

In Silico Identification of a TDP-43–AKT2 Interaction: A Novel Candidate Driver of Schwann Cell Dysfunction, Peripheral Glial Neuroinflammation and Neurodegeneration in ALS

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Introduction/Hypothesis

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of central nervous system motor neurons. A pathological hallmark of ALS is dysfunction of Transactive Response DNA-binding Protein 43 (TDP-43), an RNA-binding protein encoded by TARDBP. In ALS, TDP-43 mislocalization to the cytoplasm forms neurotoxic aggregates [1]. The role of glial cells in disease progression is under investigation, with studies finding TDP-43 inclusions in glial cells [2]. Among glial cells, the role of Schwann Cells in disease pathophysiology has gained a spotlight [3]. Demyelination has been associated with ALS progression [4], although the mechanisms by which Schwann Cell TDP-43 inclusions impair myelin protein synthesis remain obscure. Furthermore, how Schwann Cell demyelination leads to downstream neuroinflammation and neurodegeneration remains uncharacterized.

Prior to phosphorylation, TDP-43 can also be found in the cytoplasm in the form of liquid droplets [5]. Impairment of nucleocytoplasmic transport leads to passive cytoplasmic leakage of unphosphorylated TDP-43 in neurons [5][6]. Given that this impairment pathway is common for primary glial cells [7], we suggest Schwann cells are subject to the same transport vulnerabilities and hypothesize that initial cytoplasmic flooding of unphosphorylated TDP-43 in SCs harms the myelinating AKT/PI3K pathway by causing dysfunction of AKT2, a key protein in myelin synthesis [8], through interaction of the two proteins. This would lead to impaired myelin maintenance with dysfunctional lipid metabolism [9]. Dysfunctional SC lipid metabolism causes abnormalities, including mitochondrial deficits and accumulation of acylcarnitines [9]. A separate study demonstrated that acylcarnitines activate pro-inflammatory pathways [10]. Consequently, SC metabolic dysfunction may exacerbate neuroinflammation. Peripheral neuroinflammation would, in turn, enhance neurodegeneration by driving distal axonopathy [11].

Methods

I] Static Docking Analysis

Static Docking Analysis was done using HDOCK [12][13][14][15][16] and HawkDock [17][18][19][20] between AKT isoforms and TDP-43 to confirm AKT2 selectivity. Actin, GAPDH, and MAPK1 were assigned as negative controls.

Additionally, static MM/GBSA analysis was performed using the prebuilt HawkDock system [21][22][23][24][25], and the values were used to compare docking qualities with control and AKT isoform samples, since MM/GBSA accounts for Van Der Waals interactions. The top-scoring model was then run for interface residue analysis using HDOCK to check for key residues.

2] Molecular Dynamics (MD)

Following Static Docking analysis, molecular dynamics were studied using GROMACS (version 2021.7). Due to technical limitations, the production MD simulation was restricted to 40 ns, executed with the leapfrog integrator under NPT conditions. The following analyses were done:

- Root Mean Square Deviation (RMSD) was calculated to assess the conformational stability of the TDP-43–AKT2 complex over time.
- The number of intermolecular contacts between TDP-43 and AKT2 was analyzed, averaging over 40 nanoseconds, to roughly evaluate the physical interface size.
- Radius of Gyration (Rg) analysis was performed over 40 ns to assess the compactness of the AKT2-TDP-43 complex.
- Hydrogen bond analysis was performed across the 40 ns production run to monitor the persistence and dynamics of key non-covalent interactions.

Results/Figures

Target Protein	Category	Top Docking Score (Rank 1)	Top 5 Score Range
AKT2	Kinase Target	−5592.61	−5592.61 to −4322.81
AKT1	Kinase Target	−4474.48	−4474.48 to −4237.18
AKT3	Kinase Target	−4520.80	−4520.80 to −4240.90
Actin	Negative Control	−5669.41	−5669.41 to −4121.69
GAPDH	Negative Control	−5423.07	−5423.07 to −4654.90
MAPTK1	Negative Control	−4918.60	−4918.60 to −4465.76

Figure 1: Table summarizing docking scores of each Target Protein with the top 5 models

Docking Results on HDOCK and HawkDock revealed that AKT2 + TDP-43 complex was the second best by rank.

