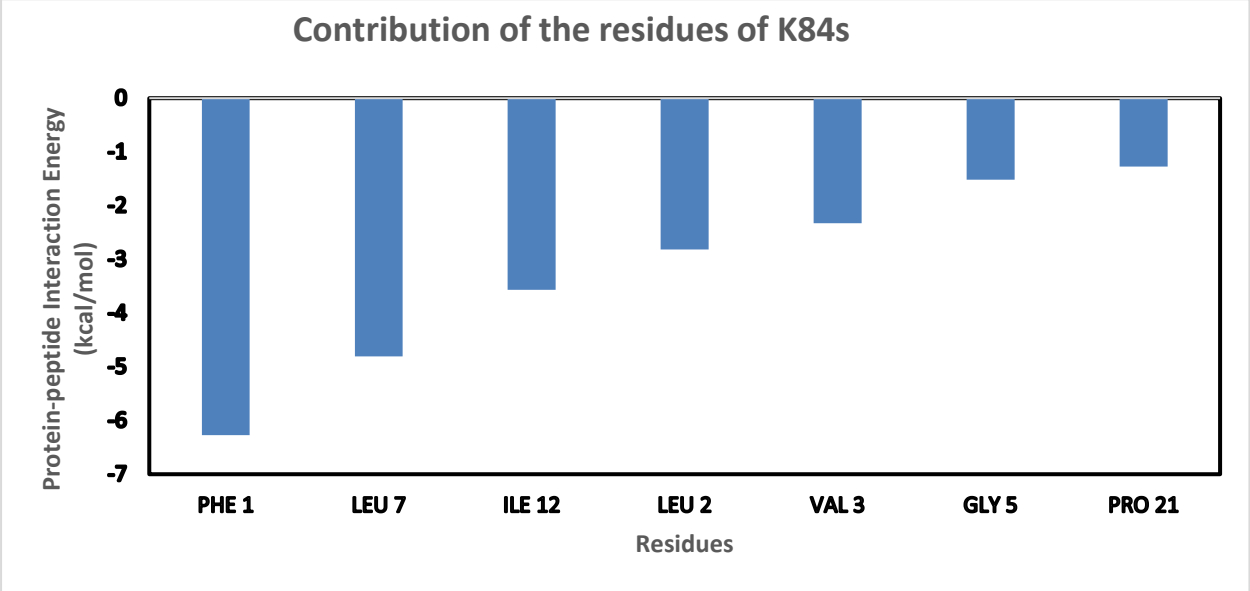

Figure 8.11. *Per-residue energy decomposition (PRED) plots for the interface residues of K84s in the (α-Synuclein-K84s) complex calculated by MM-GBSA method.*

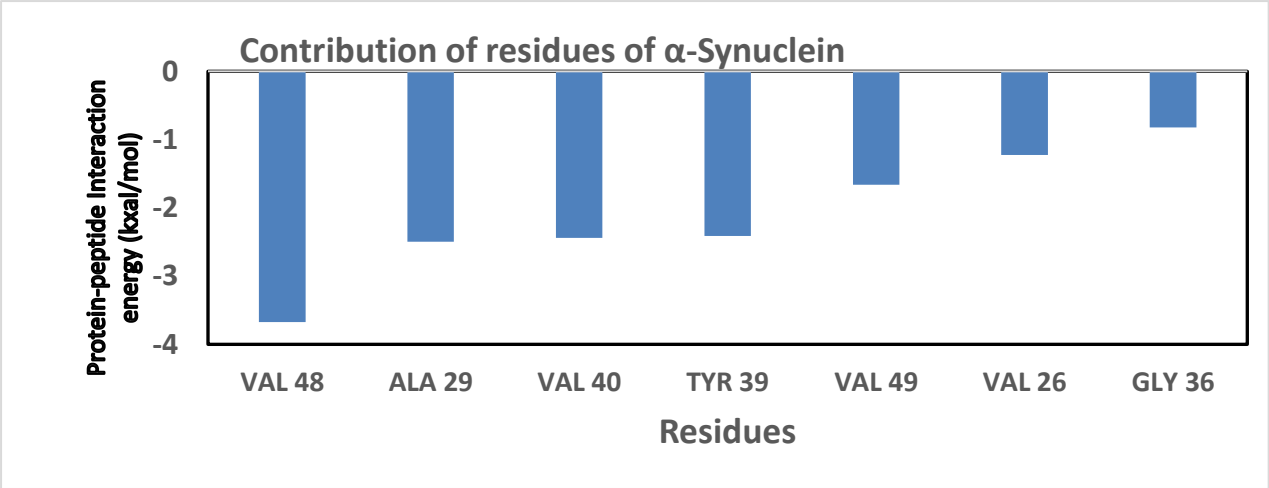

Figure 8.12. *Per-residue energy decomposition (PRED) plots for the interface residues of α-Synuclein in the (α-Synuclein-K84s) complex calculated by MM-GBSA method.*

