Coarse Grained Simulation of Amyloid Aggregators

Kenneth Osborne[1], **Michael Bachmann**[2], **and Birgit Strodel**[1]

[1] Institute of Complex Systems (ICS-6), Forschungszentrum Jülich, 52425 Jülich, Germany
E-mail: {k.osborne, b.strodel}@fz-juelich.de

[2] The University of Georgia, Center for Simulational Physics, Athens, GA 30602-2451, U.S.A
E-mail: bachmann@smsyslab.org

1 Introduction

A broad range of human diseases are known to arise as a consequence of protein aggregation and misfolding. A specific class of these so called amyloid proteins are known to generate fibrillar aggregates. Specifically these aggregates have a cross β-sheet structure, where the β-strands run perpendicular to the fiber's long axis, and the backbone hydrogen-bonds stabilize the sheets propagating along the direction of the fiber. Well known examples for diseases involving amyloid proteins include Alzheimer's disease, caused by aggregation of the Aβ peptide, and the transmissible Creutzfeldt-Jakob Disease, caused by aggregation of the human prion protein, PrP.

Although considerable progress has been made in recent years toward the elucidation of the structure and properties of amyloid fibrils, little is known about the structure and dynamics of the oligomers that are involved. The precise origin of pathogenicity in amyloid diseases remains elusive, although current evidence suggests that the soluble oligomeric precursors, rather than the fibrils themselves, are the cytotoxic species. Further studies that help to reveal the molecular mechanism of the multi-step process of amyloid aggregation are needed to find the missing link between amyloid fibrils and the disease to which they are connected.

The aim of the present work is to study the oligomerization of the peptide GNNQQNY. GNNQQNY is a polar heptapeptide from the N-terminal prion-determining region of the yeast prion protein Sup35. The atomic resolution crystal structure of GNNQQNY has recently been determined by Eisenberg and co-workers using x-ray microcrystallography[2]. There is strong evidence that the microcrystals formed by amyloidogenic peptides or proteins are closely related to their amyloid fibrils[3]. We aim to probe the self-assembled structure of GNNQQNY using peptideB, the coarse grained (CG) force field proposed by Bereau and Deserno[4]. We perform replica exchange molecular dynamics (REMD) simulations starting six monomeric GNNQQNY peptides in random starting positions, and analyze the results in terms of microcanonical and canonical quantities.

2 Methods

peptideB Force Field The GNNQQNY peptide (Gly-Asn-Asn-Gln-Gln-Asn-Tyr) was represented by the coarse grained (CG) force field peptideB[4]. The primary reason for using a coarse-grained force field was due to the time scales associated with peptide aggregation, which generally extends over a time scale much beyond a microsecond. Though molecular dynamics (MD) simulations of atomistic models have reached the microsecond time

scale, it is not possible to use standard MD simulations in explicit solvent to study aggregation processes extending over a time scale much beyond a microsecond. In the peptideB model the backbone is represented by three beads per residue, while only one bead per side chain is used. The side chain bead is the location of the C_β atom coining the name peptideB force field. The peptideB forcefield was parameterized to reproduce both local conformations and tertiary structures. The relatively high resolution of backbone beads allows for the force field to model physically relevant secondary structures, such as β-sheets, α-helicies, and random coils.

Replica Exchange Molecular Dynamics The force field is used in conjunction with the Espresso simulation package described below. All simulations were run using REMD, where multiple MD runs of the same system (replicas) are run simultaneously at different temperatures. After a specified number of time steps, replicas at neighbouring temperatures can be exchanged, provided that a Metropolis criterion is satisfied. This procedure allows high-energy structures to be accepted for the replicas at higher temperature. The associated configurational changes then migrate to the replicas at lower temperatures when exchanged with each other. We performed 20 independent REMD simulations starting from six monomeric GNNQQNY peptides in random starting positions, each at 16 different temperatures ranging from approximately $189 - 366$ K. We used a cubic box with edge length 49.5 Å, giving a concentration of $C \sim 80$mM. We let the system relax for 10 μs before collecting statistics for analysis. Production runs lasted for 40 μs.

Analysis For the identification of standard transitions and their characteristics, several impact parameters, such as the potential energy and two different orientational order-parameters, were collected during the REMD simulations and the heat capacity was calculated using the weighted histogram analysis method (WHAM)[5,7].

3 Results and Discussion

Low Energy We found the minimum energy structure in every temperature thread of every simulation. Although the minimum energy structure does not give any thermodynamic information, is does provide a clue as to what peptideB might find to be the ground state of the system. As seen in Fig. 1, peptideB has successfully sampled a β-sheet aggregate, as is expected for GNNQQNY[2].

Heat Capacity The heat capacity is a measure of the change in energy versus the change in temperature. As a system undergoes a major structural conformation change (phase transition), small changes in temperature produce proportionally larger changes in energy giving rise to a peak in the specific heat curve. In our work, we obtained the average specific heat as a function of temperature from a canonical WHAM analysis. Error in the specific heat was obtained by taking the standard deviation of the average specific heat of the system at the temperatures of the replicas over all 20 runs. As seen in the Fig. 2, the average heat capacity behaves in the manner expected for a structural transition, which transforms from the dissociated phase to the aggregated phase. The error bars tell us with certainty that the peak in the specific heat is not due to random fluctuations.

