Detection of structural and conformational changes in ALS-causing mutant profilin-1 with hydrogen/deuterium exchange mass spectrometry and bioinformatics techniques

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Abstract
The hydrogen/deuterium exchange (HDX) is a reliable method to survey the dynamic behavior of proteins and epitope mapping. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is a quantifying tool to assay for HDX in the protein of interest. We combined HDX-MALDI-TOF MS and molecular docking/MD simulation to identify accessible amino acids and analyze their contribution into the structural changes of profilin-1 (PFN-1). The molecular docking/MD simulations are computational tools for enabling the analysis of the type of amino acids that may be involved via HDX identified under the lowest binding energy condition. Glycine to valine amino acid (G117V) substitution mutation is linked to amyotrophic lateral sclerosis (ALS). This mutation is found to be in the actin-binding site of PFN-1 and prevents the dimerization/polymerization of actin and invokes a pathologic toxicity that leads to ALS. In this study, we sought to understand the PFN-1 protein dynamic behavior using purified wild type and mutant PFN-1 proteins. The data obtained from HDX-MALDI-TOF MS for PFN-1WT and PFN-1G117V at various time intervals, from seconds to hours, revealed multiple peaks corresponding to molecular weights from monomers to multimers. PFN-1/Benzaldehyde complexes identified 20 accessible amino acids to HDX that participate in the docking simulation in the surface of WT and mutant PFN-1. Consistent results from HDX-MALDI-TOF MS and docking simulation predict candidate amino acid(s) involved in the dimerization/polymerization of PFNG117V. This information may shed critical light on the structural and conformational changes with details of amino acid epitopes for mutant PFN-1s’ dimerization, oligomerization, and aggregation.

Keywords
ALS · PFN-1 · HDX · MALDI-TOF MS · MD simulations · Docking simulations

Abbreviations
ALS · amyotrophic lateral sclerosis
PFN-1 · Profilin-1
HDX · hydrogen/deuterium exchange
MALDI-TOF MS  Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
MD  Molecular Dynamics
PDB  Protein Data Bank

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive para-
litic disorder that characterized by the degeneration of both
upper and lower motor neurons (Brown and Al-Chalabi 2017, Hardiman et al. 2017). The average age of survival
for ALS patients is 2–3 years onset of symptoms (Millul et al. 2005). Currently, there are two FDA-approved drugs exist
for ALS (Riluzole and Radicava). These drugs have marginal
benefits and don’t stop the symptoms of ALS. (Kovalchuk et al. 2018, Ludolph et al. 2015). Therefore, discovery of
therapeutic strategy is an urgent unmet medical need for
ALS patients. In order to develop a novel and effective ther-
apy, understanding the mechanism(s) of toxicities caused
by mutant proteins or other factors is essential. One of the
mutant proteins linked to ALS is profilin-1 (PFN-1) (Wu et al. 2012). It is accepted that mutant profilin-1 causes ALS
(Fil et al. 2017; Sadr et al. 2021). The exact mechanism
and the specific of mutant profilin-1 toxicities aren’t fully
understood. Understanding the mechanism of PFN-1 toxicity
could be a key in unravelling how mutant proteins play a role
in the degeneration of motor neurons. Hence, we sought to
investigate the structural changes in mutant PFN-1 using in silico tools combined with hydrogen-deuterium exchange
linked with mass spectrometry (HDX-MS) techniques (Pir-
rone et al. 2017).

Mass spectrometry (MS) technique has become a power-
ful tool for the characterization of higher-order structure of protein (Englander 2006, Montalvao et al. 2008, Rozbesky et al. 2012, Hentze and Mayer 2013, Leurs et al. 2015, Xiao et al. 2015, Kochert et al. 2018, Xiao et al. 2018), protein dynamics, conformations (Lodowski et al. 2010, Majumdar et al. 2015), protein motion/flexibility (Zhou and Robinson 2017) and ligand–protein binding (West et al. 2011). Hydro-
gen-deuterium exchange linked with mass spectrometry
(HDX-MS) technique is a valuable and powerful method
for studying higher-order structure of protein (Englander 2006, Hentze and Mayer 2013, Leurs et al. 2015, Majumdar et al. 2015) that provides a read-out in the changes in solvent accessibility and hydrogen bonding exclusively and in the complex of proteins in a solution (Zhang and Smith 1993, Figueroa and Russell 1999, Villanueva et al. 2000, Englander et al. 2003, Busenlehner and Armstrong 2005, Maier and Deinzer 2005, Englander 2006, Wales and Engen 2006, Palashoff 2008, Wei et al. 2014, Weis 2016). The goal of
this study was to gather data on these and analyze the amino
acids that may be affected by the mutation and determine
the steps in the aggregation of mutant PFN-1. This kind
of pathology is proven to cause ALS in human and mouse
models (Wu et al. 2012, Fil et al. 2017).

PFN-1 is a ubiquitous protein and abundantly expressed
in the body (Witke 2004, Lee and Kim 2015, Alkam et al. 2017). PFN-1 is a small protein with 140 amino acid resi-
dues for its well-known function in converting G-actin to F-actin in actin polymerization. De novo PFN-1 func-
tion is necessary for all basic cellular activities during
development as it is involved in the formation of neuronal
cytoskeleton, development of axons and dendrites, synap-
togenesis (Kiaei et al. 2018).

