A series of statements about molecular dynamics simulations that may be true or false.

Discuss briefly a recent paper where MD is applied to study human prion proteins.

Outline our recent work with mutant insulins.
Part I: True-False statements
S: MD is a old method
Answer: Yes

- Based on Newton’s equations of motion.
- Laplace envisioned this deterministic solution to the many-body problem.
- Molecular Dynamics as we know it got started in 1957.
- Advancement in computing advances the field.
S: MD is superior to MC

Answer: No

- MC does not require force evaluations.
- MC more scalable and suitable for the Grid.
- MC is a configurational sampling method. It does not provide time information. Also, no velocity information.
- They have been combined into hybrid schemes.
S: All MD packages are commercial

Answer: No

- CHARMM
  - Chemistry at HARvard Macromolecular Mechanics
- AMBER
  - Assisted Model Building with Energy Refinement
- GROMACS
  - GROningen MAchine for Chemical Simulations
- NAMD
  - Not Another Molecular Dynamics
CHARMM

- Most popular and widely-used.
- Developed by Prof Martin Karplus, Dept of Chemistry & Chemical Biology, Harvard University.
- Latest release: c29b1
- Academic licensing available: US$500.
- Commercial version available from Accelrys.
AMBER

- Developed under leadership of Prof Peter Kollman, UC San Francisco.
- Set of force fields* as well as a suite of programs.
- Academic/non-profit: US$400
- AMBER 7 is dedicated to memory of Prof Kollman.
- After Prof Kollman’s death, development now headed by Scripps Research Institute, one of the lead partners in the collaboration.
GROMACS

- Developed by Berendsen group, dept of Biophysical Chemistry, Groningen University, Netherlands.
- Current release: 3.1.5 beta
- Contributors around the world, since GNU GPL.
- No scripting language needed.
- Optimized for processors with SSE/2 or Altivec.
- Parallel version uses standard MPI calls for distributed computers.
NAMD

- Developed by Theoretical & Computational Biophysics Group
  UIUC Beckman Institute, led by Prof Klaus Schulten.

- Latest release: 2.5b1

- Free to download and use but redistribution prohibited.

- Portable to any platform.

- Provides scalable performance through parallel C++ library:
  over 300K atoms on 1000 processors. (http://www.ks.uiuc.edu/
  Research/namd/performance.html)
UIUC: Theoretical & Computational Biophysics Group investigates the possibility of interactive MD (http://www.ks.uiuc.edu/Research/smd_imd/)

UPenn: Center for Molecular Modeling studies conformational changes associated with phosphorylation of a HIV accessory protein. (http://www.cmm.upenn.edu/research/index.html)

Scripps: The Brooks Group explores role of dynamics in enzyme catalysis. (http://www.scripps.edu/brooks/)

Oxford: Laboratory of Molecular Biophysics uses MD to study membrane proteins. (http://biop.ox.ac.uk/www/lj2002/msps/msps_journal.html)
S: MD codes are very scalable

Answer: No

- MD Benchmarks at Scripps (http://www.scripps.edu/brooks/Benchmarks/) for Lemieux at Pittsburgh Supercomputing Center:
S: MD is all powerful

Answer: No

- MD makes a couple of approximations.
- These allow study of up to 100,000 atoms.
- ‘Classical’ method.
- Cannot account for chemical reactions/enzyme activity: need QM or QM/MM to describe electrons more accurately.
Part II: Case Study
Molecular Dynamics Simulation of Dimeric and Monomeric Forms of Human Prion Protein: Insight into Dynamics and Properties

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ABSTRACT A central theme in prion protein research is the detection of the process that underlies the conformational transition from the normal cellular prion form (PrP\textsuperscript{C}) to its pathogenic isoform (PrP\textsuperscript{Sc}). Although the three-dimensional structures of monomeric and dimeric human prion protein (HuPrP) have been revealed by NMR spectroscopy and x-ray crystallography, the process underlying the conformational change from PrP\textsuperscript{C} to PrP\textsuperscript{Sc} and the dynamics and functions of PrP\textsuperscript{C} remain unknown. The dimeric form is thought to play an important role in the conformational transition. In this study, we performed molecular dynamics (MD) simulations on monomeric and dimeric HuPrP at 300 K and 500 K for 10 ns to investigate the differences in the properties of the monomer and the dimer from the perspective of dynamic and structural behaviors. Simulations were also undertaken with Asp178Asn and acidic pH, which is known as a disease-associated factor. Our results indicate that the dynamics of the dimer and monomer were similar (e.g., denaturation of helices and elongation of the β-sheet). However, additional secondary structure elements formed in the dimer might result in showing the differences in dynamics and properties between the monomer and dimer (e.g., the greater retention of dimeric than monomeric tertiary structure).

INTRODUCTION
Prion proteins have been found to be the cause of spongiform encephalopathies, a group of brain-attacking disease, which includes BSE in cattle and Creutzfeldt-Jakob disease in humans.

Abnormal forms of the prion proteins enter the brain and convert normal versions into the pathological form. An avalanche of destruction then results.

Source: http://apu.sfn.org/content/Publications/BrainBriefings/prion_protein.html
Normal cellular prion (PrP\textsuperscript{C}) gets converted to the pathogenic prion (PrP\textsuperscript{Sc}), which share identical sequences (isoforms).