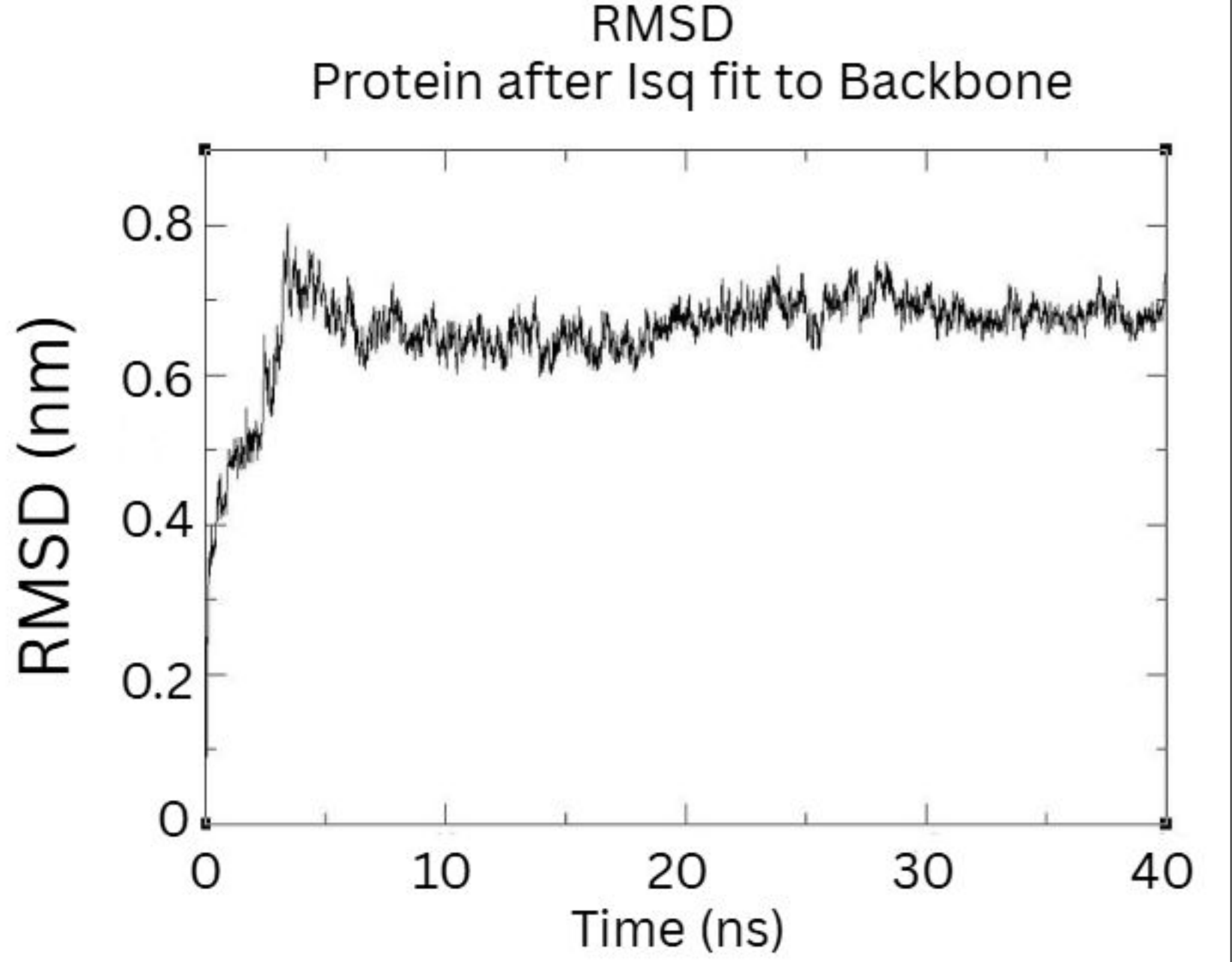


Figure 4: RMSD in nanometers (nm) averaging over 40 nanoseconds

Following up with the Molecular Dynamics (MD), RMSD increased during the first ~ 3 ns, reflecting structural relaxation of the docked complex, before reaching a relatively stable plateau around 0.66–0.70 nm. For a big complex that is AKT2+TDP, a 0.7 nm plateau is common, confirming structural stability of the complex.