Eight known mutations in PFN-1 (A19T, C70G, T108M,
M113T, E116G, G117V, R136W, and Q139L) are associ-
ated with human ALS (Wu et al. 2012, Chen et al. 2013, Kiaei et al. 2018). The wild type PFN-1 (PFN-1WT) tertiary
structure is formed by seven antiparallel β-sheets and five
α-helices that two of these are located at the termini (Schutt et al. 1993, Metzler et al. 1995, Krishnan et al. 2009, Alkam et al. 2017). The glycine to valine (PFN-1G117V) substitution
and threonine to methionine (PFN-1T108M) substitution are
two mutations that cause the most changes in the protein
stability and actin binding ability of PFN-1. Therefore, we
sought to understand the changes and the impact of these
mutations. We and others previously reported that hydro-
phobicity of the interactive regions increases and the flex-
bility between α-helix and β-sheet decreases due to glycine
to valine substitution, which leads to the reduction or the
loss of actin binding (Alkam et al. 2017, Zhang et al. 2017).

The dimerization/polymerization of PFN-1WT and PFN-
1G117V was the subject of several recent studies (Lambrechts et al. 2002, Nekouei et al. 2018). We analyzed the purified
PFN-1WT and PFN-1G117V protein samples expressed in E. Coli and probed to determine the structural and con-
formational changes by identification of accessible amino
acids from HDX-MALDI-TOF MS. We then used molecular
dynamic simulation as a reliable approach that enables
understanding the structure-to-function relationships of pro-
teins (Hospital et al. 2015) to determine the number and type
of amino acids involved in HDX and the changes impacted
the structure of mutant PFN-1.

Materials and methods

Recombinant PFN-1 proteins expression
and optimization of expression conditions

Wild type PFN-1 and mutant PFN-1G117V cDNA were
cloned into pET21a (Nekouei et al. 2018). The PFN-1
constructs transfected into E. Coli BL21 host cells and
grew in LB medium (Liofilchem, Italy) containing 100 µg/

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mL ampicillin and to obtain higher yields of recombinant PFN-1 proteins, the expression conditions optimized. IPTG concentration (0.2, 0.4, 0.6, 0.8 and 1 mM) was used to induce expression and the times for induction were (2 h, 4 h and overnight) in 20 °C to obtain optical density at 600 nm of 0.6 (Fedorov et al. 1994). Cells were pelleted down by centrifugation, resuspended, and sonicated in Tris-HCl 50 mM and NaCl 150 mM, pH 7.4. Initially, this solution was centrifuged for 5 min (2,000 rpm), then for 15 min (13,000 rpm). This protocol is according to reference (Lambrechts et al. 2002) and modified for IPTG (Isopropyl-β-D-thiogalactoside) concentrations, induction times, and temperatures used to increase the efficiency and yield of PFN-1 protein.

### PFN-1 purification by poly-L-proline Sepharose Column

In this study, we prepared and used the buffers as described in Nekouei et al., (Nekouei et al. 2018) with minor modifications to improve the coupling/binding process. So, 0.1 gram of CNBr activated sepharose and allowed it to be hydrated in 30 ml of 1 mM HCl until settled down. Sepharose resin was centrifuged for 3 min and washed with deionized water. Poly-L-proline (Sigma-Aldrich, USA) was dissolved in the coupling buffer (0.5 M NaCl, pH 8.5), mixed with the CNBr activated Sepharose resin, and stirred for 20 h at 4 °C. Next, Poly-L-proline was added and allowed to bind to the resin and the mixture was washed with 0.5 M NaCl and 0.1 M Tris-HCl (pH 8.0) for 2 h to remove any unbound poly-L-proline. The resin was washed with 3 ml of 0.1 M HCl (pH 8.0) and stirred for 2 h. The Poly-L-proline-Sepharose column was equilibrated with a wash buffer containing (20 mM Tris, 0.2 mM NaN3, 0.1 mM CaCl2, and 2 mM DTT, pH 7.3). This supernatant was diluted with the wash buffer and placed in an ice bath with moderate stirring for 2 h. The PFN-1 was eluted from the Poly-L-proline-sepharose column with the wash buffer without adding Urea. Ultimately, Vivaspin® centrifugal concentrators (5 kDa molecular weight cutoff) tubes were used to purify and concentrate PFN-1 protein samples.

### Polyacrylamide gel electrophoresis (PAGE) analysis

Tris glycine SDS-PAGE (15%) with Laemmli buffer (Lambrechts et al. 2002) was used to separate the protein samples. The sample buffer (3 M glycerol, 10% SDS, 0.5 M Tris-HCl pH 6.8, 0.05% bromphenol blue) was added to the protein samples. Protein samples were loaded on SDS-PAGE precast gel and the interior chamber of the gel tank filled with running tris-glycine buffer at pH 8.5 and electrical power applied to start electrophoresis. The gel was removed from the cast and stained with silver nitrate or Coomassie Brilliant Blue G-250 staining to visualize the bands. The protein marker used in this study was Prestained Protein Ladder, (10–250 kDa) purchased form SINACLONE Biosciences, sinaclone.com.

