Learning about Ehler-Danlos Syndrome and Alzheimer's Disease using Molecular Dynamic (MD) Simulations

Apichart Linhananta

Department of Physics

Lakehead University

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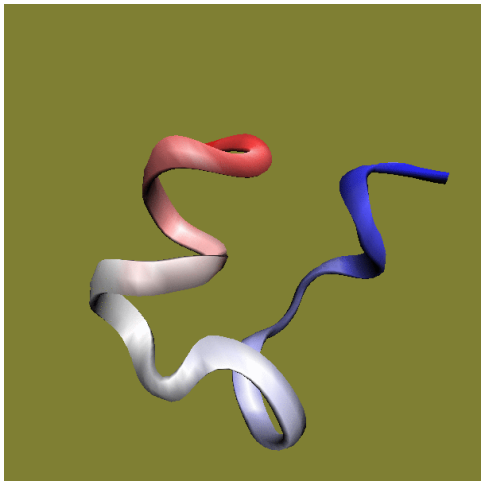

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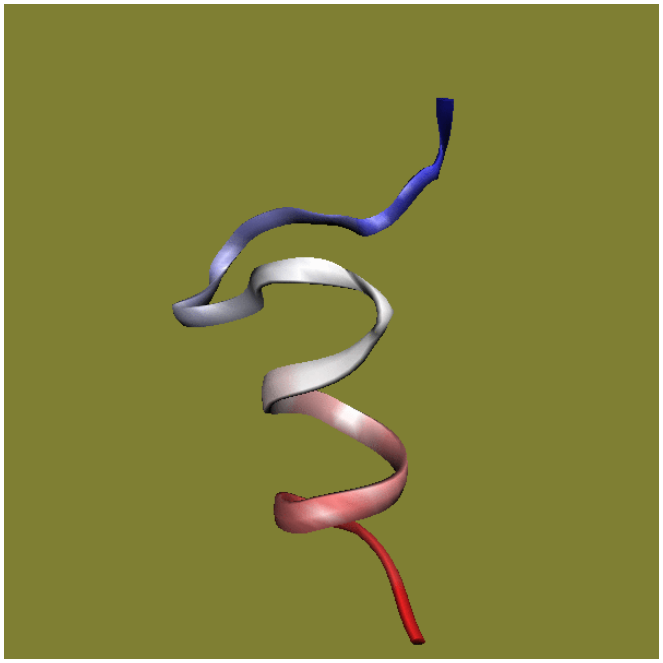

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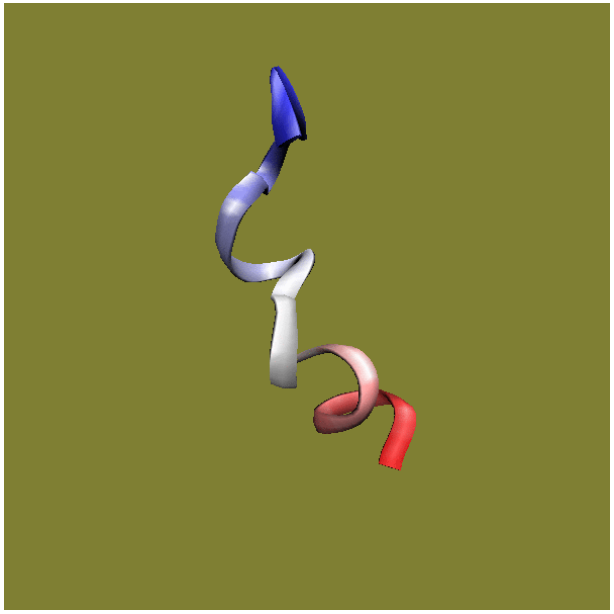

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First Molecular Dynamics (MD) Simulation in 1953

- N Metropolis, AW Rosenbluth, MN Rosenbluth, AH Teller, and Edward Teller (1953), *Journal of Chemical Physics,* 21:1087.
- Monte Carlo (Metropolis) method on the MANIAC computer to obtain 2D equation of states.

Nobel Prize in Chemistry 2013

- The development of **multi-scale models** of complex chemical systems
- Michael Levitt, Martin Karplus, Ariel Warshel

A Bit of History

The birth of computational structural biology

Michael Levitt

Like Sydney Altman[1], I too was initially rejected by the renowned Medical Research Council (MRC) Laboratory of Molecular Biology in Cambridge, England. The year was 1967 and I was then in my final year of a B.Sc. degree in Physics at Kings College in London. Enthralled by John Kendrew's BBC 1964 television series "The Thread of Life", I wanted desperately to do my Ph.D. at the MRC in Cambridge. Alas there was no room for any new postgraduate students in 1967!

After some negotiations, I was accepted for the following year. More importantly, John Kendrew said that I should spend the intervening period at the Weizmann Institute in Israel with Shneior Lifson. Kendrew had just heard of Lifson's initial ideas[2] on the consistent force field (CFF), which was an attempt to simulate the

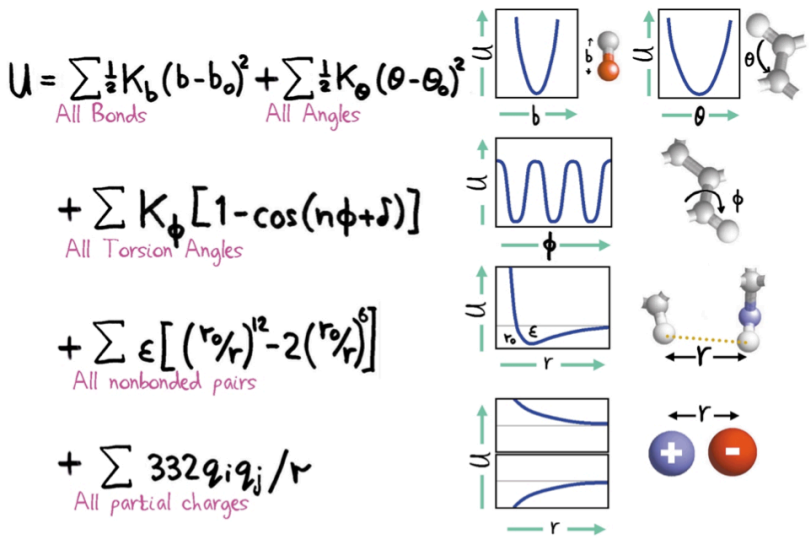
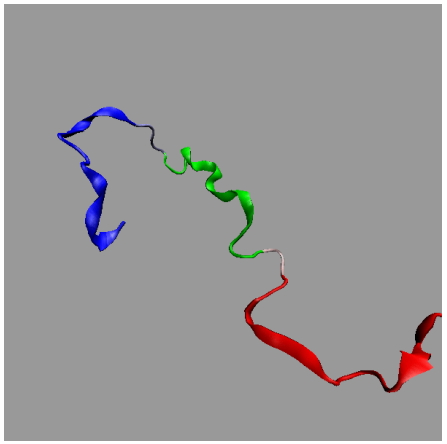


Fig. 1 The total potential energy of any molecule is the sum of terms allowing for bond stretch-

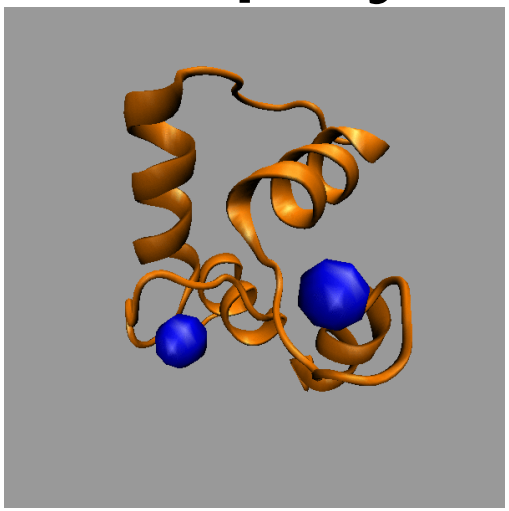
A Bit of History

- Programing mostly by **Bruce Gelin**
- Translated **M. Levitt's IBM Fortran II** (subroutine,end, .., but no loop) to **Fortran 77** (IF, ENDIF, Do,…)
- First version of MD simulation software CHARMM
- Follow by simulation software, AMBER, GROMACS, GROMOS