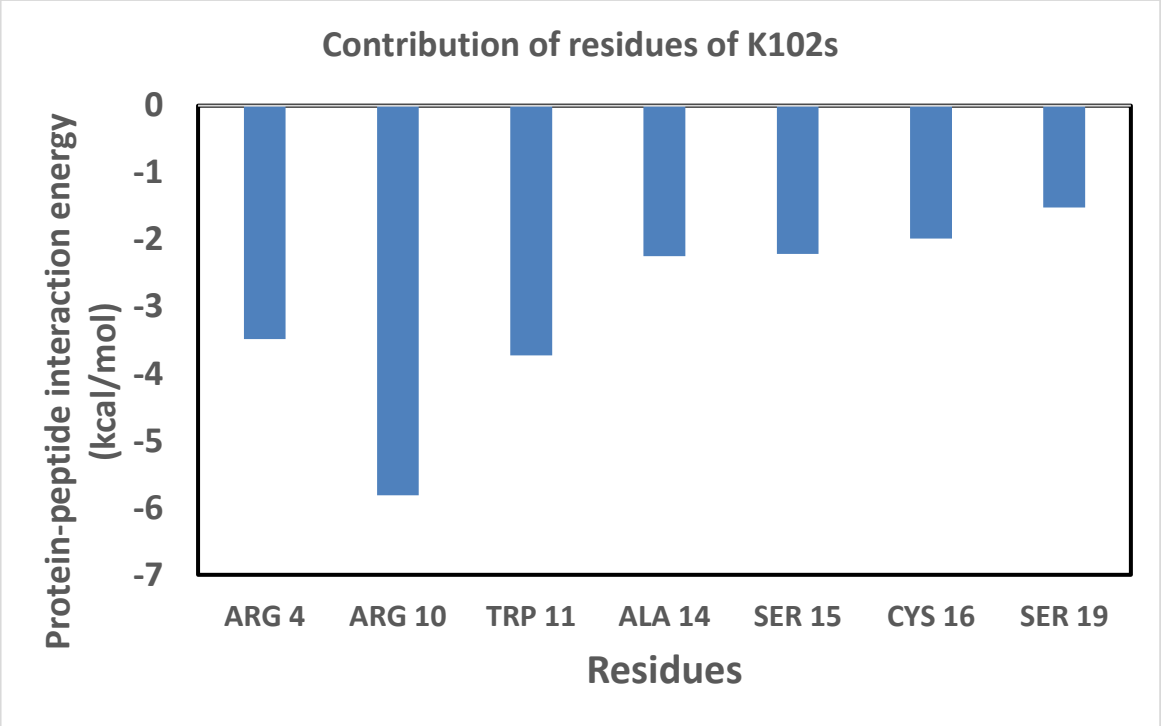

Figure 8.13. *Per-residue energy decomposition (PRED) plots for the interface residues of K102s in the (α-Synuclein-K102s) complex calculated by MM-GBSA method.*