### Identification of protein using MALDI-TOF MS

Intact PFN-1 protein samples were analyzed by MALDI-TOF MS (Applied Bio systems model 4800 MALDI-TOF MS, Waltham, Massachusetts, USA) according to the protocol described in (Lambrechts et al. 2002) using α-cyano-4-hydroxycinnamic acid matrix solution containing 50% acetonitrile containing 0.1% trifluoroacetic acid. To calibrate the system, equine cytochrome c (average mass 12,362 Da) as internal standards and Equine Apomyoglobin (average mass 16,952 Da) as external standards were used. The protein samples were mixed with matrix at a ratio of 1:2 and then 1 µL of mixture was spotted onto the chilled MALDI plate and placed under a chiller until spots dried. The matrix and samples were incubated on ice and vortexed before use. MALDI-TOF MS analysis was done in linear positive mode. Also, PFN-1 proteins were identified by in-gel trypsin digestion. Spots/bands of protein of interest were selected and excised. To wash out the stain from the excised bands, they were washed with a bicarbonate buffer and acetonitrile. IAA alkylating agent was used to reduce disulfide bonds (Shevchenko et al. 2006). Trypsin used to digest the protein samples at the peptide bonds from carboxyl terminal where arginine and lysine can be found. The peptides samples and α-cyano-4-hydroxycinnamic acid matrix were mixed at a ratio of 1:2 and then 1 µL aliquot was spotted onto the MALDI plate and analyzed in reflector positive mode. The Swiss-Protein database was used to search the data generated in this study.

### Preparation of protein sample for HDX by MALDI-TOF MS

Experiments were carried out to evaluate rates of deuterium (D2O) insertion into the amide positions in PFN-1WT and PFN-1G117V proteins. For non-deuterated procedure, samples of PFN-1 (1000 ppm) were diluted in 150 mM NaCl plus 8.3 mM Tris pH 7.3 at a ratio of 1:3 in H2O solution at 0 °C for 1 min. Then, 36 µl of the quenched solution was added to 24 µl of protein sample and incubated for 1 min on ice. Protein samples were incubated in a D2O buffer (150 mM NaCl pulse 8.3 mM Tris, pH 7.3, in D2O) at a ratio of 3:1 in for 10 s and 2, 4, 10 and 420 min.
Analysis for HDX used the MALDI-TOF MS operated in MS Linear positive mode with the optimized source settings factors for HDX and intact samples: mass range 10,000–20,000 Da and detector voltage 0.84 with the laser intensity 5700. Also, the exact mass of PFN-1\textsuperscript{WT} and PFN-1\textsuperscript{G117V} was calculated in the https://web.expasy.org/compute_pi tool to calculate the mass changes for before and after deuteration.

Molecular dynamics simulation technique

The PFN-1\textsuperscript{G117V} mutation, which is the same as G118V for rodents, structure has not yet been resolved in the protein data bank (rcsb.org), and in this study a point mutation (G117V) was inserted using the Swapaa command by the Chimera program software (Ferrin et al. 1988, Pettersen et al. 2004). Molecular dynamics (MD) simulation for 200 ns was carried out to obtain protein structure profile. The

![Diagrams of structural changes in 200ns MD simulation of PFN-1 in the wild and mutated form. (a) Root-mean-square deviation (RMSD) is one of the fundamental properties for exploring structural changes and the dynamic behavior of PFN-1 structure that we obtained after 200ms for wild and mutant structure. The average RMSD for wild and mutant PFN-1 were calculated and found to be 0.23 nm and 0.24 nm, respectively (b) Rg analysis of PFN-1 indicates the introduction of compactness in the mutant PFN-1 (red) as compared to the wild state (black). The Rg data is measured in Å. (c) RMSF analysis shows increased fluctuation in the mutated residue area in and around the loops 5 (green marked zone) in the mutant form (red plot). (d) Conformational changes in PFN-1 structure are the result of the varying degrees of dynamic residual secondary structure. The secondary structure elements such as α-helix, β-sheet, and turn of PFN-1 were broken into individual residues at each time step, and the average number of residues forming secondary structure was plotted as a function of time. (e) Solvent accessible surface area (SASA) of wild PFN-1 (black) and mutant PFN-1 (red) of the PFN-1 versus time at 300 K. (f) Time evolution and stability of hydrogen bonds formed. Intramolecular hydrogen bonds formed within wild PFN-1 and mutant in 200 ns]
appropriate PDBID: 1FIK structure was obtained from the Protein Data Bank (rcsb.org) and prepared by UCSF Chimera program. The Swapaa command of chimera program was used to create G117V point mutation on PFN-1, and the final structure of the mutated PFN-1 (PFN-1G117V) was obtained by performing 200 ns of MD simulation by the Gromacs 2018 program (Lemkul 2018) with the OPLS-AA Force Field (Robertson et al. 2015). The structure of the PFN-1WT was also simulated for 200 ns by Gromacs (Figs. 1 and 2). The conformation of crystal structure data of the studied protein (WT and mutants) was optimized and used at the initial steps of MD simulations. The topology parameters of proteins were created by using the Gromacs program. The structures were immersed in a cubic box of simple point charge water molecules. The solvent system (protein and water) was neutralized by adding two Cl- ions in both cases. To equilibrate the system, the solutes (Protein domains, ions, and solvent) were

![Image](image_url)