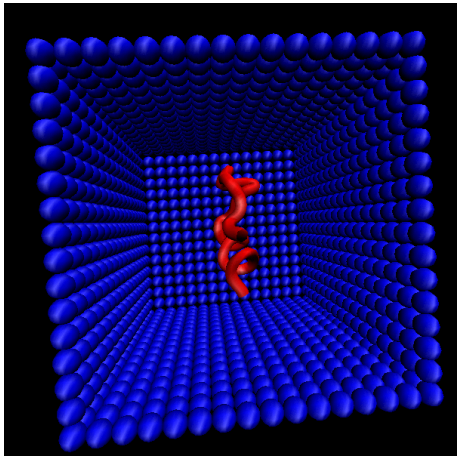
Theoretical Biophysics



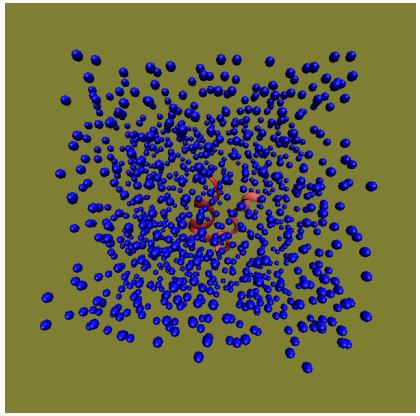
Protein Folding: Folding of Protein G



Protein Binding: Fragment of Calmodulin



Confined Systems: Chaperonin, GroEL-ES

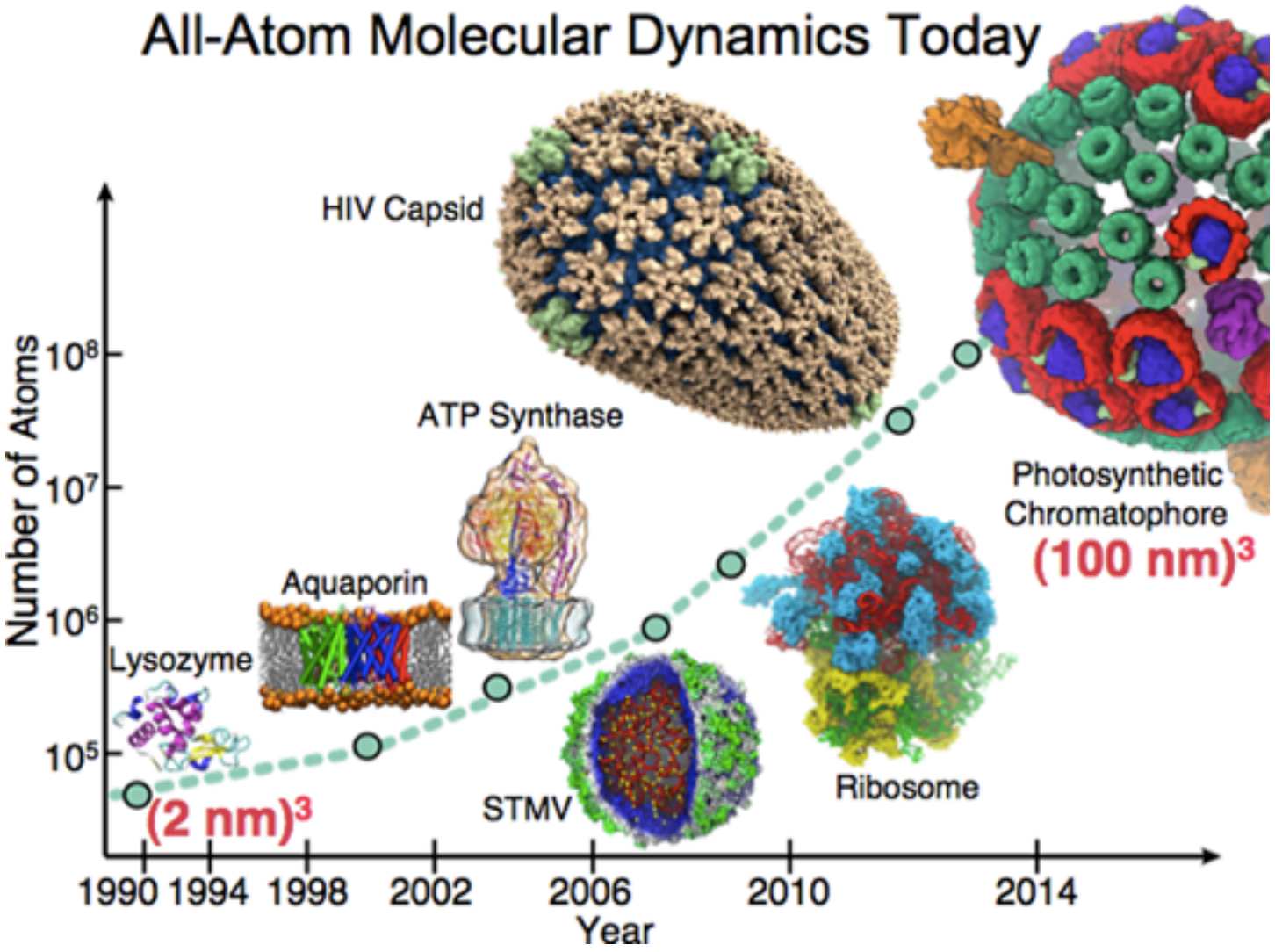


Macromolecular Crowding Effects: Protein Stabilization by Osmolytes

MD of HIV-1 Virus Capsid

- Klaus Schulten et al (2013), *Nature*, 497: 643-646
- ≈ 50nm, > 30 million atoms

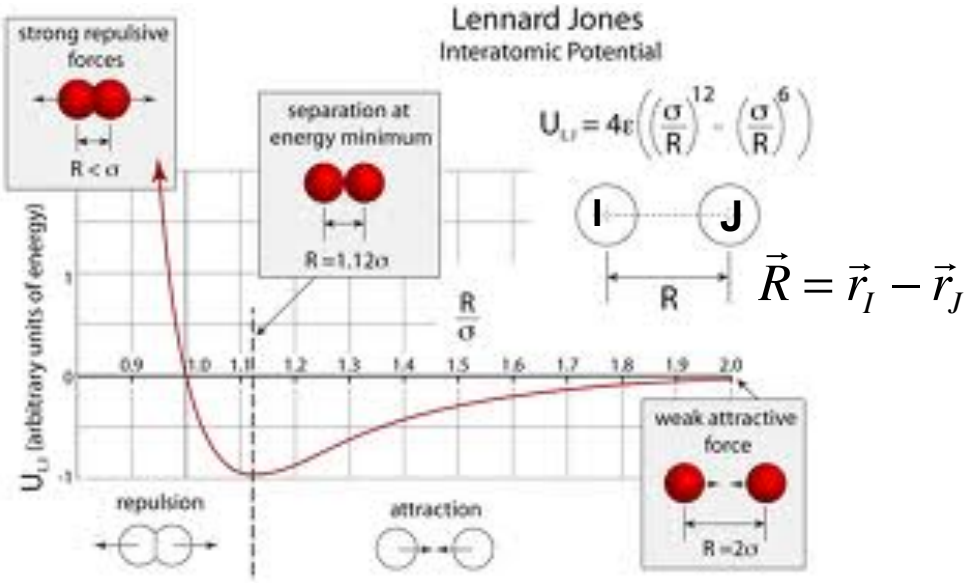
State-of-the-Art MD



Molecular Dynamics (MD) Models from Scratch

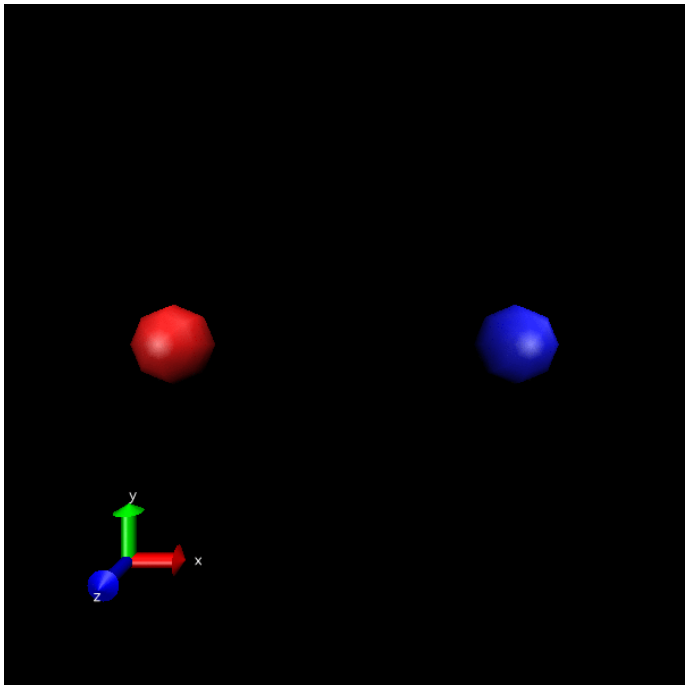
- **Specificity** of proteins, DNA, solvents, and membranes, requires the solution of **Newton's equations**.
- **Large number** of **particles** requires **Statistical Mechanics**.
- Certain systems (for example light harvesting plants) require QM to model chemical reactions and quantum tunneling.

Two particles: Lennard-Jones (LJ) Interaction



$$U_{LJ} = 4\varepsilon\left(\left(\frac{\sigma}{R}\right)^{12} - \left(\frac{\sigma}{R}\right)^{6}\right)$$

$$\vec{R} = \vec{r}_I - \vec{r}_J$$



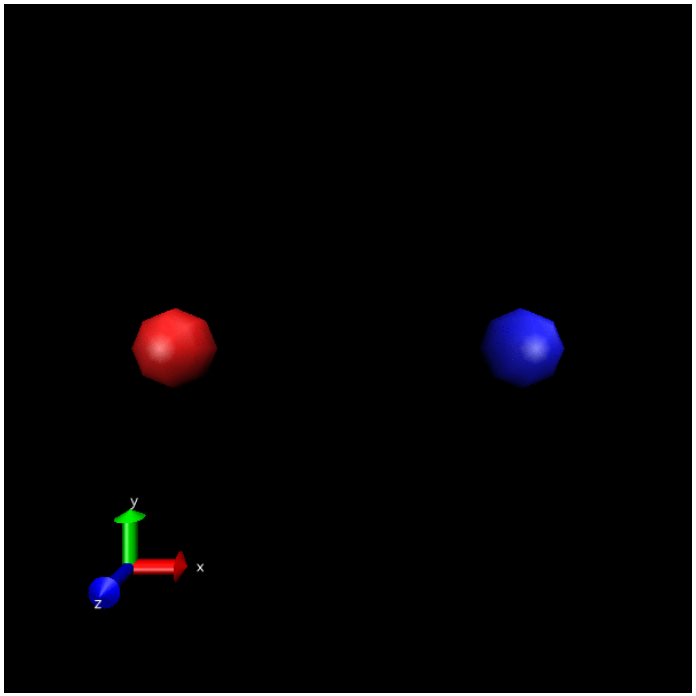
Force

Ith Particle $\vec{F}_I = -\left(\frac{\partial U_{LJ}}{\partial \vec{r}_I}\right)$