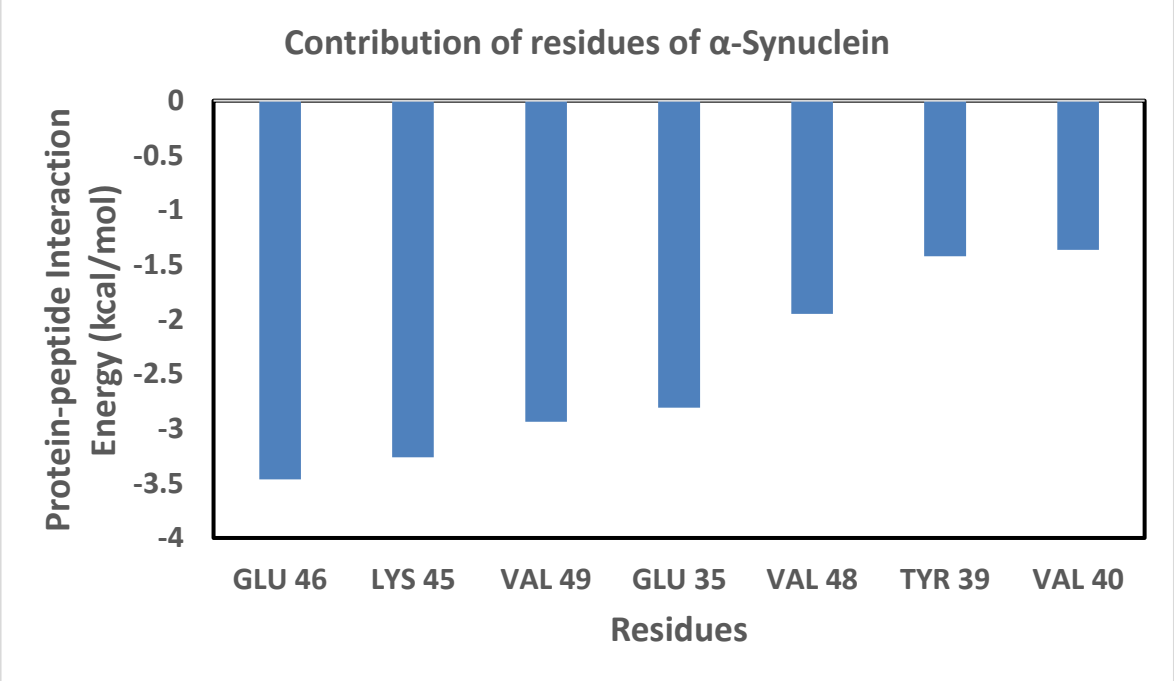

Figure 8.14. *Per-residue energy decomposition (PRED) plots for the interface residues of α-Synuclein in the (α-Synuclein-K102s) complex calculated by MM-GBSA method.*

8.5. Conclusion:

This computational study has provided information on the interaction of the residues of αS with the two novel peptides K84s and K102s. MD Simulation study performed on the (α-Synuclein-K84s) and (α-Synuclein-K102s) complexes have helped to understand the stability of the two complexes and also study in detail the fluctuation of the residues in both the complexes. From the BFE analysis, the binding affinity was found to be indeed high for both the complexes: BFE for (α-Synuclein-K84s) complex is -33.61 kcal mol^{-1} and (α-Synuclein-K102s) complex is -40.88 kcal mol^{-1}. From the PDBsum server analysis, the residues involved in the interaction in the (α-Synuclein-K84s) complex were found to be GLY 25, ALA 29, VAL 49, LEU 38, VAL 40, GLU 28, GLY 47, LYS 32, GLU 35, GLY 36, TYR 39, VAL 48 and VAL 26 (from αS) and SER 23, LEU 7, ILE 12, HIS 25, PHE 1, HIS 18, CYS 6, ARG 24, PRO 21 and ARG 8(from K84s peptide). The residues involved in interaction in the (α-Synuclein-K102s) complex were found to be VAL40, GLY36, GLU35, TYR39, LYS45, LEU38, LYS43, VAL37, THR44, VAL49, VAL48 and GLU46 (from αS) and ARG 10, GLY 12, GLY 18, SER 15, THR 13, SER 19, TRP 11, ALA 14, CYS 16, ARG 7,ARG 4 and GLY 17 (from K102s peptide). The PRED analysis using MM-GBSA algorithm showed that their intermolecular interaction can be credited to the residues PHE 1, LEU 7, ILE 12, LEU 2, VAL 3, GLY 5 and PRO 21 of K84s peptide and residues VAL 48, ALA 29, VAL 40, TYR 39, VAL 49, VAL 26and GLY 36 present in the αS of (α-Synuclein-K84s) complex. In case of the (α-Synuclein-K102s) complex, the inter-molecular interaction can be credited to the residues ARG 4, ARG 10, TRP 11, ALA 14, SER 15, CYS 16 and SER 19 of K102s peptide and residues GLU 46, LYS 45, VAL 49, GLU 35, VAL 48, TYR 39 and VAL 40 of αS. It has also been noticed that the αS has retained the helical content to significant extent when it is in complex form with the K84s and K102s peptides. Our findings from this study have added information in better understanding the intermolecular interaction of the residues of αS with the two novel peptides.