Table 1  Molecular formula, CID, CAS#, and 2D, 3D structure of benzaldehyde (flat) and acetaldehyde (not flat)

| Structures   | Molecular Formula | 2D Structure | 3D Structure |
|--------------|-------------------|--------------|--------------|
| Acetaldehyde | C2H4O or CH3CHO   | ![](2D_structure_image) | ![](3D_structure_image) |
|              | CID :177          |              |              |
|              | CAS: 75-05-0      |              |              |
| Benzaldehyde | C7H6O or C6H5CHO  | ![](2D_structure_image) | ![](3D_structure_image) |
|              | CID : 240         |              |              |
|              | CAS : 100-52-7    |              |              |

The gaussian Ver. 03 used to determine the 3-D structure of these molecule and found only benzaldehyde is flat.
subjected to the position-restrained dynamics simulation at constant temperature and pressure with the following specifications (299.177 K at 1000 picoseconds). Finally, the full system was subjected to an MD production run for 200 ns at 300 K temperature and 1 bar pressure. The simulation results were analyzed using the backbone RMSD values of PFN-1, the H-bonding between PFN-1 and solvent, as well as some parameters such as RMSF, DSSP and Rg being analyzed (Fig. 1a-f) also structural comparison (Fig. 2). View of the final 5 nanoseconds MD simulation of wild and mutant structures and presentation of this data is available in supplementary data section (Movie 2, 3).

Small fragments such as acetaldehyde and benzaldehyde were used to find the hotspots of a protein used. Once the structure of two small fragments were downloaded from the PubChem data bank (e.g. Kim et al. 2019), we began to optimize their energy structure by Gaussian03 optimized fragment structures (Gaussian03 2004) were randomly probed 220 times around PFN-1WT and PFN-1G117V by the Autodock 4 program. The regions of tendency to be coupled with the corresponding binding energy were finally clustered and identified (Table 1Sa-4Sa). It is important to clarify that we investigated the ligand finding position in PFN-1 protein at possible lowest energy at a certain number of runs that a cluster of events at a lowest possible energy where a ligand and protein bind. For example, for acetaldehyde and wildtype PFN1, out of 220 runs a cluster of 183 events at -2.67 Kcal/Mol were detected for acetaldehyde (the ligand) and the wild type PFN-1 (Table 1S).

Small fragments such as acetaldehyde and benzaldehyde are used to find the hotspots of a protein in a method known as FTMAP (Ngan et al. 2012). Spots that group molecular fragments predict the drug’s sites of interest for binding. In this study, we used two small molecular structures called acetaldehyde and benzaldehyde.

Obtaining, modeling and optimization of structures

Small fragments

In bioinformatic research the preparation of the initial data set is highly important. It is critical that the preparation of starting data set for ligand and protein must be optimized in order the final data set can be properly used and interpreted.

| Sample       | MW (Da) | Amino acids | 10 s | 2 min | 4 min | 10 min | 7 h  |
|--------------|---------|-------------|------|-------|-------|--------|------|
| PFN-1WT      | 16,763  | 140         | 23   | 40    | 86    | 97     | 238  |
| PFN-1G117V   | 16,662  | 140         | 14   | 40    | 65    | 133    | 242  |

Table 2 Experimental data of accessible amino acid hydrogens in protein samples
Therefore, Gaussian software program Ver. 03 was utilized to help in the optimization and to detect structural deviation. The primary structures of acetaldehyde and benzaldehyde were obtained from PubChem structural database with unique chemical identification (CID) 177 and 240, respectively. These were optimized for geometrical energy and spatial structure. The Gauss View program provided the necessary outputs for the Gaussian03 program while making initial changes (Frisch et al. 2004). In this program, using the DFT/B3LYP protocol, we obtained the optimal structure by the command line, thus providing the acetaldehyde and benzaldehyde small ligand model for further use (Table 1).

**Molecular docking simulation**

Molecular docking simulation by Autodock 4.2 software was used to identify the hotspots of PFN-1 protein. In the first phase, acetaldehyde and benzaldehyde were randomly displaced at the PFN-1 surface, took 220 runs to find their ideal PFN-1 binding site as hotspots. The adequacy of 220 runs for the purpose of binding site and hotspots has been previously demonstrated (Morris et al. 2009). Also, we used two additional molecular docking software called autodock vina (Trott and Olson 2010) and molegro virtual docker (MVD) (Thomsen et al. 2006) to confirm Autodock 4 results. (Table 1Sb,c-4Sb,c) Otherwise, it would need a larger number of repeated times with molecular docking simulation to achieve the same results.

**Insertion of point mutation on PFN-1 and docking simulation to identify hotspots**

To find the amino acids involved in the hotspot of mutant protein (G117V), the protein structure with the mutation needed to be generated first, since the structure of the mutant protein was not found in the protein database. To obtain this structure, we used the method of inserting a point mutation by the Swapaa command line by UCSF-Chimera. The position of residue 117 was changed from glycine to valine. A change in any amino acid can affect the overall structure of any protein which appears to be the case for PFN-1. To observe the changes in the tertiary structure of the mutant PFN-1, the structure was simulated by a molecular dynamics program for 200 ns. This condition of MD simulation will show the amino acid residues in a most optimal dynamic state. The simulation of the molecular dynamics residue 117 reveals its effects on the structure. To further investigate the effects of G117V mutation on PFN-1, we carried out molecular dynamic simulations using the Gromacs 2018 package. The x-ray crystal structures data of PFN-1 (PDB ID:1FIK) is from wild type protein and G117V mutation introduced, and molecular dynamic simulations ran for 200 ns in three replicates.