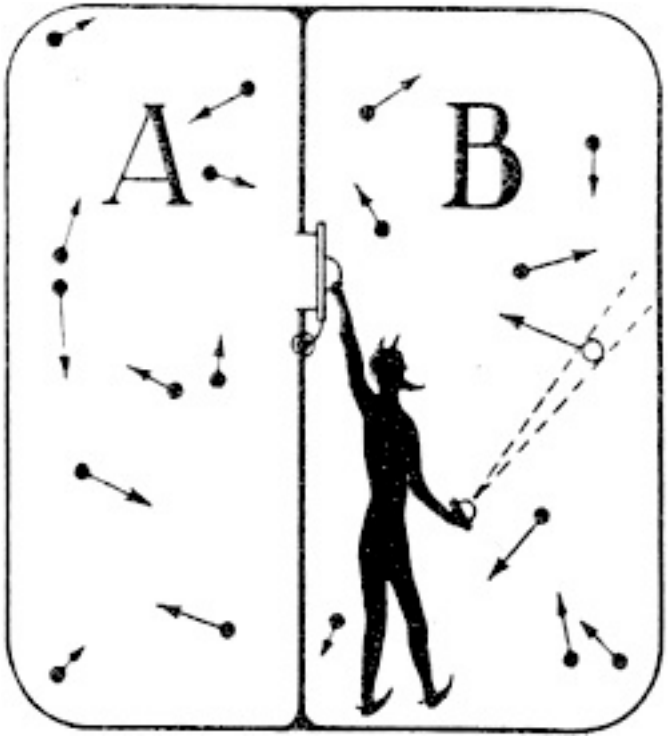
Jth Particle $\vec{F}_J = -\left(\frac{\partial U_{LJ}}{\partial \vec{r}_J}\right)$

Molecular Dynamics (MD): Numerical Solution of Newton's Equation

Thermal Equilibration and the Maxwell's Demon

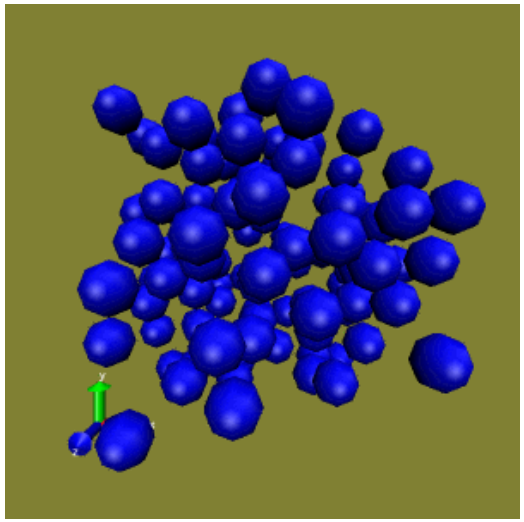


Random Collisions with **Ghost Particles** maintain system at **constant temperature**

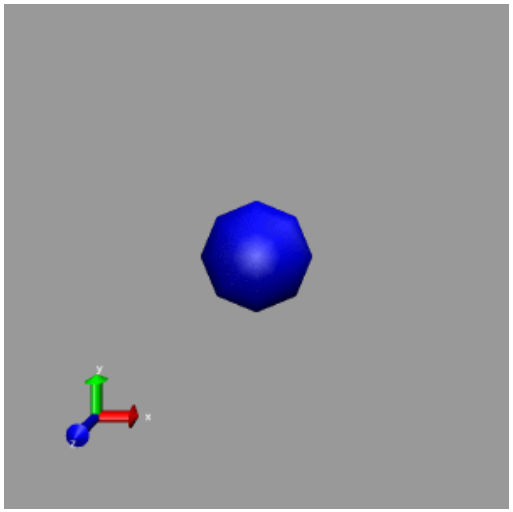


Maxwell's Demon

MD of 108 Argon and Periodic Boundary Condition

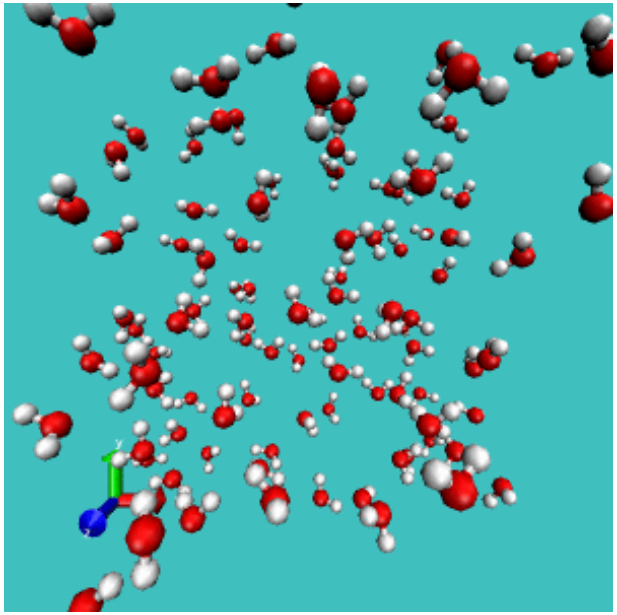


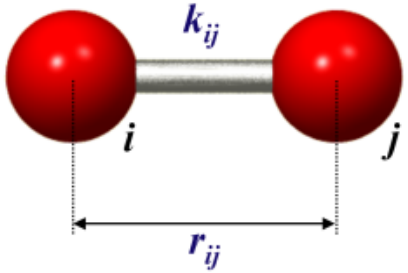
108 argons interacting by LJ potential



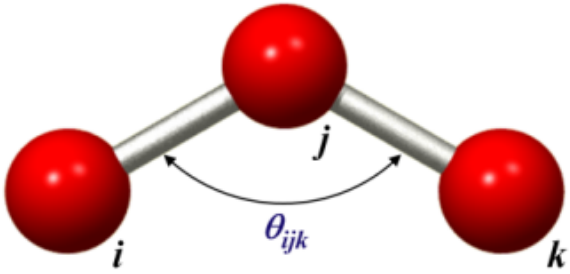
Periodic Boundary condition minimizes the effects of surface

Tri-atomic Molecules: H_2O

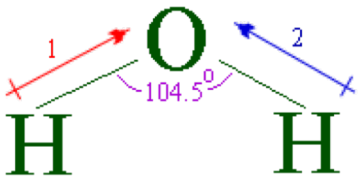




$$V_{harmonic} = k_{ij}\left(r_{ij} - R_{bond}\right)^2$$



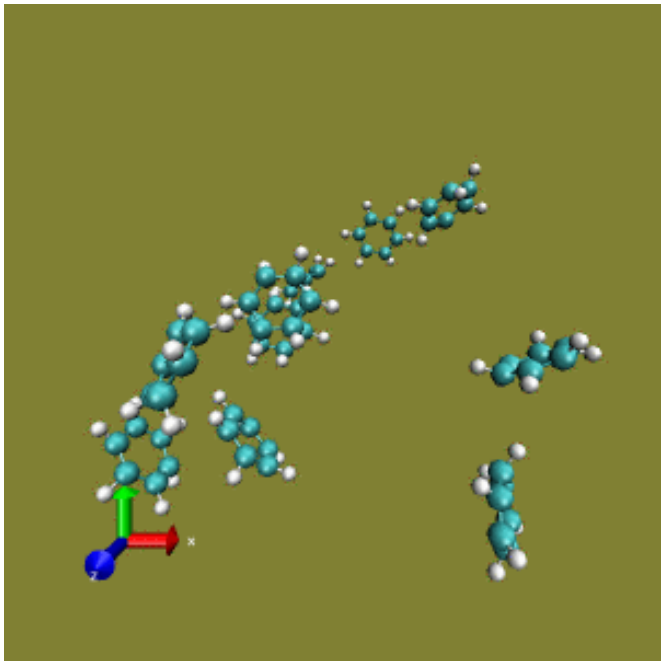
$$V_{angle} = k_{ijk}\left(\theta_{ijk} - \theta_0\right)^2$$

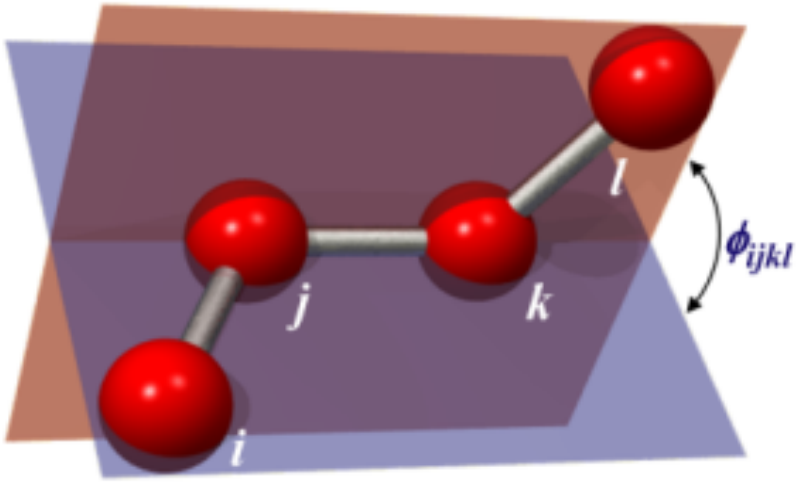


For water $\theta_0 = 104.45^\circ$

Force Calculation: $\vec{F}_I = -\vec{\nabla}_I V$

Benzene





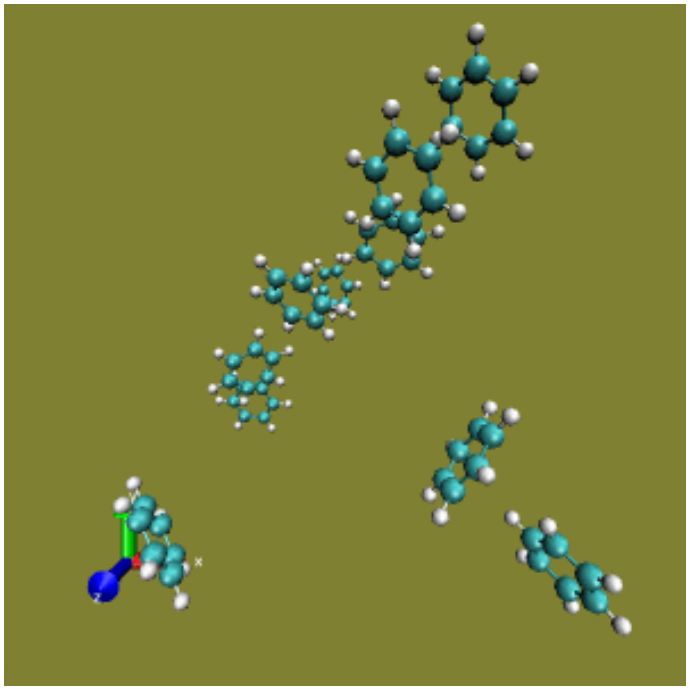
$$V_{dihedral} = k_{ijkl}\left(\theta_{ijkl} - \theta_0\right)^2$$

