A Molecular Dynamic Study of TAR DNA binding protein TDP-43 Diagnostic Peptides

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Abstract

In human cells, the TAR DNA binding protein, TDP-43, serves multiple functions with regulation of gene expression and mRNA splicing being the primary. An abundant amount of research has linked this protein to neuronal-degeneration in cases of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD), due to its tendency to form stress-induced inclusions. Using a study in which phage display technology was used to select for two peptides that were shown to have a high binding affinity for TDP-43, we performed a computational docking and molecular dynamics simulation to identify theoretical binding sites on the TDP-43 protein that were favored by the two peptides. These results coupled with the experimental data suggests that these peptides function as an effective biomarker in TDP-43 detection.

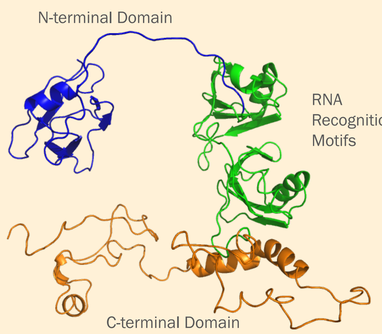
Stages

Some colleagues of ours at the *Chemistry and Research Institute of Natural Sciences* in Hanyang University have developed Polyvalent-directed peptide polymers (TDPRP1 and TDPRP2) that are highly sensitive toward and show a high binding affinity for TDP-43. The goal of the computational studies is to locate a binding site on the TDP-43 protein to which these peptides may bind. And to approximate how stable this binding interaction may be.

Structures

TDP-43:

The first step in this study was to generate accurate structures. The TDP-43 protein consists of three domains, for each of which an NMR structure was found.

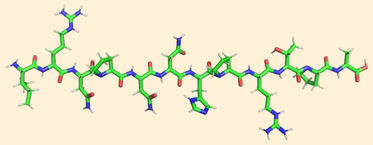


TDPRP1 + TDPRP2:

The peptides were synthesized using the program Avogadro

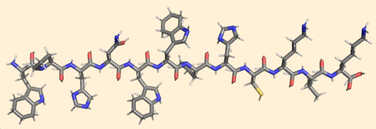
TDPRP1: (K_d = 2.29 x 10^{-12} M)

Iso-Arg-Asn-Pro-Asn-Asn-His-Pro-Arg-Thr-Pro-Ala-GCGK-FITC



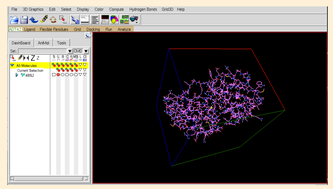
TDPRP2: (K_d = 4.81 x 10^{-12} M)

Trp-Pro-His-Asn-Trp-Trp-Pro-His-Cys-Lys-Val-Lys-GCGK-FITC



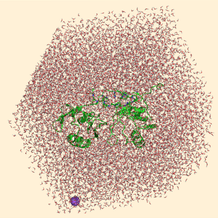
Docking

Docking of the peptides was done using a computer program to provide us with potential binding sites that the peptides showed a preferation for. The binding sites with the most stable and consistent conformations, were then carried on to dynamics.



Dynamics

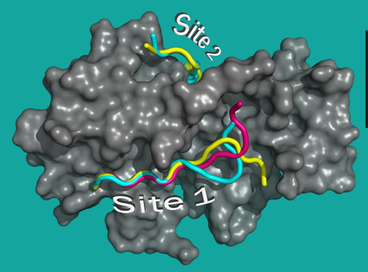
The importance of dynamics comes in understanding how stable the interaction between the protein and ligand really is. Using the protein-ligand complexes obtained from the docking process, a molecular dynamics process was performed. This showed us which of the potential binding sites and conformations were to be best determined as the theoretical binding sites for the TDPRP peptides.



Docking Results

TDPRP1-TDP43:

After performing the docking of TDPRP1 to each domain of TDP-43, we determined there to be two potential binding sites for this peptide, both on the RNA recognition motifs.