| Sample              | MW, (Da) | Amino acids found in the crystal structure data | Amino acids that participated in PFN-1-acetaldehyde interaction (according to the lowest binding energy) | Accessible hydrogen in molecular docking simulation in PFN-1-acetaldehyde complex | Total number of accessible hydrogens |
|---------------------|----------|-----------------------------------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------|
| PFN-1WT             | 16,763   | 140 139 Q79, T64, K25, N99, L78                | 22 T31, R74, R135                                                                                  | 18                                                                               | 40                                  |
| PFN-1G117V          | 16,662   | 140 139 R55, Y24, K25, T59, V60, F83           | 16 V118, K104, D54, N124, I123, Y59, D106, D25                                                   | 37                                                                               | 53                                  |

| Sample              | MW, (Da) | Amino acids found in the crystal structure data | Amino acids that participated in PFN-1-benzaldehyde interaction (according to the lowest binding energy) | Accessible hydrogen in molecular docking simulation in PFN-1-benzaldehyde complex | Total number of accessible hydrogens |
|---------------------|----------|-----------------------------------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------|
| PFN-1WT             | 16,763   | 140 139 Q79, T64, K25, N99, L78                | 22 T31, R74, R135                                                                                  | 18                                                                               | 40                                  |
| PFN-1G117V          | 16,662   | 140 139 R55, Y24, K25, T59, V60, F83           | 16 V118, K104, D54, N124, I123, Y59, D106, D25                                                   | 37                                                                               | 53                                  |
generated trajectories were evaluated according to their RMSD, RMSF, RG, intramolecular Hb characteristics.

**Results and Discussion**

**Protein expression optimization, affinity purification and mass spectrometry**

Bacterial culture from transfected cells were used to express wild type and mutant PFN-1 proteins (Fig. 1S). The protein synthesis induction time was from 4 to 20 h (Table 4S), and overnight at 20 °C (Fig. 2S), and the recovery and purification of PFN-1 protein improved significantly (10–20 %). To extract PFN-1 protein from *E. Coli* bacterial culture, bacterial cells were separated from the media at low speed (2000 rpm), to prevent lysis initially and then pelletized at 13,000 rpm. The suspended pellet was separated into water-soluble and insoluble proteins fractions. A small volume from each fraction was prepared to load on SDS-PAGE to analyze the expressed proteins and confirmed the protein in the band around size (14–15 kDa) as PFN-1, as it is close to the size of the recombinant PFN-1 (Fig. 3S).

This was further confirmed by MALDI-TOF MS peaks (Fig. 3). The purification of PFN-1<sup>WT</sup> and PFN-1<sup>G117V</sup> were performed without urea, to maintain protein folding and conformation, and eliminate the need of dialysis. The bands were excised, digested, and analyzed with MALDI-TOF MS. The peptides sequences were obtained and searched in Swiss-Prot database. Among the mixture of peptide sequences, 14 peptides obtained for the complete digestion of PFN-1<sup>WT</sup> (71 % sequence coverage) and 9 peptides from the incomplete digestion of PFN-1<sup>G117V</sup> (47 % sequence coverage).
HDX analysis using MALDI-TOF MS

The idea of using HDX was a promising approach to examine the active sites of PFN-1<sup>WT</sup> in comparison to PFN-1<sup>G117V</sup>. This assay allows the monitoring of the speed of dimerization/polymerization using HDX and MALDI-TOF MS as useful parameters in understanding the mechanisms in controlling/developing ALS. This approach allowed us to predict possible hotspots regions in the PFN-1 protein to introduce suitable candidates to indicate binding with these points. We calculated molecular weights of 16,763 Da for PFN-1<sup>WT</sup> and 16,662 Da for FPNI<sup>G117V</sup> by using the spectra related to PFN-1<sup>WT</sup> and PFN-1<sup>G117V</sup> samples. The ratio of the masses for PFN-1<sup>WT</sup> in 10 s, 2 min, 4 min, 10 min and 7 h were 23, 40, 86, 97 and 238 Da and for PFN-1<sup>G117V</sup>, were 14, 40, 65, 133 and 242 Da, respectively. These differences indicate that the active hydrogens in the first minutes and the other hydrogens with less activity in the following minutes would participate in the exchange reaction (Table 2).

These results are consistent with our previous findings reported in Nekouei et al. (2018) that the dimerization/polymerization of PFN<sup>G117V</sup> related to PFN-1<sup>WT</sup> was rather rapid MALDI-TOF MS showed dimer and polymer form of PFN<sup>G117V</sup> in the samples of mutant PFN-1 as observed by the appearance of cloudiness in the solution indicating spontaneous oligomerization. The lower values in HDX for PFN<sup>G117V</sup> compared with PFN-1<sup>WT</sup> samples can be related to speed of dimerization and reduced available hydrogen for exchange than in the PFN-1<sup>WT</sup> that is in monomer form.