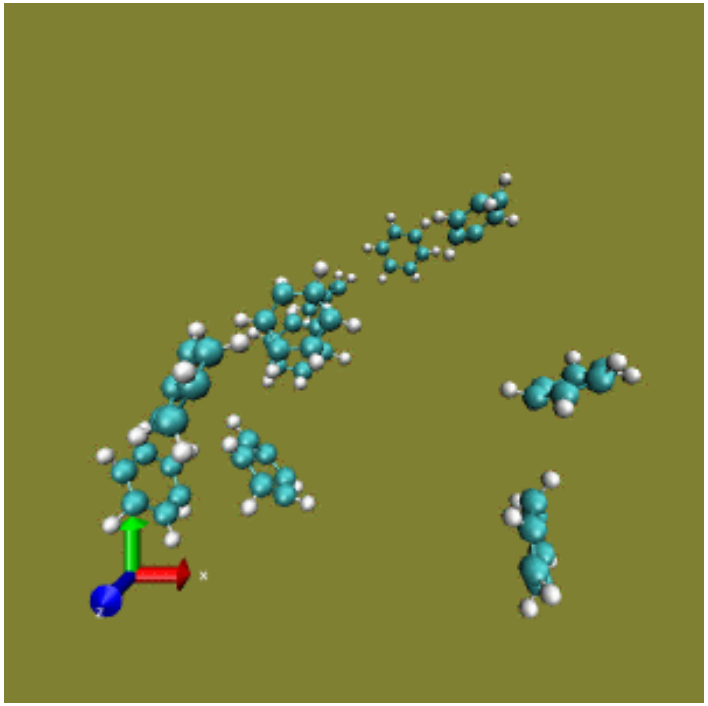
Too Flexible!!

4-body interaction

For Benzene $\theta_0 = 0, 180^\circ$

Benzene with Dihedral

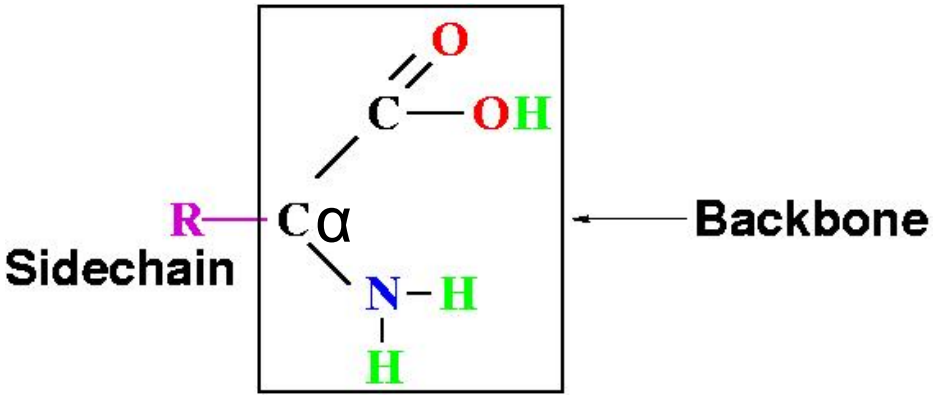




With Dihedral. Rigid!! No Dihedral. Floppy!!

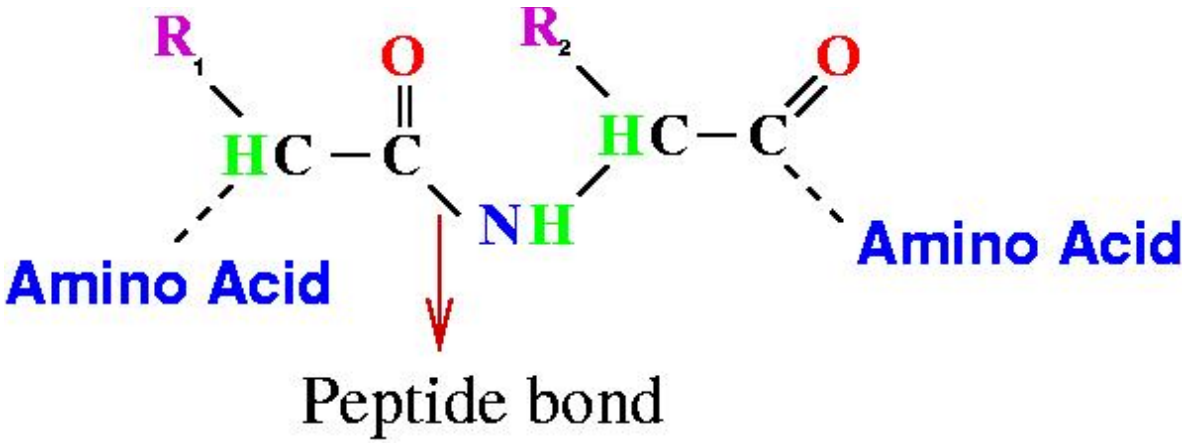
If we can model benzene, we can model proteins, DNA, lipid membrane…

Proteins:Amino Acids



Amino acid

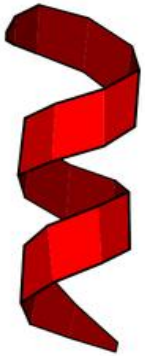
1. C, N, O, H or S
2. 20 types
3. Distinguished by R
   - Nonpolar
   - polar
   - charged

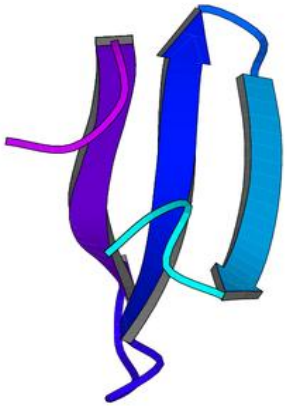


Amino acid residue

•Denoted by A,T …

Proteins:Structure of Proteins



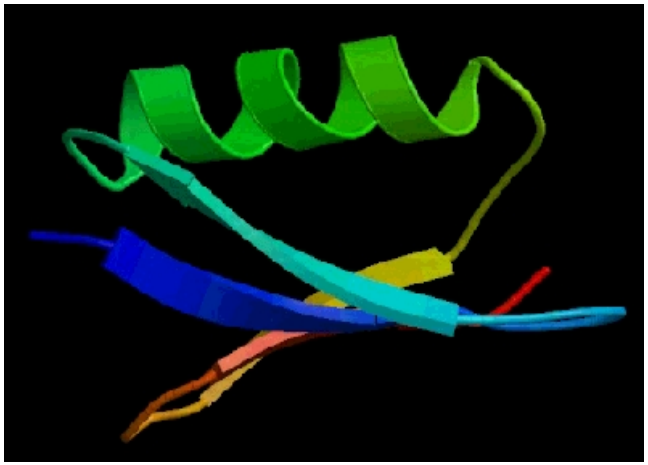


helix β-sheet loop/turn

Primary structure
•40 to 10000 residues

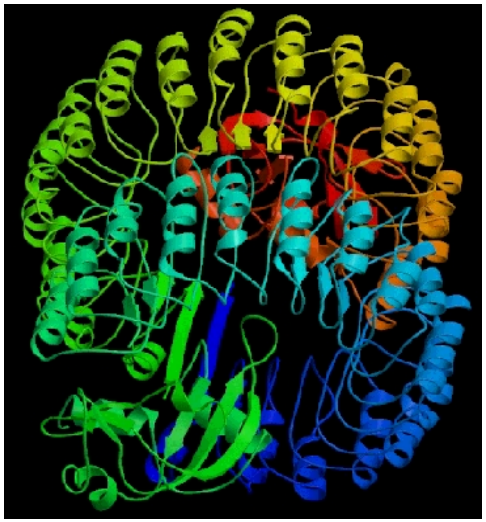
Secondary structure
•Backbone hydrogen bonds

Proteins: Tertiary Structure



Non-covalent Contacts

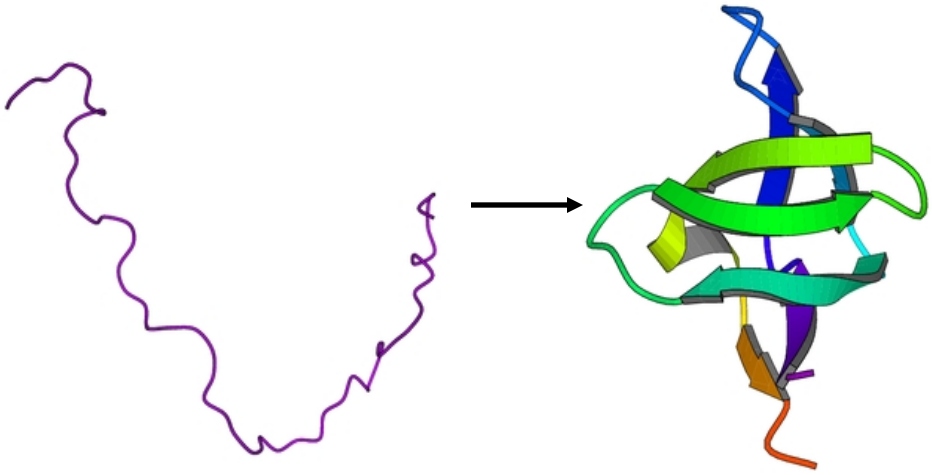
- side-chain packing
- side-chain hydrogen bonds
- salt bridges
- Hydrophobic Core
- Hydrophilic Surface