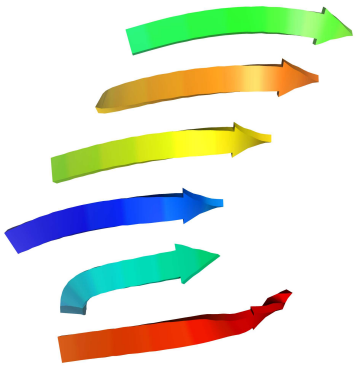

Figure 1. A minimum energy structure from one of the 20 GNNQQNY runs. This structure was sampled at $\sim$ 189 K. We see that although GNNQQNY forms β-sheets with parallel β-strands, the strands are not in-register.

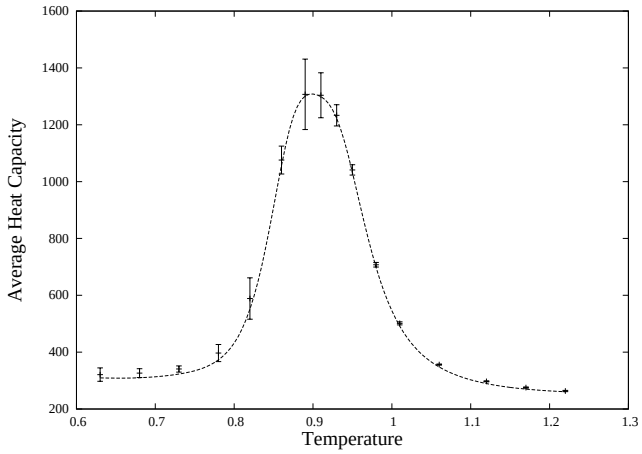

Figure 2. The heat capacity is plotted against temperature as obtained from our REMD simulations of six GNNQQNY peptides in a box. Temperature in CG simulations is not as easy to define as temperature in atomistic simulations, but 1 temperature unit in the peptideB model is approximately equal to 300 K. Thus the peak at ~ 0.9 temperature units indicates a phase transition at $\sim$ 267 K.

Orientational Order Parameters We also looked at the orientational order parameters $\overline{P_1}$ and $\overline{P_2}$, which are widely used for studying the properties of anisotropic fluids, and are

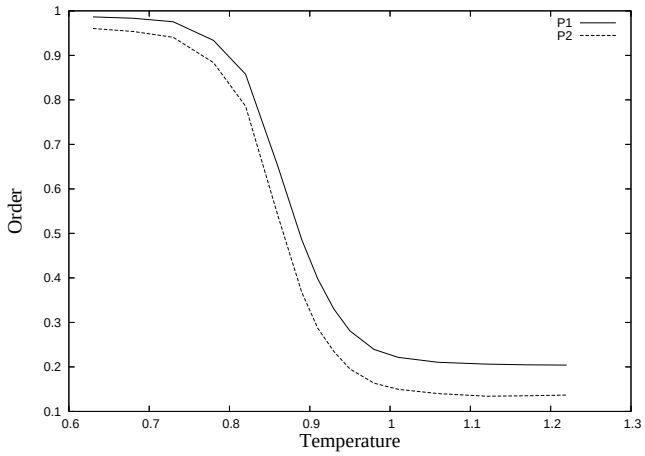

Figure 3. The average $\overline{P_1}$ and $\overline{P_2}$ as a function of temperature show how β-strand orientation evolves with temperature. Temperature in CG simulations is not strictly proportional to temperature outside of the simulation, but 1 Temperature Unit is approximately equal to 300 K.

defined as[6]:

$$\overline{P_1} = \frac{1}{N}\sum_{i=1}^{N}\left(\hat{\mathbf{z}}_i \cdot \hat{\mathbf{d}}\right) \tag{1}$$

$$\overline{P_2} = \frac{1}{N}\sum_{i=1}^{N}\frac{3}{2}\left(\hat{\mathbf{z}}_i \cdot \hat{\mathbf{d}}\right)^2 - \frac{1}{2} \tag{2}$$

Here, the director $\hat{\mathbf{d}}$ is a unit vector defining the preferred direction of alignment, $\hat{\mathbf{z}}_i$ is defined as unit vectors linking the peptides termini from the N to the C terminus, and N is the number of molecules in the simulation box, i.e., six peptides in this study. The director is defined as the eigenvector of the ordering matrix, that corresponds to the largest eigenvalue. $\overline{P_2}$ describes the orientational order of the system and discriminates between ordered and disordered conformations, i.e., $\overline{P_2}$ will be 1 if the monomers are arranged in either a parallel or antiparallel conformation and will vanish for the system in a fully isotropic state. The polar $\overline{P_1}$ describes the polarity of the system, i.e., how much the molecular vectors $\hat{\mathbf{z}}_i$ point in the same direction. It will be 1 if the monomers are arranged in a parallel conformation. $\overline{P_1}$ thus allows to discriminate between parallel and antiparallel mixed ordered aggregates. At low temperature, we see that $\overline{P_1}$ and $\overline{P_2}$ are high, whereas at high (~ 1) Temperature $\overline{P_1}$ and $\overline{P_2}$ are low (~ 0). At low temperature, we see that $\overline{P_1}$ and $\overline{P_2}$ are high, whereas at high (~ 1) Temperature $\overline{P_1}$ and $\overline{P_2}$ are low (~ 0).

With this knowledge, we can conclude that that peptideB has the capability of aggregating randomly structured monomers into ordered parallel beta-sheets.

Acknowledgments

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