Next, we performed molecular docking simulation and identified the amino acids involved in the binding of PFN-1<sup>WT</sup> and PFN<sup>G117V</sup> to the small ligands like acetaldehyde and benzaldehyde (Table 3).
Based on predictions by molecular docking simulation and according to the lowest binding energy, in the binding of PFN-1\(^{WT}\) and PFN\(^{G117V}\) to acetaldehyde ligand, the amino acids Gln 79, Thr 64, Leu 78, Gln 79 and Asn 99, respectively. We found 16 and 22 exchangeable hydrogen, comparing them to experimental change of mass to charge related to PFN-1\(^{WT}\) and PFN\(^{G117V}\), 14 and 23 Da changes after 10 s of incubation with deuterium based HDX method. Therefore, we conclude that these amino acids are hotspot residues on the surface of the PFN-1\(^{WT}\) and PFN\(^{G117V}\) that begin to be exchanged in the first minutes. It is possible to propose that these amino acids and related hydrogens are the initial steps of the dimerization/polymerization of PFN\(^{G117V}\) related to PFN-1\(^{WT}\). The experimental and in silico data are consistent and confirm the hotspots by mass-spectroscopy, MD simulations and molecular docking simulations.

Also, the change of mass to charge related to PFN-1\(^{WT}\) and PFN\(^{G117V}\) was 40 Da after 2 min incubation in both cases. This is consistent with the pre-folded structures of PFN-1\(^{WT}\) and PFN-1\(^{G117V}\) where β-sheet and α-helices have

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**Fig. 5** Molecular docking simulations of binding of PFN-1\(^{WT}\) to Acetaldehyde and Benzaldehyde. (a) The hotspots amino acids on the surface of PFN-1\(^{WT}\) related to PFN-1\(^{WT}\)/Acetaldehyde by Lys25, Thr64, Leu78, Gln79 and Asn99. (b) The amino acids involved in the hotspots on the surface of PFN-1\(^{WT}\) related to PFN-1\(^{WT}\)/Benzaldehyde are Trp31, Arg74 and Arg135. An animated presentation of this data is available in supplementary data section. (Supp. Movie 2)

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**Fig. 6** Molecular docking simulations of binding of PFN-1\(^{G117V}\) to Acetaldehyde and Benzaldehyde. (a) The amino acids involved in the hotspots on the surface of PFN-1\(^{G117V}\) related to PFN-1\(^{G117V}\)/Acetaldehyde by Tyr24, Lys25, Arg55, Tyr59, Val60 and Phe83. (b) The amino acids involved in the hotspots on the surface of PFN-1\(^{G117V}\) related to PFN-1\(^{G117V}\)/Benzaldehyde are Tyr24, Lys25, Asp54, Tyr59, lys104, Asp106, Val118, Ile123, and Asn124. An animated presentation of this data is available in supplementary data section. (Supp. Movie 3)
not formed yet for their special dimension structures. Therefore, PFN-1^{WT} and PFN-1^{G117V} show similar HDX behavior. In molecular docking simulation, surface amino acids which participate in binding with PFN-1^{WT} to benzaldehyde and acetaldehyde and PFN-1^{G117V} to benzaldehyde complexes are Trp 31, Arg 74, Arg 135, Asn 99, Gln 79, Thr 64, Lys 25, Leu 78 and Val 118, Lys104, Asp 54, Asn 124, Ile 123, Tyr 59, Asp106, Lys 25, Tyr 54 with 40 and 37 accessible hydrogens, respectively (Table 3).

In the longer incubation, the PFN-1 proteins incubated for 4 min with deuterium. The changes of mass to charge for PFN-1^{WT} and PFN-1^{G117V} in mass spectrometry results were 86 and 65 Da, Respectively. Based on native mass spectrometry data of PFN-1, we discover the second type of hydrogens after 4 min of incubation which do not appear in the first wave to HDX, which could be because they were hidden in the folded structures of PFN-1.

In molecular docking simulation, time isn’t taken as a parameter during interactions analysis and benzaldehyde and acetaldehyde interactions followed as the next step, with PFN-1 and looked for any correlation between the docking data and experimental HDX data. The amino acids located on the surface of PFN-1 that may participate in binding to benzaldehyde and acetaldehyde complexes were found to be Arg 55, Tyr 24, Lys 25, Phe 83, Val 118, Lys104, Asp 54, Asn124, Ile 123, Tyr 59, Asp106, Lys 25, Tyr 54. The surface amino acids which participate in binding with PFN-1^{WT} to benzaldehyde and acetaldehyde complexes found to be Trp 31, Arg 74, Arg 135, Asn 995, Gln 79, Thr 64, Lys 25, Leu 78 with the intra-amino acids with lower activity than surface amino acids with 54 and 86 accessible hydrogens, respectively (Tables 1S-S4). The molecular docking simulation of PFN-1^{G117V} using computer programs is presented in the form of figures (Figs. 4, 5 and 6) and tabulated data presented in Table 3 and further demonstrated with a short animation (Supp. Movie 1, 2, 3).

According to the results and comparison of the experimental and molecular docking simulation data, the amino acids identified as hotspots on the surface of the PFN-1^{WT} and PFN-1^{G117V} have been located.

**Conclusions**

In this study we optimized the method to express recombinant wild type and mutant PFN-1 proteins for HDX MALDI-TOF MS analysis. The optimization enabled higher yield and recovery by varying the time and IPTG concentration. HDX MALDI-TOF MS results were analyzed using computation modelling to delineate structural changes and ligand-binding in the PFN-1^{WT} and PFN-1^{G117V}.