Important Points

1. **Unique Native Structure**
2. **Must Fold to Function**

Proteins:Levinthal Paradox (1968)



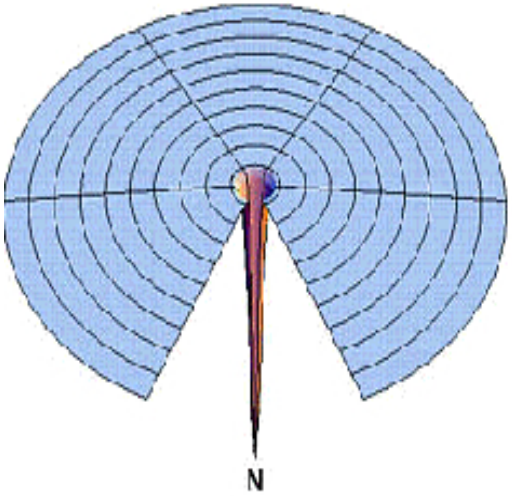
- 100 amino acid residues
- 2 conformation/residue
- $2^{100} \approx 10^{30}$ conformations
- 1 picosecond $\approx 10^{-12}$ s to convert between conformations

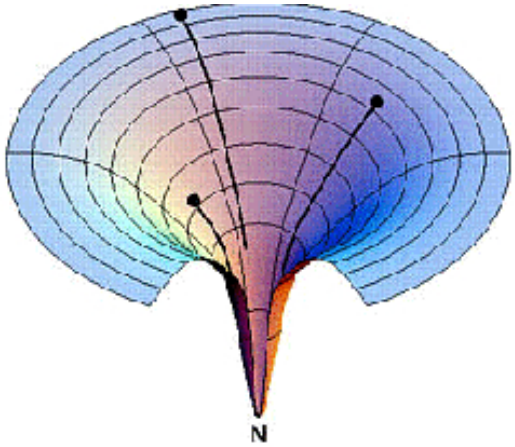
Random search to unique native state

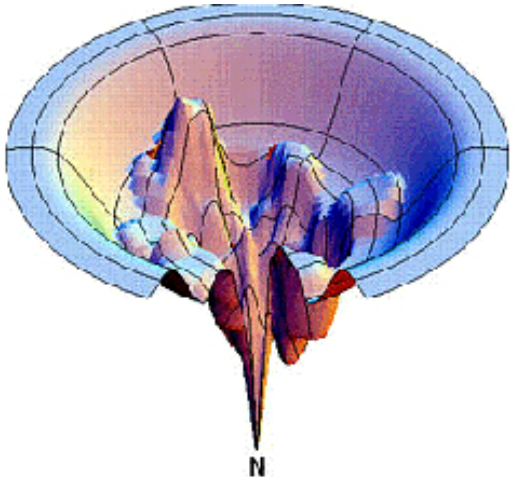
$\sim 10^{-12} \text{x } 10^{30} = 10^{18}$ s $\sim 10^{11}$ years

Actual Folding time 1μs to few seconds

Proteins: Folding Free-Energy Landscape







Uniform landscape
- Levinthal paradox

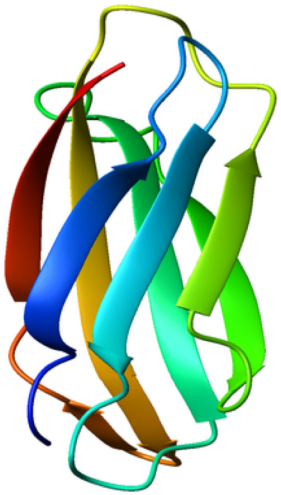
Funnel landscape
- Guided Pathways

Rough landscape
- Funnel-like
- Traps

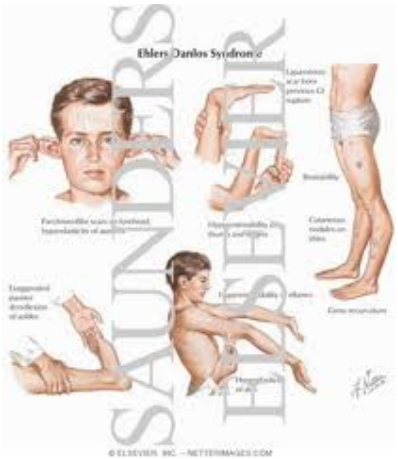
- Dill and Chan, **Nature Struct. Biol. 4**, 10 (1997)

Part I: Ehler-Danlos Syndrome

- Molecular-level investigations of **genotype** and **phenotype**

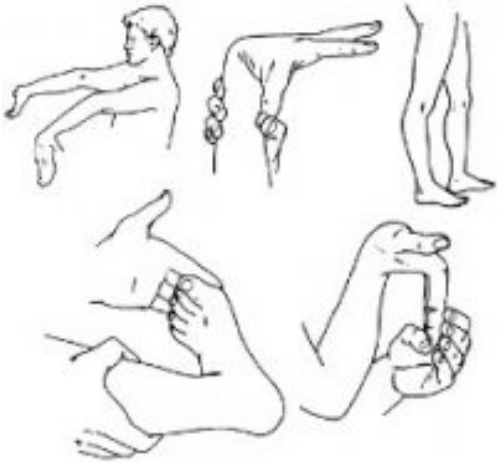


Tenascin



Ehler-Danlos Syndrome

Ehlers-Danlos Syndrome (EDS)

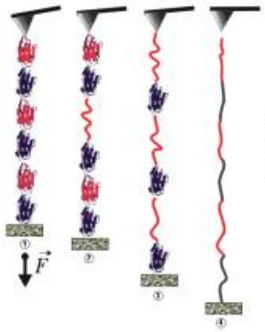


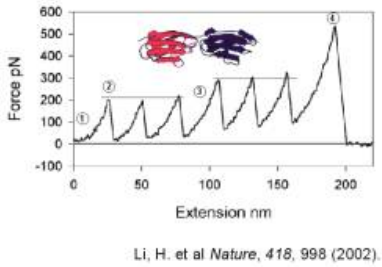
EDS is a group of **inherited disorder** of connective tissue, which can leads to **extreme flexibility**

Tenascin-X and its V1195M Mutant, and EDS

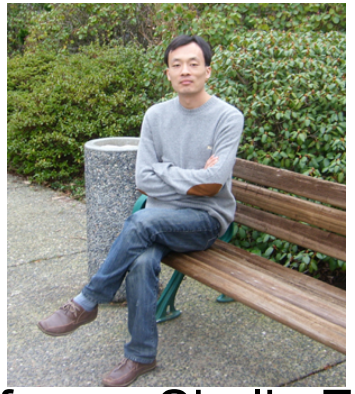
- Tenascin-X (TNX) is an exracellular matrix protein essential for healthy skin
- Deficiency of TNX causes Ehlers-Danlos syndrome (EDS)
- V1195M mutation of TNX is associated with EDS

Professor Hongbin Li





Li, H. et al *Nature*, *418*, 998 (2002).

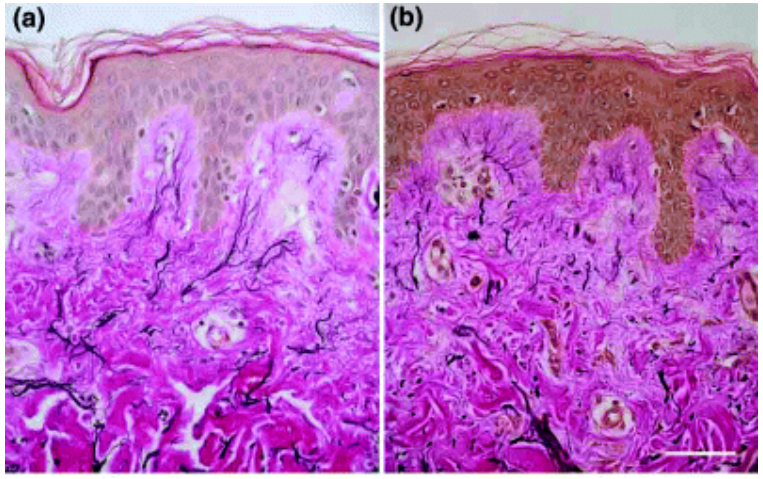


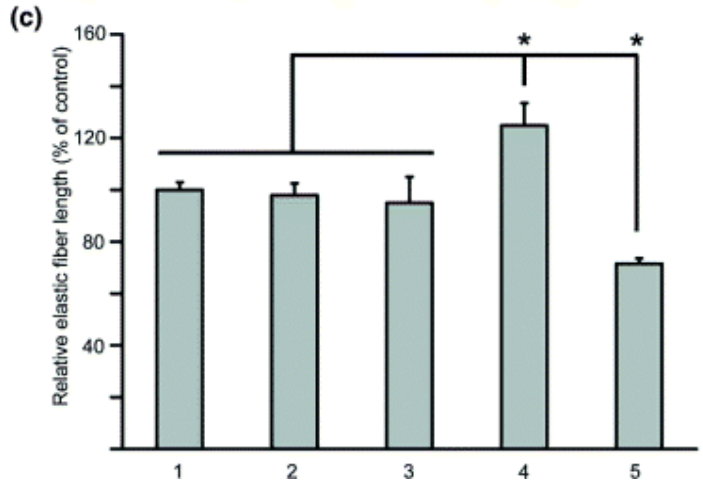
Professor Shulin Zhuang

CHEMISTRY @ UBC

ZJUT Zhejiang University of Technology

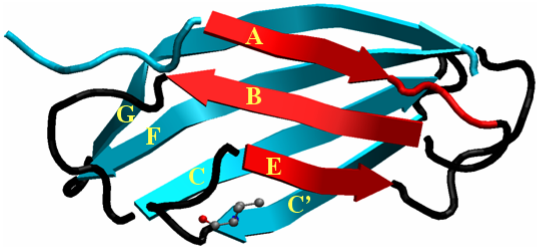
Ehlers-Danlos Syndrome (EDS)