Site 1:

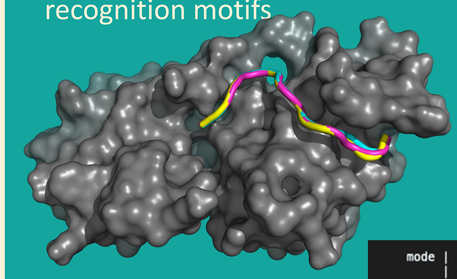
mode	affinity (kcal/mol)
55	−8.5
110	−8.0
108	−7.2

Site 2:

mode	affinity (kcal/mol)
63	−8.2
79	−8.2

TDPRP2-TDP43:

The docking results for this peptide reflected only one potential binding site that was also within the RNA recognition motifs



mode	affinity (kcal/mol)
73	−8.9
74	−8.8
64	−8.8
10	−8.7
136	−8.4
118	−8.1
55	−8.0

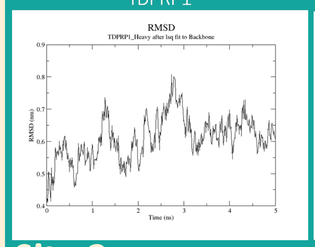
Dynamics Results

TDPRP1-TDP43:

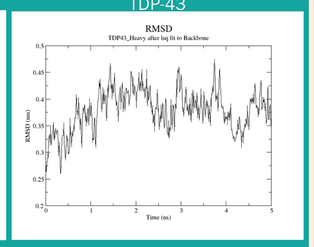
A dynamics study was performed for a peptide-protein complex at each site, and then compared to one another.

Site 1:

TDPRP1

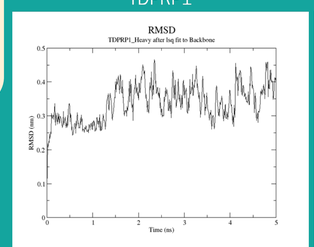


TDP-43

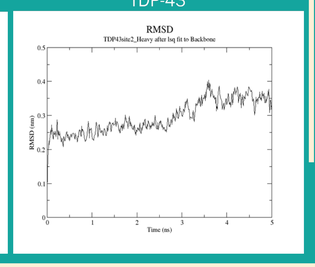


Site 2:

TDPRP1



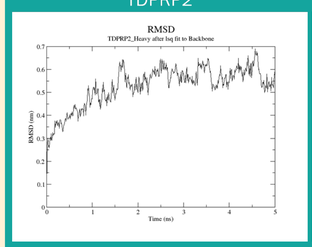
TDP-43



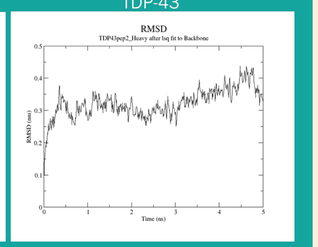
TDPRP2-TDP43:

The dynamics study performed for this peptide-protein complex showed us that some sense of stability was maintained.

TDPRP2



TDP-43



Programs Used

Generating Structures

To create the model structure of the TDP-43 protein the NMR structure for each of its domains was obtained from the Protein Data Bank. Because there has yet to be a determined crystallized structure for TDP-43 as a whole, the structures of each domain had to be tested separately. The TDPRP peptides were simply synthesized using the Avogadro program

The program Pymol was used to prepare all of the structures for docking.

Peptide Docking

To perform the docking, the program AutodockVina was used. The accuracy of this program has shown to be competitive with many other docking programs, and a substantial improvement from its predecessor Autodock 4.

Performing Dynamics

The dynamics of the protein-ligand complex was carried out using the Gromacs program with CHARMM forcefields applied to both the protein and peptides.

Discussion

Analyzing the dynamics for both sites of TDPRP1, it becomes a bit more clear that site2 is potentially more stable than site1 is. Knowing that site1 overlaps with the binding site of TDPRP2, reflects a measure of inaccuracy, as according the original study, this shouldn't be the case. Considering the dynamics for both TDPRP1 and TDPRP2, the binding sites we have determined can serve as strong theoretical binding sites for the two peptides to TDP-43.