**Known:** 3D structures of monomeric and dimeric forms of the human prion protein (HuPrP) via experiments.

**Unknown:** process underlying conformational change. Theory suggests dimerization to play an important role.

PrP\textsuperscript{Sc} has much more beta-strands than PrP\textsuperscript{C}. 
Aims of paper:

- Assess differences in the functions and dynamics of the PrP monomer and dimer.
- Provide more simulation data on dimer dynamics for PrP\textsuperscript{C}.
- Look at effects of mutations and pH.
- Perform longer runs with newer force field than previous work on monomers.
What is done?

- Series of 10-ns long MD simulations using AMBER 7 carried out on dimer and monomer forms of human PrPc under various conditions: 300K and 500K, acidic pH and Asp178Asn mutant.

- 1040-processor Magi parallel cluster running SCure 4.1 at the Computational Biology Research Center is employed for the study.

http://www.cbrc.jp/cbrc/cluster/comp.eng.html
Materials & Methods - a recipe

- Built the dimer model based on a PDB model using Insight II.
- Surround protein with a 20 Angstroms layer of water.
- Neutralize by adding positively-charged sodium ions.
- Do 1000 CG energy minimization steps for equilibration.
- Production run of 10 ns done at constant V and T.
Simulation Results

FIGURE 2  RMSD values of Ca from the initial structures. (a–c) Red and blue lines indicate RMSD values of the dimer and the monomer at 300 K, respectively. (a) Green and pink lines indicate RMSD values of the dimer and the monomer at 500 K, respectively. (b) Green and pink lines indicate RMSD values of the dimer and the monomer at D178N, respectively. (c) Green and pink lines indicate RMSD values of the dimer and the monomer at acidic pH, respectively.

Monomer denatures faster

BII structural & functional genomics group
Observations

- C-alpha RMSD from initial structures:
  - monomer deviates significantly while dimer does so gently.
  - effect more pronounced at higher temperatures.
  - little conformational change for mutant or acidic pH.

- Additional secondary structures formed in the dimer:
  - Dimer formed extra helices and beta-sheets per ‘monomer’.
  - During denaturation at 500K, dimer retained more secondary structures. Monomer has helices turned into strands, which corresponds to increase in RMSD above.
Conclusions

- Monomer started to denature faster than dimer.
- Not sufficient evidence to confirm or rule out importance of dimerization in the transition from $\text{PrP}^C$ to $\text{PrP}^\text{Sc}$.
- More work is currently being carried out.
Part III: Our work
Conformational study of insulin mutants

Motivation: Insulin is a medicinally important molecule. Experimentalists from China and Denmark (Zeng, 2000) looked at how structures of mutants affect insulin’s biological activity.

We hope to gain a better understanding of residue interactions through molecular simulations.
Experimental study by Zeng et al.

- C-terminal residue of insulin A chain is conserved across all known insulin molecules.

- Paper looked at crystal structures of 4 human insulin mutants where Asn21 is replaced by Asp, Glu, Gly and Ala.

- Authors observed conformational correlation and coupled motion between A21 and B25.

- B25 is important in receptor binding:
  - Phe → Leu caused diabetes.
  - Other substitutions lead to inactive mutants.
A21A’s side-chain has only 1 C-beta and occupies same orientation as native. Empty space created occupied by B25’s Phenol group.
Inferences from the findings

- Phe$^{B25}$ is critical for insulin’s bioactivity via moving into a spatial position to expose the pharmacophore of insulin. A21 is there to assist in the reorienting & stabilizing ‘active’ conformation of B25 during receptor binding.

- Ala, having shorter side-chain, gives Phe$^{B25}$ more freedom and hence enhances ‘active’ form formation. Potency increased 36% compared to native insulin!
Our research focus

Title: Understanding the role of mutations in protein structure-function-dynamics -- a computational study of insulin

- Aim:
  - “MD simulations will be used to examine the role of position A21 in influencing the motion of residue B25 which is implicated in receptor binding.” [Dr Chandra Verma, joining in Oct]

Team: Dr Chandra (PI)
  Sandeep
  Me
Fig: Wild-Type Insulin monomer, edited from PDB entry 4INS. [figure generated via PyMol]
Materials & Methods

- All simulations and analyses use GROMACS.
- Protein placed into a cubic box and filled with water.
- Neutralize by adding 2 positively-charged sodium ions.
- Energy minimization followed by 30 ps of position-restrained MD.
- Production run of 5000 ps done at constant pressure of 1 atm and temperature of 300K (27°C).
- Takes 25 hours with 8 processors of Mamba cluster at BII.
Scaling of Insulin simulation on Mamba

Short 20ps runs

Scalability, Sp/S1

GROMACS Lysozyme Benchmark Scaling on Mamba
Simulation results

end of 4ns

end of 5ns
Observations

- Temperature and pressure values approach desired values, indicating proper simulations.
- Total energy fluctuates around a constant value: required.
- Need to restrict linear motion of protein in simulations.
- Rotations still observed: ?
- B25 and A21 does move around in each other’s vicinity. (need more quantitative data)
Work pending

- Refinements to X-ray crystal data prior to simulations.
- Get more quantitative measures.
- Mutate A21 into 4 other residues and gather simulation data.
- Still many things to learn!
References
Papers


Softwares

- E. Lindahl and B. Hess and D. van der Spoel. 


  DeLano Scientific LLC, San Carlos, CA, USA. http://www.pymol.org