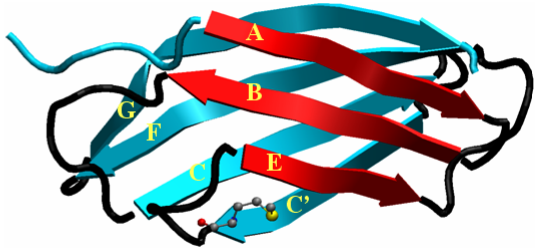


- Panel A shows is a biopsy of healthy skin
- Panel B is a biopsy of EDS skin with fragmented elastic fibers
- EDS skin are **deficient** in the protein **Tenascin-X** (TNX) or due to **mutation** of **TNX**

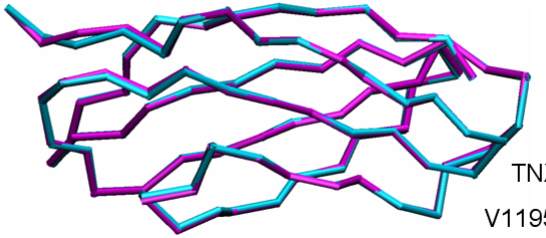
Modeling of Tenascin-X (TNX) and Mutant V1195M



TNXfn7



V1195M

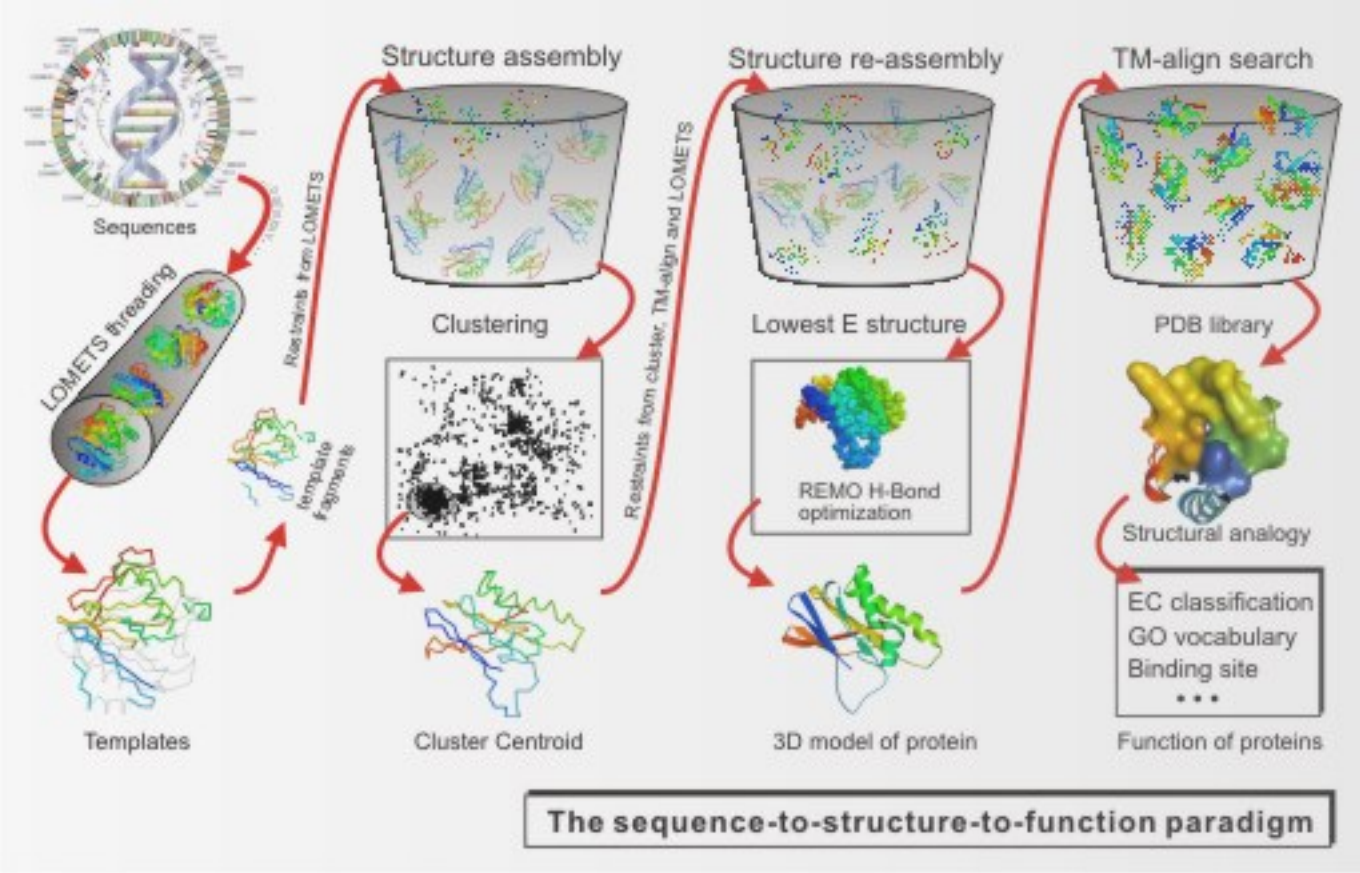


TNXfn7:cyan

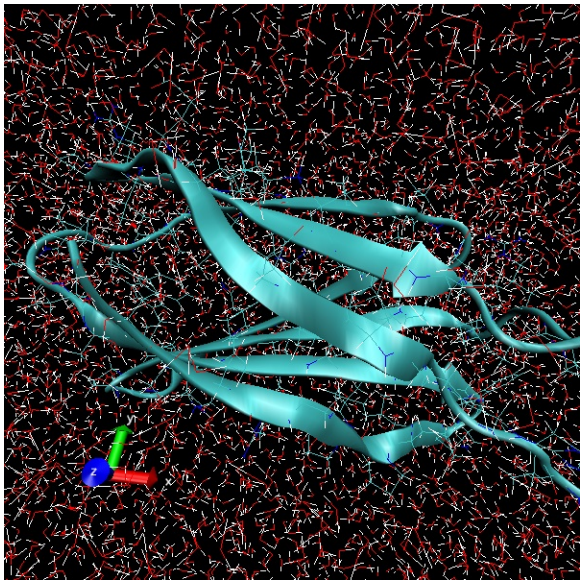
V1195M:magenta

- Structure **of wild-type** TNXfn7 is known
- Structure **of mutant V1195M** predicted by **I-TASSER SERVER** of Professor Y. Zhang (Kansas State University)

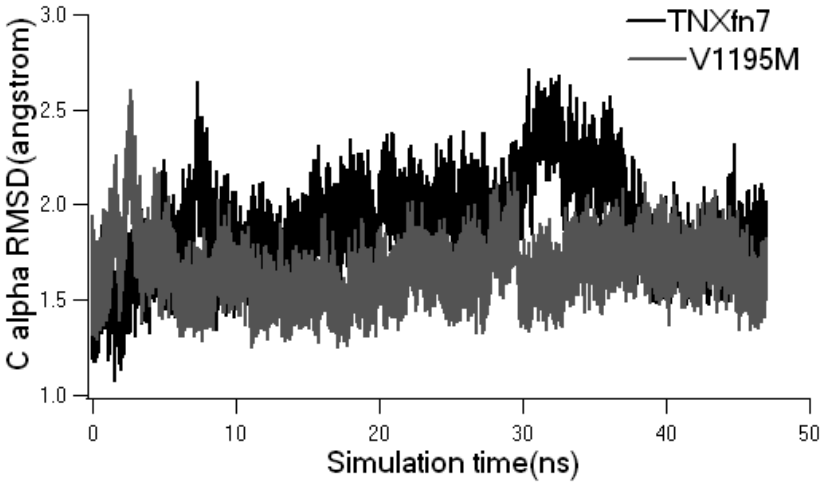
Predicting Structure of V1195M mutant with Professor Y. Zhang I-TASSER



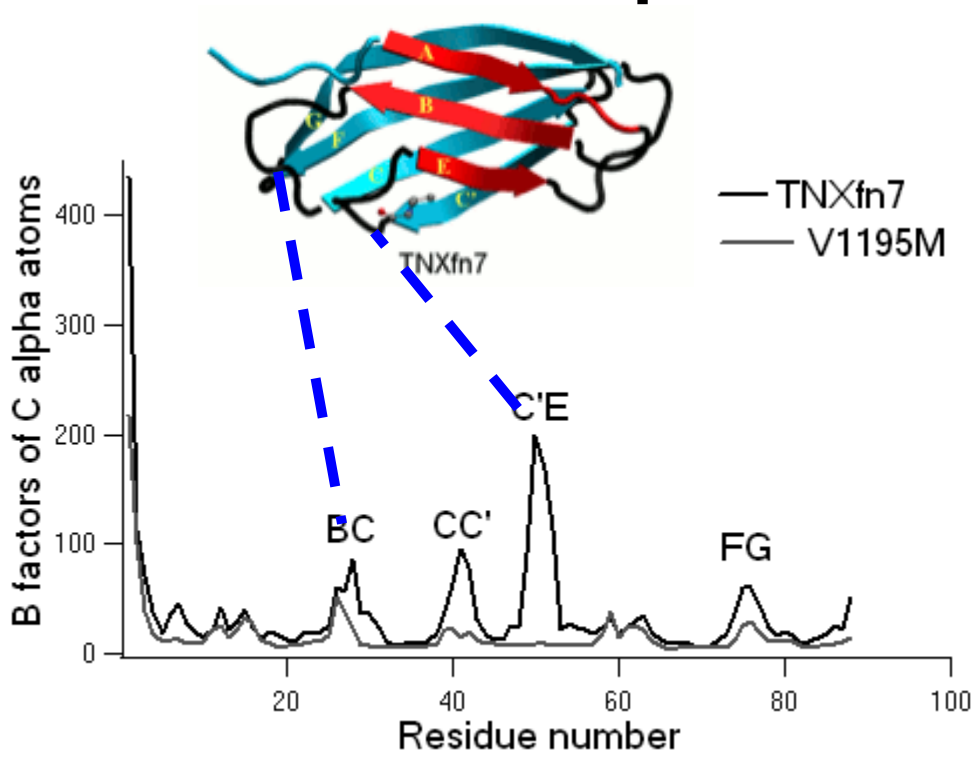
Massively Parallel Computer Simulation



- Models of wild-type and its mutant in TIP3P water uses AMBER, ≈ 25 000 atoms
- Constant temperature and pressure
- 16 processors parallel jobs