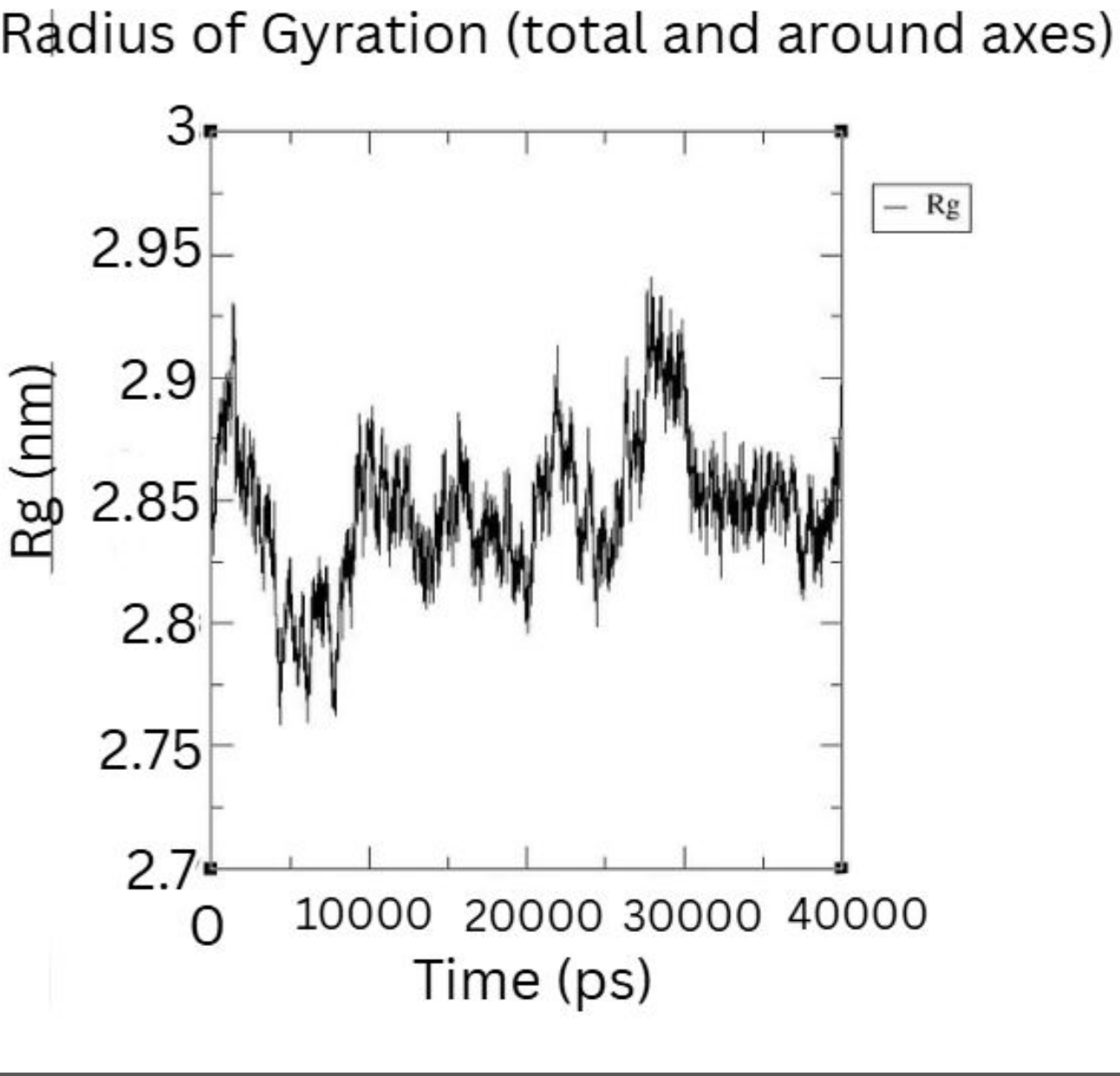


Figure 7: Radius of Gyration (Rg) in nanometers (mn) over 40 000 picoseconds (~40 ns)

The TDP-43-AKT2 complex exhibited high structural compactness throughout the 40 ns trajectory, with Rg values fluctuating minimally between 2.76 nm and 2.94 nm. Following initial interfacial packing during the first 7 ns, the complex maintained a highly stable baseline 2.85 nm, confirming that no global unfolding or complex dissociation occurred.

