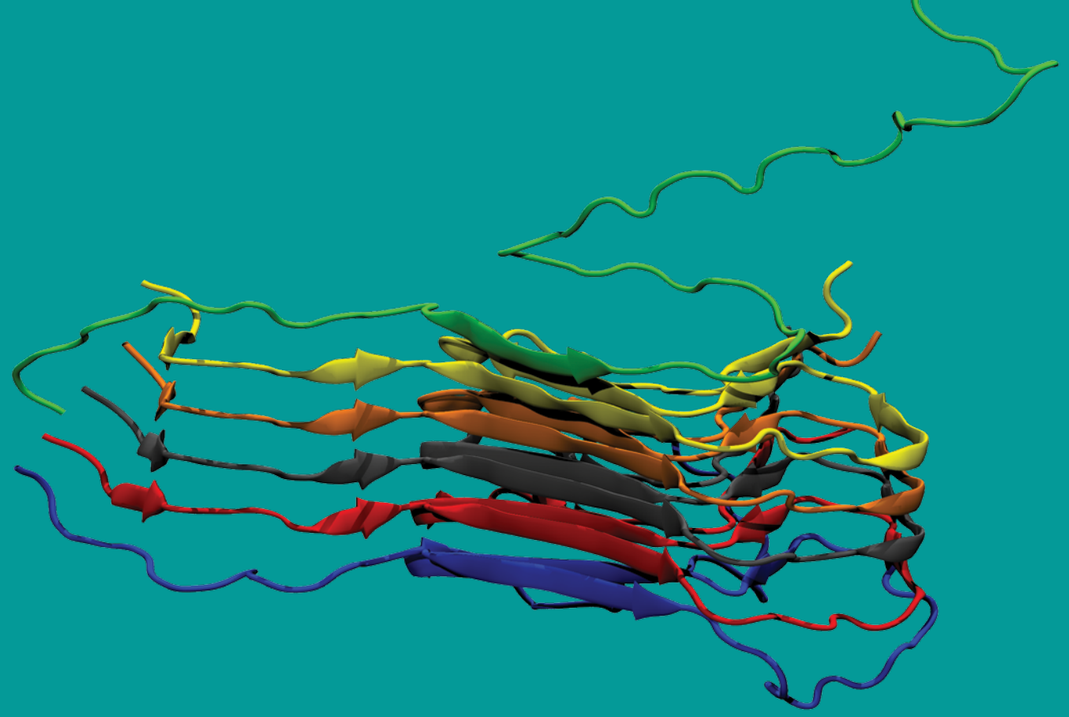

PROTEIN STRUCTURE DETERMINATION AND REFINEMENT FROM RAW NMR DATA

FIGURE 1: NAMD-GO simulation: folding and docking of monomer to alpha-synuclein fibril, 2.5 ns simulation on NAMD platform with GROMACS force field.

Allocation: Illinois/50 Knh
PI: Chad M. Rienstra[1]

[1]University of Illinois at Urbana–Champaign

EXECUTIVE SUMMARY:

Nuclear magnetic resonance (NMR) is a very powerful technique for investigating protein structure, dynamics, and interactions, and in the present project we are developing new computational methods for this purpose. One protein of special interest is alpha-synuclein, which forms protein aggregates as part of the neurodegenerative cascade of Parkinson's disease (PD) [1]. Thus, the understanding of this pathway may assist in the diagnosis and treatment of PD. We investigate the free-energy profile of alpha-synuclein aggregation using bias-exchange metadynamics, with collective variables that mimic the beta sheet structure, hydrogen bonding, and hydrophobic contacts present in the fibril, using GROMACS and PLUMED.

INTRODUCTION

Alpha-synuclein helps regulate the supply of synaptic vessels in the presynaptic terminals in the neuron, as part of the regulation of dopamine release. Misfolded alpha-synuclein can aggregate in the brain to form insoluble fibrils under pathological conditions, giving rise to disorders like Parkinson's disease [1]. The mechanism of aggregation is unknown and hence it is not clear if the protofibrils or the fibrils themselves are toxic to our brain. Using high resolution solid-state nuclear magnetic resonance (NMR), our lab recently determined the structure of the final form of the fibril. Using this structure, we aim to elucidate the pathway of aggregation by constructing a free-energy profile, including the intermediates formed during the process. This will help us determine the toxic species and eventually help identify a cure to Parkinson's disease.

METHODS & RESULTS

A trajectory on the pathway of a disordered monomer getting linked to a shorter fibril to form the final structured fibril was considered to be the model for generating free energy profile. The system was biased with GO-Potential [2] based on the final fibril structure and the NAMD-GO [3] technique was used with the GROMACS [4] force field. The nucleation was carried out in 2.5 ns.

A 20 ns equilibration simulation was performed in NAMD [6] using the CHARMM36 [7] force field on the experimental structure of the fibril to verify its stability. Next, we successfully simulated a 2.5 ns trajectory of an unfolded disordered monomer folding and docking to a shorter fibril to form a larger fibril (fig. 1). Three sharp changes in the RMSD value of the trajectory indicated that a topological barrier was present at that region in the pathway of the protein folding and docking.

As the NAMD-GO path proved to be too short for analysis of the trajectory, we simulated the reverse process of fibril unfolding with the biasing potential of the metadynamics [5] method in an explicit solvent environment. This simulation was run for 60 ns with appropriate collective variables using the NAMD2 [6] molecular dynamics code and the CHARMM36 [7] force field.

Several metadynamics [5] simulations were carried out on the initial system of a large fibril (haxamer), where a pentamer portion was kept fixed and the last monomer was made to unfold under the metadynamics [5] potential. The collective variables used were: (a) hBond [10] (calculates the hydrogen bonding present between the fibrils), and (b) coordNum [10] (calculates the amount of hydrophobic contacts present in between fibrils). Thus far, these collective variables have not been able to mimic the unfolding of the protein.

These results showed that better collective variables and technique for performing the free-energy calculations are needed. We decided to use the Bias-Exchange Metadynamics [8] technique that worked best on the GROMACS molecular dynamics code with PLUMED [9] software for analysis. The work of implementing the software and setting the simulation is in progress. Future work will include collective variables that leverage the experimental NMR shift values (via CAMSHIFT [9]).

WHY BLUE WATERS?

Blue Waters was essential to our project since we needed a supercomputing facility to run our long simulations on fairly large systems (~10,000 atoms) in a convenient amount of time. Also, the installation of required software is convenient on Blue Waters because all the prerequisites like compilers are available for easy use.