After the incubation of PFN-1^{WT} and PFN-1^{G117V} within 10 s, 2, 4 min with deuterium, changes of mass to charge were investigated, and we found that in 10 s, PFN-1^{WT} and PFN-1^{G117V} showed a change of 23 and 14 Da, respectively. This indicates that only the active amino acids on the protein surface were exchanged during this short period of time. During the 2 min incubation, the mass-to-charge ratio of PFN-1^{WT} and PFN-1^{G117V} changed to 40 Da for both proteins, and in 4 min, that increased to 86 and 65 Da, respectively. This indicates that other active amino acids on the surface have exchanged. Also, in the study of molecular docking simulation and comparison of results based on the lowest binding energy, surface amino acids Gln 79, Thr 64, Lys 25, and Arg 55, Tyr 24, Lys 25 that have participated in the binding of PFN-1^{WT} with acetaldehyde and PFN-1^{G117V} with acetaldehyde have 22 and 16 hydrogen exchangeable, respectively. We have noticed consistency between the theoretical data obtained using in silico programs and experimental data using wet laboratory devices, and they happen to be in agreement with each other in identifying the amino acids that are most likely involved and may be affected with Gly117Val mutation.

This study highlights the value of combining two powerful tools when HDX MALDI-TOF MS to detect the “peaks” from the protein solution, cross analyze and validate the data generated with computation modelling.

We believe this report is novel in using this approach to detect the major and even minor changes in PFN-1 due to mutation that we have presented in this report. The new results certainly will add new knowledge in understanding the reasons for the alteration in the protein tertiary and quaternary structure of PFN-1 and binding ability with ligands. All of these together build a helpful picture of underlying mechanism in the protein toxicity of PFN-1 in ALS.

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**Author contribution** The concept and idea of the study was designed by MK, CE, ZA, MN, ASS, AG. The experiments were designed and carried out by ZA, AA, MN, ASS. The data were analyzed and evaluated by MK, CE, ASS, AG. The manuscript was written by MK, CE, ASS, ZA, VK, AG.

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**Data availability** All data generated or analyzed during this study are included in this published article and its supplementary information files as a movie can be found in the link shown here: https://doi.org/10.6084/m9.figshare.13551218

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Declarations

Disclosure of potential conflicts of interest Dr. Kiaei is the founder, President and CEO of RockGen Therapeutics, LLC., and have a financial interest in the technology discussed in this publication. These financial interests have been reviewed and approved in accordance with the RockGen conflict of interest policies. Other authors have no conflict.

Research involving human participants and/or animals There were no human participants or animals used in this study.

Informed consent N/A.

