Scientific Report

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Project title: Investigation on mutations in prion protein
Starting date of the visit: 16/10/2010
Duration: 10 days
Host Institute: German Research School for Simulation Sciences, Jülich, Germany,
Host Contact: Prof. Paolo Carloni

Introduction

Prion diseases are a group of fatal and incurable neurodegenerative disorders of mammals. They uniquely manifest as sporadic, genetic and infectious maladies. The agent responsible for prion diseases is the prion. A prion is defined as a proteinaceous infectious particle, which is solely constituted by an alternate folded form of the prion protein (PrP) [1]. In diseased animals and humans PrP exists in two forms, the physiological, cellular form of PrP, and the pathological prion form, denoted as PrPSc. The mechanism through which nascent PrPSc is generated is currently unknown. Structural studies of either isoforms are of great importance in the biology of prion diseases since they may shed light into the molecular mechanism responsible for these pathologies.

Purpose of the visit

The purpose of the visit was to participate to the analysis and interpretation of the results of Molecular Dynamics (MD) simulations performed by the applicant in collaboration with Prof. Carloni and coworkers. These activities are set in the framework of a common project between the German Research School for Simulation Sciences of Jülich, the International School for Advanced Studies of Trieste and the Institute of Crystallography of Bari, aiming at investigating how mutations in human prion protein are related to the presence and locations of instabilities in the 3D protein structure.

Prion diseases are caused by the conversion of the cellular prion protein into an altered infectious conformation. In human, familiar forms of the disease are linked to specific mutations in the PrP gene, which may facilitate the conversion reaction by destabilizing the native structure of PrP [2]. Despite over 40 point mutation in PrP are known, our understanding of the pathogenic mechanisms by which these mutation cause the disease remains limited.

The application of a recent simulation protocol on a limited number of PrP variants in aqueous solution sheds new light on the molecular basis of inherited prion disease, introducing novel structural features, which are of great importance for our understanding of the earliest molecular events leading to the conformational transition [3].

The common project foresees the implementation of a high-throughput approach for a comprehensive investigation of the structural facets of the PrP disease–causing mutations, based on the above mentioned simulation protocol.

Description of the work carried out during the visit

During the visit the following issues were tackled:

1) Use of NAMD program for molecular dynamics simulations;
2) Use of supercomputer facilities at the host institution;
3) Analysis of the trajectories generated;
4) Interpretation of the results.

A description of the undertaken activity for each issue will be described in the following:

1) Point mutations were introduced on the PrP globular domain, consisting of residues 125-228. A specific model (the third) from the NMR structure at pH 7.0, deposited in PDB with entry 1HJN, was used [4]. It is shown in Figure 1, with a cartoon representation were a color is associated to each residue type. It has be done by the program JaV, included in the software package IL MILIONE [5], made and distributed by the Institute of Crystallography and devoted to protein crystal structure solution. A total of 30 mutations were considered. It was decided to simulate each one with two different programs and force fields. I dealt with simulations with the program NAMD [6] done with the CHARMM force field [7]. The input files for each mutation, corresponding to the simulation protocol followed in [3], have been produced. The original pdb file of the NMR structure has been also considered as input for a reference simulation, denoted wild type (WT).
2) Three computer facilities were used in the host institution: the grsjuc cluster hosted at the German Reserch School for Simulation Sciences and two supercomputers hosted at the Jülich supercomputer center: jugene and juropa. Grsjuc is a local clusters of 16 compute nodes, equipped with 128 cores. Jugene is currently the fastest computer in Europe, with a computing power of one Petaflops, while juropa, which is designed for jobs to be run with a smaller number of processors, hence with moderate parallelization, has a peak performance of 207 Teraflops. I received training on the use of such facilities. After an analysis of the scaling of NAMD runs, the proper strategy for large scale computations has been set, which resulted to be different for the three computer facilities. The contemporary use of three computer facilities allow me to perform 50ns MD simulations on about 20 mutations in a relatively short time (about a week).
3) The analysis on the generated trajectories has been performed by means of VMD [8]. Several tcl scripts have been written on purpose to perform the analysis in batch mode (without using the graphic display) and shell scripts to perform the calculations on all the generated mutations and recover the results in automatic way.
4) The results obtained from the considered mutations have been related with the features of the WT simulation. Moreover they were compared with analogous results obtained with the different program (GROMACS) and the different force field (AMBER) by other members of the team. The interpretation of the results has been done in collaboration with all the members of the team.