V1195M Mutant has reduced loop flexibility

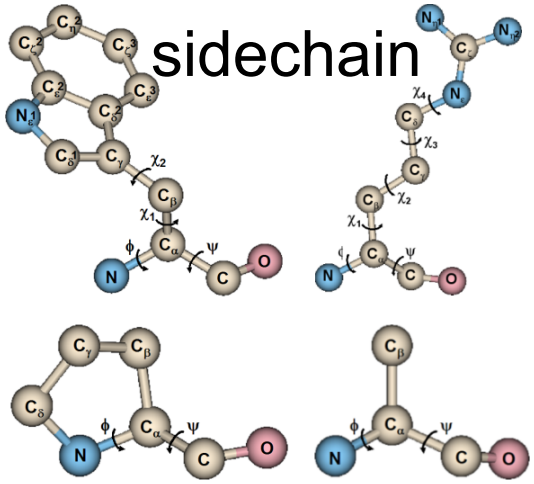


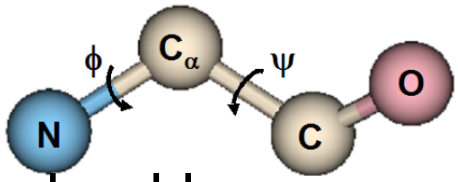
- Loss of flexibility associated with loss of functions
- Hypothesize that loss of loop flexibility causes EDS

Gerstein M, Krebs W (1998) A database of macromolecular motions. Nucleic Acids Res 26:4280–4290

C-beta branch amino acids

Most amino acids contains only **one non-hydrogen** connected to C_β , but C-beta branch contains **two carbons**, making **the side chain bulky**.

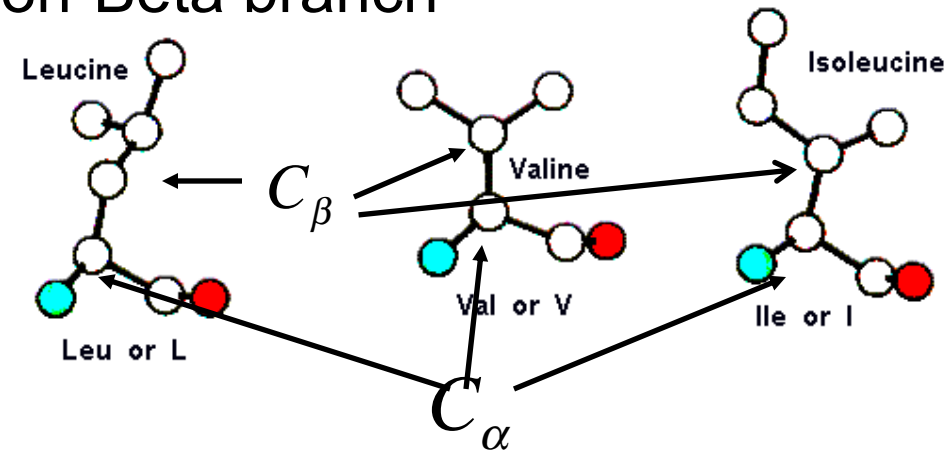




backbone

Non-Beta branch

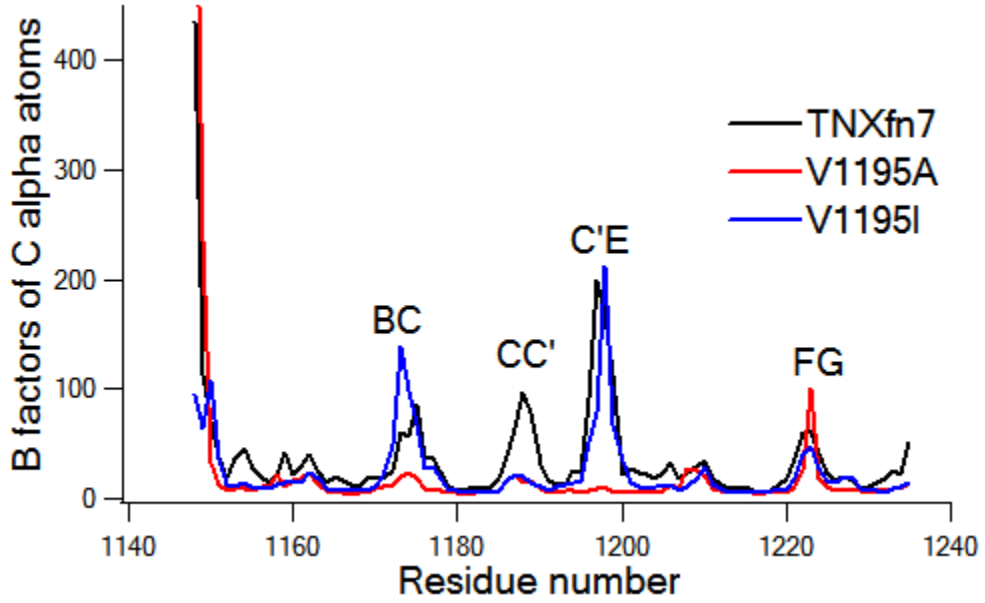
Beta branch



Carbon is white in above

Beta-branch to non-beta branch mutation causes EDS

Beta-branch to non-beta-branch: V1195M and V1195A
Beta-branch to beta-branch: V1195I

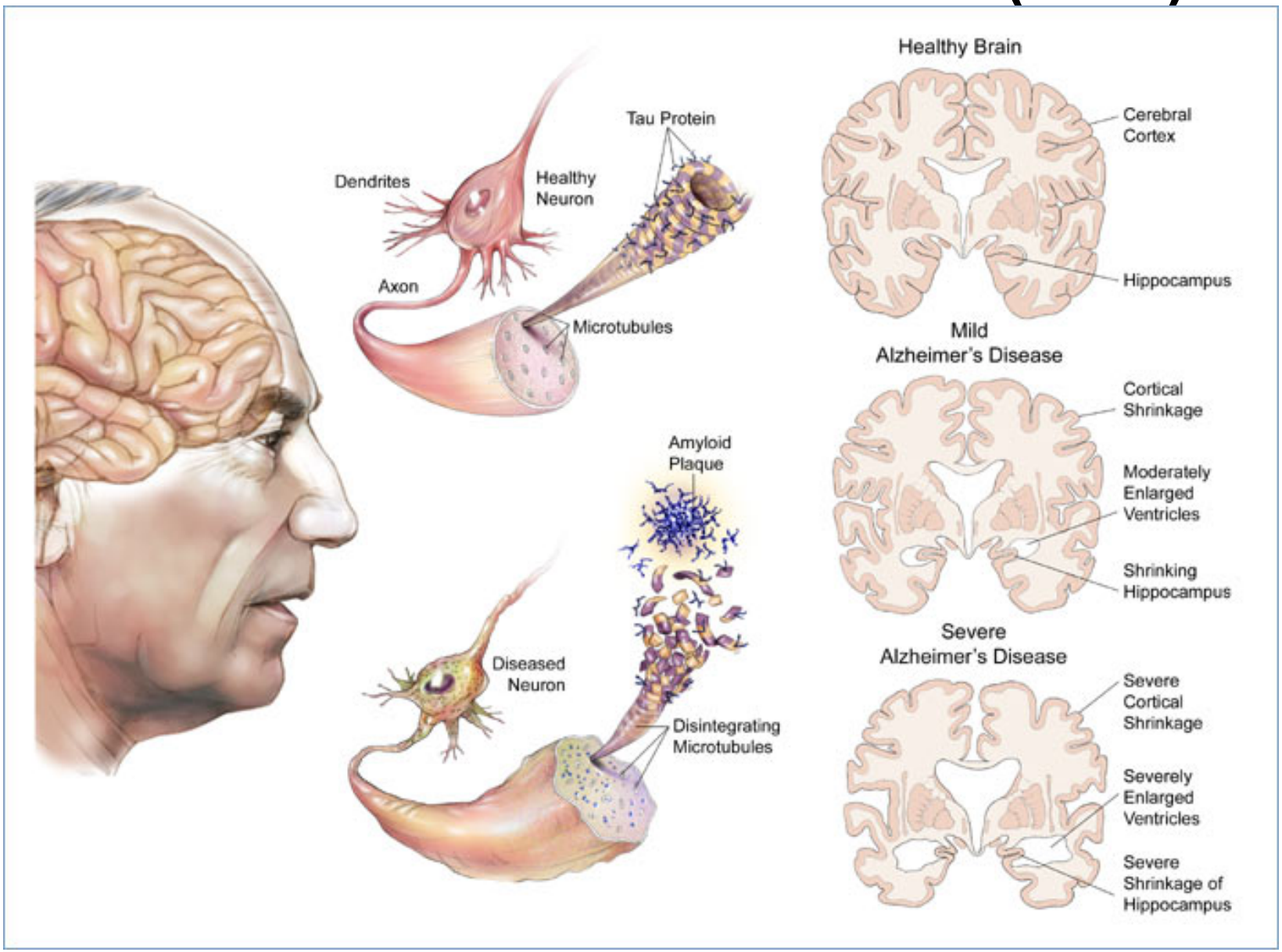


S. Zhuang, A. Linhananta, H. Li (2010) *Phenotypic effects of Ehlers-Danlos syndrome-associated mutation on the FnIII of tenascin-X* **PROTEIN SCI**, 19, 2231-2239.