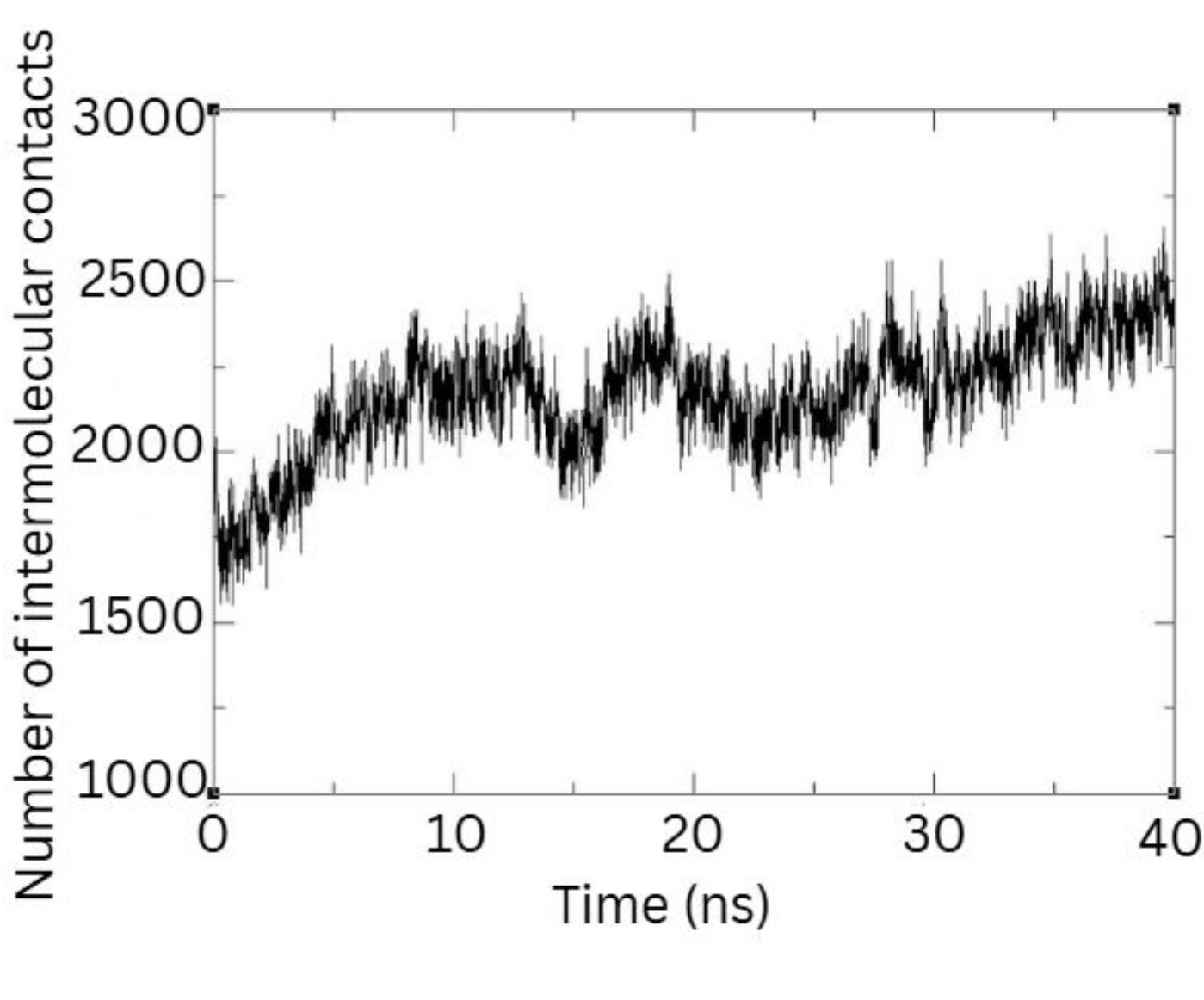


Figure 6: Number of intermolecular contacts averaging over 40 nanoseconds

The results of the number of intermolecular contacts analysis revealed persistent intermolecular association throughout the 40 nanoseconds trajectory.