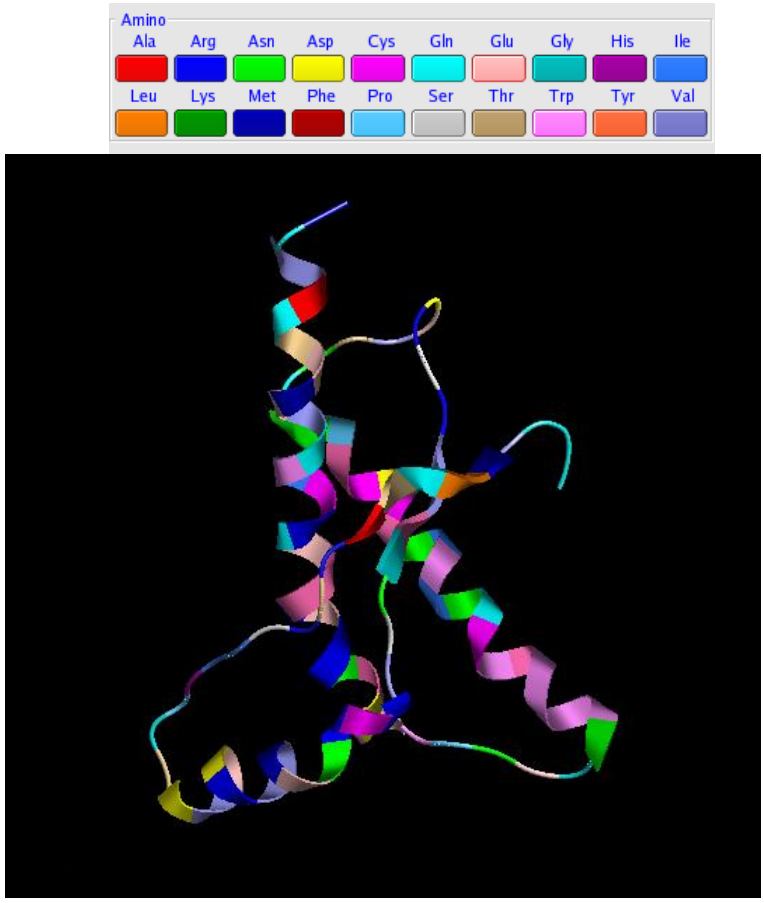

Figure 1 Cartoon representation of the Prion protein globular domain, obtained by NMR at pH 7.0 (model n.3 of the PDB code 1HJN). The colors represent the different residue types, as shown in the legend. The figure has been done by the viewer JaV [5].

Description of the main results obtained

The following results were achieved:

1) A common protocol for MD simulations of prion protein has been established;
2) The protocol has been applied on all the observed familiar mutations of PrP. A total of 20 mutations have been simulated for 50ns.
3) The following analysis has been performed on each mutation:
    a. Distances between relevant residues;
    b. Solvent accessible area of relevant residues;
    c. Root mean squared distance (RMSD) among the C_α atoms of the protein structure in each frame of the trajectory with respect to those of the WT reference structure;
    d. Residue-by-residue root mean squared fluctuation (RMSF) of the C_α atoms;
    e. Clusterization of the protein structures in each trajectory;
    f. Analysis of the salt bridges in the representative structures of the more populated clusters;
4) The results have been compared with those obtained by the team of Prof. Carloni, by using another simulation program (GROMACS) and force field (AMBER). The main findings are the following: three regions of the globular domain show an increased flexibility in the mutants: the α_1 helix, the α_2-α_3 region and the β_2-α_2 loop. Notably, these features are common to both force fields.

Future collaboration with host institution

Apart from finalizing the analysis on prion mutants, the short visit has been used to establish future collaborations with the group of Prof. Carloni. This will be facilitated by the fact that one of my graduate students will follow the doctoral program at the German School for Simulation Sciences, under the supervision of Prof. Carloni. She will act as a bridge between the activities of my group, at the Institute of Crystallography in Bari and those at the German School for Simulation Sciences in Jülich.

An agreement was reached to follow the seminars and the lectures organized by the Computational Biophysics sector of the German School for Simulation Sciences by using video-conference facilities.

Projected publications resulting from the grant

The work carried out during the short visit grant has been reported in a publication which is going to be submitted (with ESF properly acknowledged).

References

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