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References

Alkam D, Feldman EZ, Singh A, Kiaei M (2017) Profilin1 biology and its mutation, actin (g) in disease. Cell Mol Life Sci 74(6):967–981
Brown RH, Al-Chalabi A (2017) Amyotrophic lateral sclerosis. N Engl J Med 377(2):162–72
Busenlehner LS, Armstrong RN (2005) Insights into enzyme structure and dynamics elucidated by amide H/D exchange mass spectrometry. Arch Biochem Biophys 433(1):34–46
Chen Y, Huang R, Chen K, Song W, Zhao B, Chen X, Yang Y, Yuan L, Shang H-F (2013) PFN-1 mutations are rare in Han Chinese populations with amyotrophic lateral sclerosis. Neurobiol Aging 34(7):1922. e1921-1922. e1925
Englander SW (2006) Hydrogen exchange and mass spectrometry: A historical perspective. J Am Soc Mass Spectrom 17(11):1481–1489
Englander JJ, Del Mar C, Li W,Englander SW, Kim JS, Stranz DZ, Hamuro Y, Woods VL (2003) Protein structure change studied by hydrogen-deuterium exchange, functional labeling, and mass spectrometry. Proc Natl Acad Sci 100(12):7057–7062
Fedorov A, Pollard T, Almo SC (1994) Purification, characterization and crystallization of human platelet profilin expressed in Escherichia coli. J Mol Biol 241(3):480–482
Ferrin TE, Huang CC, Jarvis LE, Langridge R (1988) The MIDAS display system. J Mol Graph 6(1):13–27
Figuerola ID, Russell DH (1999) Matrix-assisted laser desorption ionization hydrogen/deuterium exchange studies to probe peptide conformational changes. J Am Soc Mass Spectrom 10(8):719–731
Fil D, DeLoach A, Yadav S, Alkam D, Mac Nicoll M, Singh A, Campdre CM, Goeller JJ, O’Brien CA, Fahmi T (2017) Mutant Profilin1 transgenic mice recapitulate cardinal features of motor neuron disease. Hum Mol Genet 26(4):dw2429
Frisch M, Trucks G, Schlegel H, Scuseria G, Robb M, Cheeseman J, Montgomery J, Vreven T (2004) Gaussian03 R.B. Gaussian Inc, Wallingford
Fedorov A, Pollard T, Almo SC (1994) Purification, characterization and crystallization of human platelet profilin expressed in Escherichia coli. J Mol Biol 241(3):480–482
Ferrin TE, Huang CC, Jarvis LE, Langridge R (1988) The MIDAS display system. J Mol Graph 6(1):13–27
Figuerola ID, Russell DH (1999) Matrix-assisted laser desorption ionization hydrogen/deuterium exchange studies to probe peptide conformational changes. J Am Soc Mass Spectrom 10(8):719–731
Fil D, DeLoach A, Yadav S, Alkam D, Mac Nicoll M, Singh A, Campdre CM, Goeller JJ, O’Brien CA, Fahmi T (2017) Mutant Profilin1 transgenic mice recapitulate cardinal features of motor neuron disease. Hum Mol Genet 26(4):dw2429
Frisch M, Trucks G, Schlegel H, Scuseria G, Robb M, Cheeseman J, Montgomery J, Vreven T (2004) Gaussian03 R.B. Gaussian Inc, Wallingford
Gaussian03 R.B. (2004) 05, Frisch MJ et al. Gaussian Inc, Wallingford
Hardimon O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, Shaw PJ, Simmons Z, Van Der Berg LH (2017) Amyotrophic lateral sclerosis. Nat Rev Dis Primers 5(1):1–9
Hentze N, Mayer MP (2013) Analyzing protein dynamics using hydrogen exchange mass spectrometry. J Proteome Res 12(7):3066–3069
Hospital A, Gotri JR, Orozo M, Gelpi JL (2015) Molecular dynamics simulations: advances and applications. Adv Appl Bioinform Chem 8:37
Kiaei M, Balasubramaniam M, Kumar VG, Reis RJS, Moradi M, Vanaghese KI (2018) ALS-causing mutations in profilin-1 alter its conformational dynamics: a computational approach to explain propensity for aggregation. Sci Rep 8(1):1–10
Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B (2019) PubChem 2019 update: improved access to chemical data. Nucleic Acids Res 47(D1):D1102–D1109
Kochert BA, Iacob RE, Wales TE, Makriyannis A, Enger JH (2018) Hydrogen-deuterium exchange mass spectrometry to study protein complexes. Protein Complex Assembly, Springer, Berlin, pp 153–171
Kovalchuk MO, Heuberger JA, Sleutjes BT, Ziegkos D, van den Berg LH, Ferguson TA, Franssen H, Groeneveld GJ (2018) Acute effects of riluzole and retigabine on axonal excitability in patients with amyotrophic lateral sclerosis: a randomized, double-blind, placebo-controlled, crossover trial. Clin Pharmacol Ther 104(6):1136–1145
Krishnan K, Holub O, Gratton E, Clayton AH, Cody S, Moens PD (2009) Profilin interaction with phosphorylidyinositol (4, 5)-bisphosphate destabilizes the membrane of giant unilamellar vesicles. Biophys J 96(12):5112–5121
Lambrecht A, Jonckheere V, Dewitte D, Vandekerckhove J, Ampe C (2002) Mutational analysis of human profilin I reveals a second P1 (4, 5)-P 2 binding site neighbourg the poly (L-proline) binding site. BMC Biochem 3(1):12
Lee S, Kim H-J (2015) Prion-like mechanism in amyotrophic lateral sclerosis: are protein aggregates the key? Exp Neurobiol 24(1):1–7
Lemkul J (2018) From proteins to perturbed Hamiltonians: A suite of tutorials for the GROMACS-2018 molecular simulation package [article v1. 0]. Living J Comput Mol Sci 1(1):5068
Leurs U, Mistarz UH, Rand KD (2015) Getting to the core of protein pharmaceuticals–Comprehensive structure analysis by mass spectrometry. Eur J Pharm Biopharm 93-95–109
Lodowski DT, Palczewski K, Miyagi M (2010) Conformational changes in the G protein-coupled receptor rhodopsin revealed by hidride hydrogen – Deuterium exchange. Biochemistry 49(44):9425–9427
Ludolph A, Drory V, Hardiman O, Nakano I, Ravits J, Robberecht W, Shefner J (2015) A revision of the El Escorial criteria-2015. Amyotrophic Lateral Scler Frontotemporal Degener 16(5–6):291–2
Maier CS, Deinzer ML (2005) Protein conformations, interactions, and H/D exchange. Methods Enzymol 402:312–360
Majumdar R, Middaugh CR, Weis DD, Volkin DB (2015) Hydrogen-deuterium exchange mass spectrometry as an emerging analytical tool for stabilization and formulation development of therapeutic monoclonal antibodies. J Pharm Sci 104(2):327–345
Metzler WJ, Farmer BT, Constantine KL, Friedircks MS, Mueller L, Lavoie T (1995) Refined solution structure of human profilin I. Protein Sci 4(3):450–459
Millul A, Beghi E, Logroscino G, Micheli A, Vitelli E, Zardi A (2005) Survival of patients with amyotrophic lateral sclerosis in a population-based registry. Neuroepidemiology 25(3):114–119
Montalvao RW, Cavalli A, Salvatella X, Blundell TL, Vendruscolo M (2008) Structure determination of protein – protein complexes using NMR chemical shifts: case of an endonuclease colicin – immunity protein complex. J Am Chem Soc 130(47):15990–15996
Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4; Automated docking with selective receptor flexibility. J Comput Chem 30(16):2785–2791
Nekouei M, Ghelzellou P, Alihamadi A, Arjmand S, Kiaei M, Ghasempour A (2018) Changes in biophysical characteristics of PFN-1 due to mutation causing amyotrophic lateral sclerosis. Metabolic Brain Dis 33(6):1975–1984