Model 1		
#	Receptor interface residue(s):	
TRP	80A	4.360
VAL	140A	4.324
ARG	144A	3.479
LYS	146A	4.552
ASP	192A	3.393
VAL	194A	3.456
ALA	195A	3.147
VAL	198A	4.032
THR	199A	3.177
ARG	202A	3.354
VAL	203A	4.123
ASN	206A	2.770
THR	207A	3.702
ARG	208A	2.493
HIS	209A	3.905
SER	261A	4.176
GLU	264A	2.698
TYR	265A	2.030
SER	268A	2.776
ARG	269A	2.245
ASP	270A	4.090
VAL	271A	4.245
GLU	299A	4.715
SER	302A	3.243
ASP	303A	2.435
ARG	329A	4.777
LYS	401A	2.055
MET	404A	2.915
TRP	414A	3.291
GLN	415A	2.267
ASP	416A	4.458
VAL	418A	2.719
GLN	419A	3.434
THR	468A	2.441
HIS	469A	4.175
GLN	472A	3.412
SER	474A	2.818
TYR	475A	3.448
SER	476A	2.144
ALA	477A	3.741
SER	478A	2.990

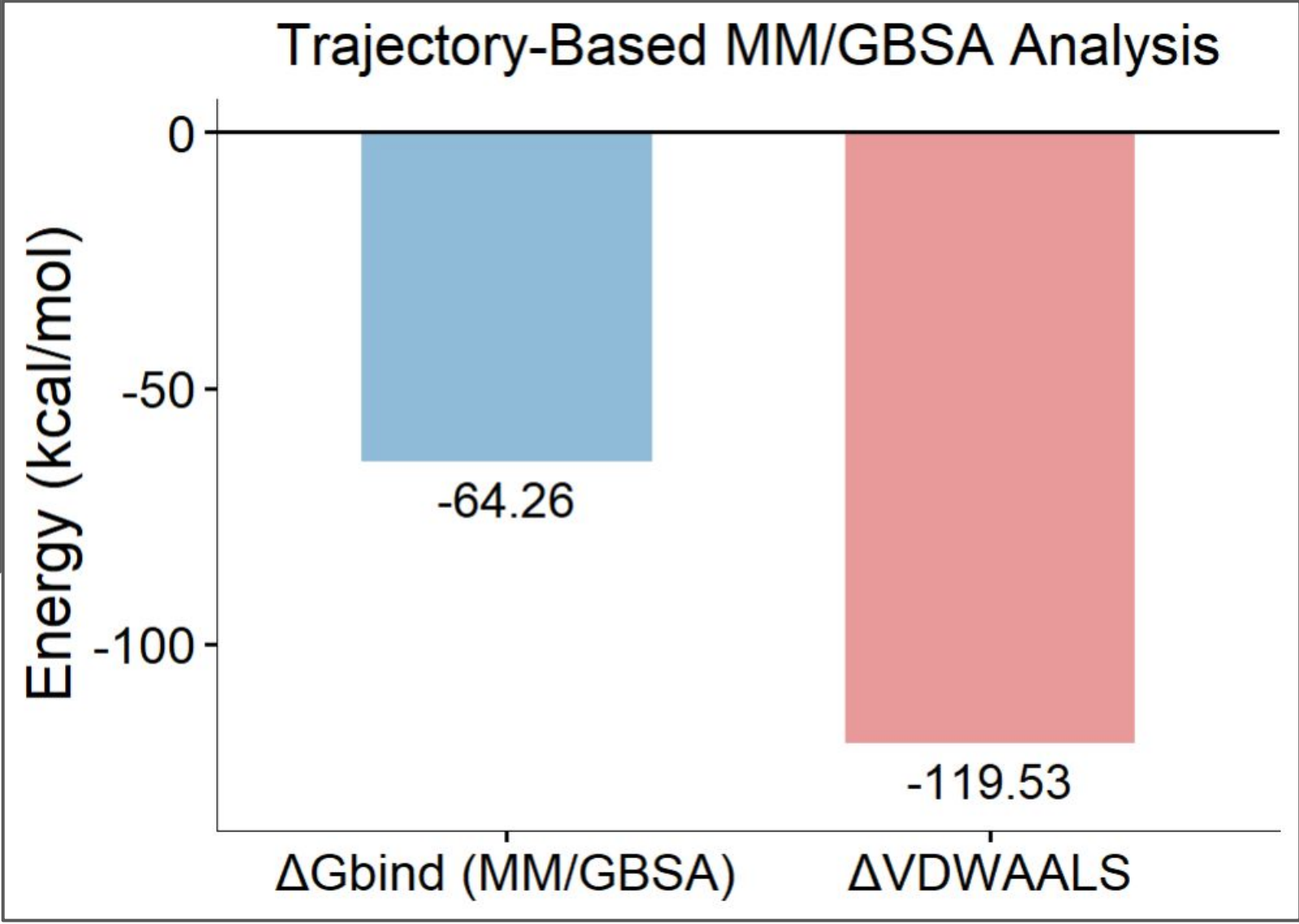


Figure 5: Trajectorial MM/GBSA with free binding energies (G) and Van der Waals energies (V) in kcal/mol

Results show -64.26 kcal/mol for MM/GBSA and -119.53 for Van Der Waals forces, supporting favorable complex stability and association.

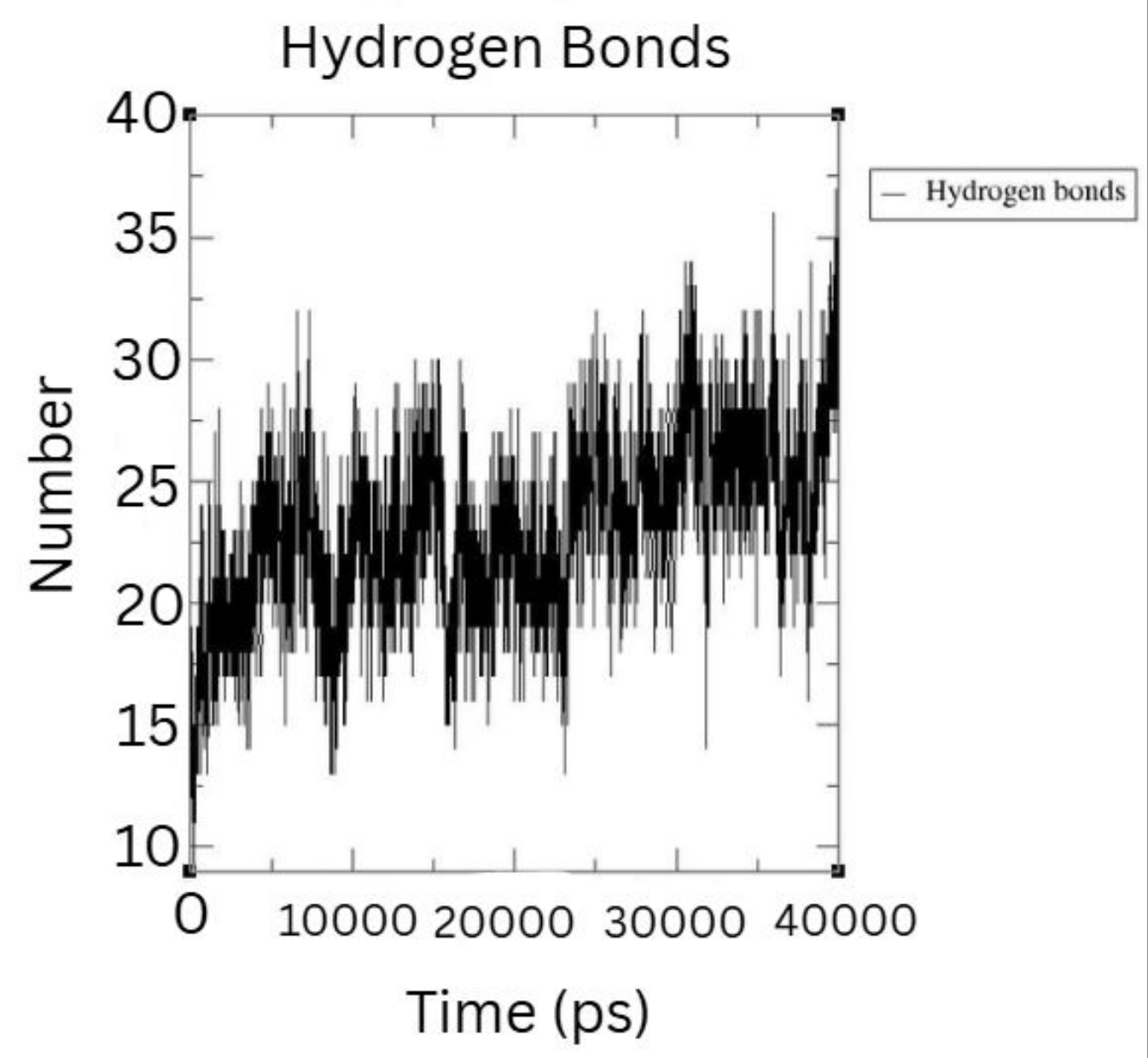


Figure 8: Number of Hydrogen Bonds over 40 ns

Following an initial equilibration period, the average number of hydrogen bonds increased, reaching 23.11 ± 3.73, suggesting that structural relaxation of the docked complex allowed optimization of the binding interface. The absence of prolonged losses in hydrogen bonding indicates that the interaction interface remained continuously engaged.

Figure 3: List of AKT2 interface residues for the complex

TDP-43 binds directly across the catalytic protein kinase domain (residues 152 to 409) and the C-terminal regulatory tail of AKT2. Crucially, TDP-43 establishes a direct interaction with Ser-474 (measured at an interatomic distance of 2.818 Angstroms), required for AKT2 activation. Suggesting that TDP may prevent its activation and inhibit its kinase function.

Results and Figures

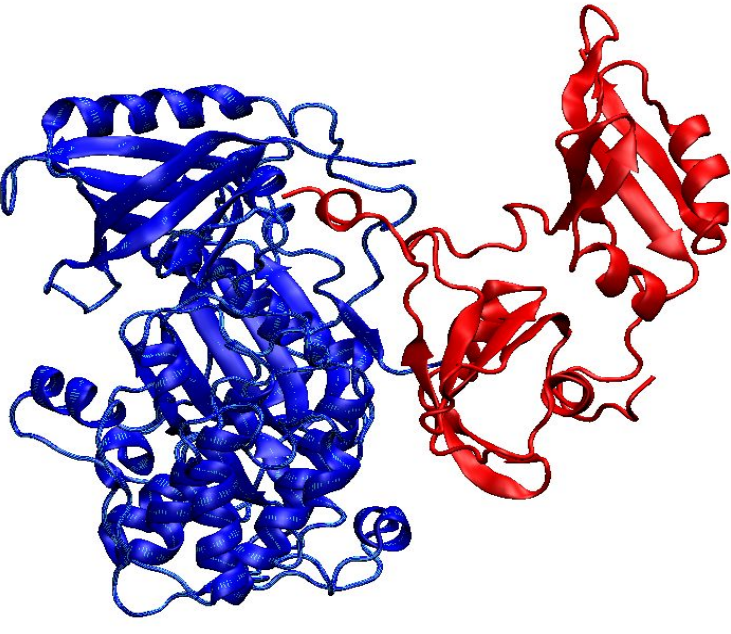


Figure 2 : AKT2 + TDP-43 Complex. Red = AKT2 Blue = TDP-43. 642 residues in total.

Static MM/GBSA revealed : AKT2 + TDP-43 show -46.01 kcal/mol , -21.44 kcal.mol for AKT1 + TDP-43 ; -30.01 for AKT3 + TDP-43 , -33.01 for Actin + TDP-43 ; -15.17 for GAPDH + TDP-43 ; -38.89 for MAPTK1 + TDP-43A. AKT2 + TDP-43 presented the highest score among control and other AKT isoforms with TDP-43, suggesting a favorable static association.

Conclusion

Rise in cytoplasmic unphosphorylated TDP-43



AKT/PI3K Dysfunction via AKT2 dysfunction



SC Lipid Metabolism Dysfunction



Acylcarnitines-mediated Neuroinflammation



Distal Axonopathy



Neurodegeneration

Both the static docking analysis and Molecular Dynamics provide preliminary evidence that the AKT2–TDP-43 complex is stable over short timescales. These findings support the hypothesis that AKT2 and TDP-43 can form a stable interaction, at least in the short term, which raises the possibility that longer-term interactions could be relevant to pathways of neuroinflammation and neurodegeneration, following the pathway detailed with the figure 9, supported by literature.

Figure 9 : Visual representation of the hypothesized pathway

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