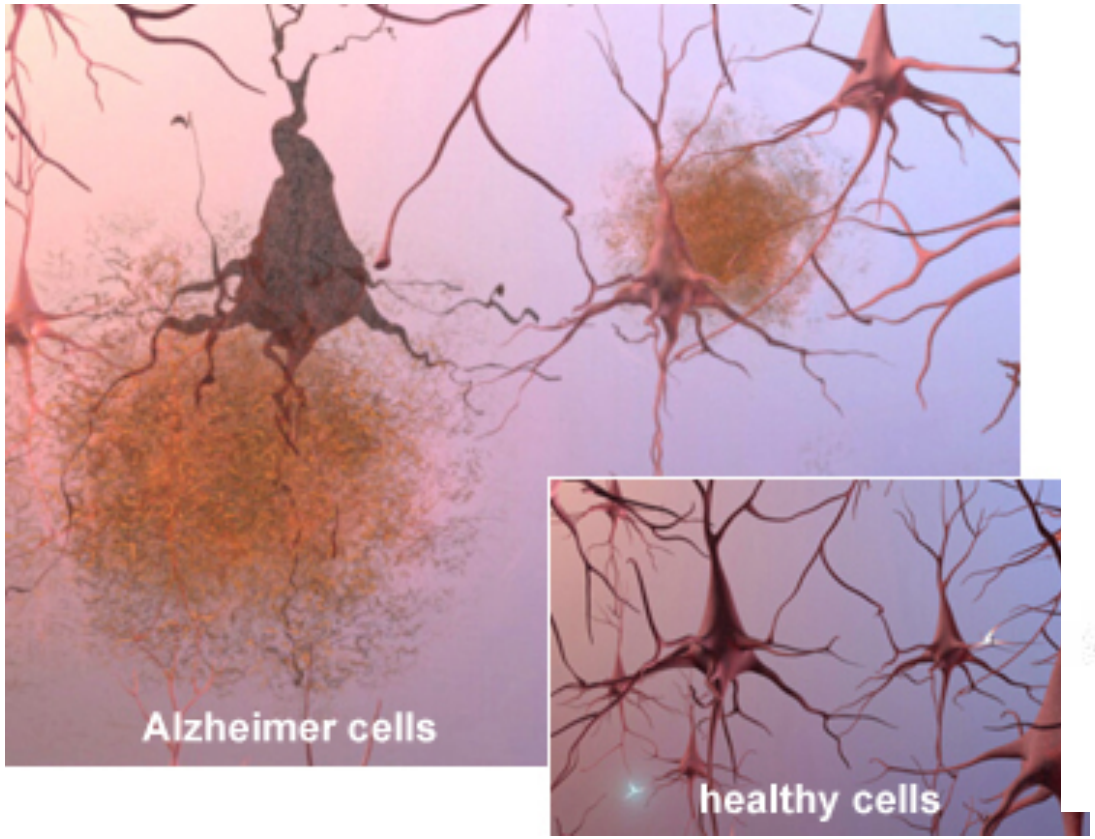
Conclusion on Ehlers-Danlos (ED) MD Study

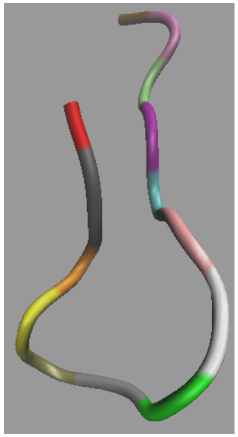
- MD simulation shows that mutation-induced ED syndrome is associated with loss of loop flexibility in mutant TNXs, leading to loss of full protein function
- MD simulation suggests that beta-branch to non-beta-branch mutations, V1195M and V1196A, induce loss of flexibility
- Direct link of these results with phenotype requires experiments

Alzheimer's Disease (AD)

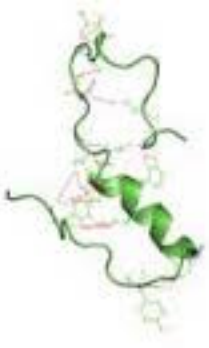


Plaques and Tangles





Tau Proteins
form **Tangles**

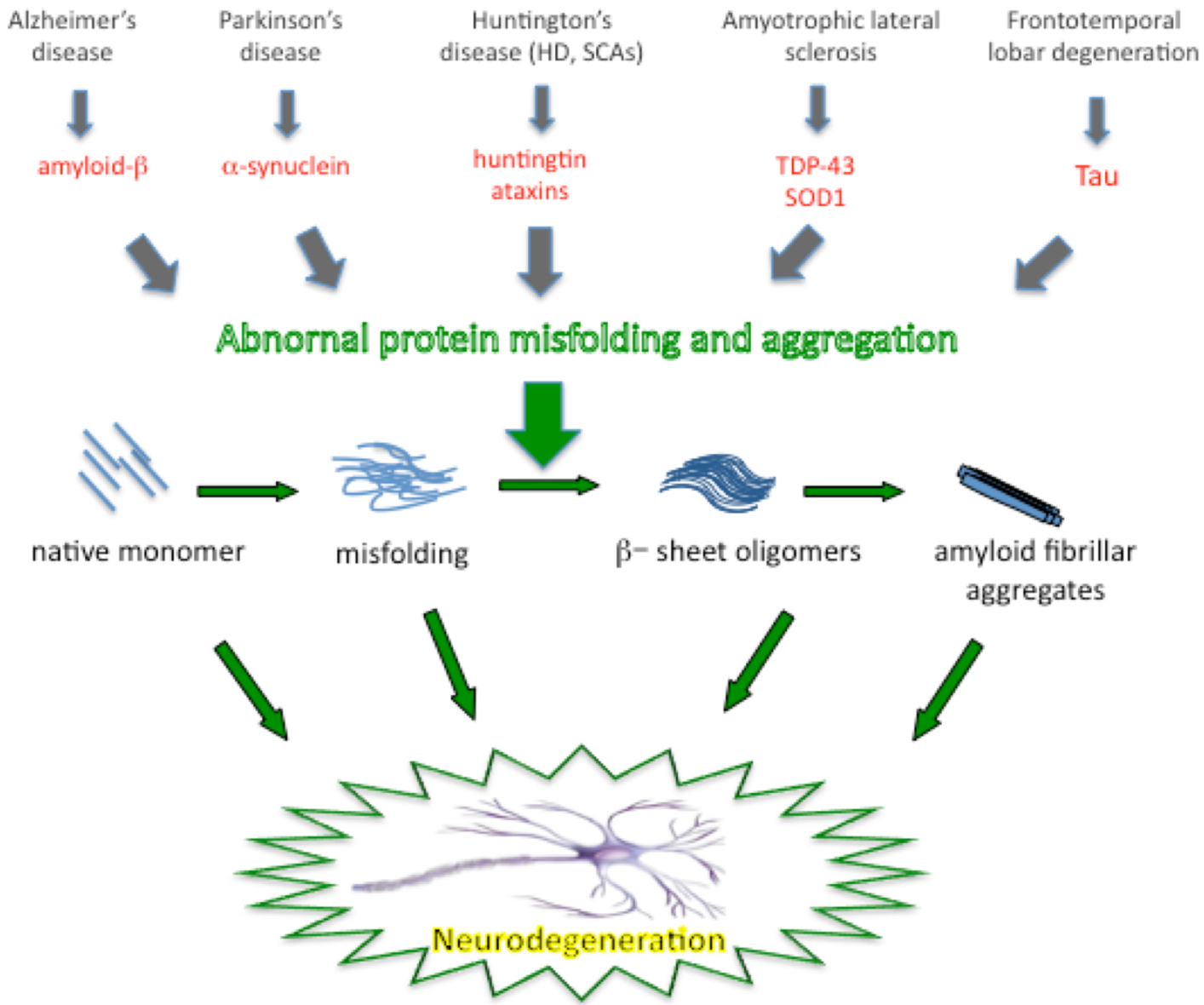


Beta-Amyloid (Aβ)
peptides form
Plaque

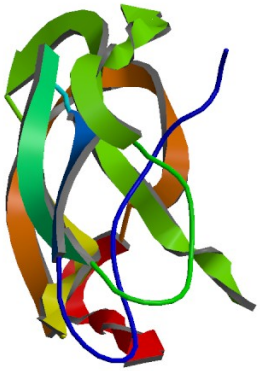
Beta-Amyloid Cascade Hypothesis

- The **beta-amyloid cascade hypothesis** states that Alzheimer's Disease (AD) is caused by the build up of extracellular neuritic (senile) plaques.
- AD's plaques are insoluble clusters (aggregates) of Beta-Amyloid (Aβ) peptides
- **Plaques** are also called **fibrils** or **amyloids**
- The formation of plaques is also referred to as **fibrillation** or **amyloidosis**

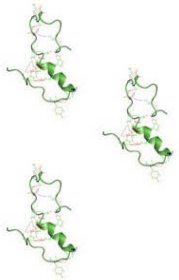
Protein-Folding Diseases



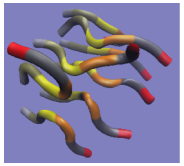
Amyloidosis in AD



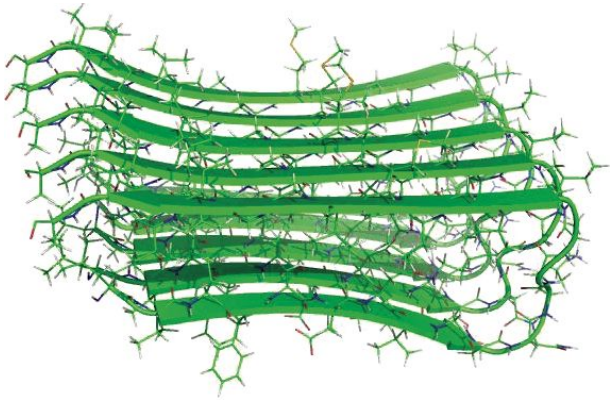
Amyloid Precusor Protein (APP)



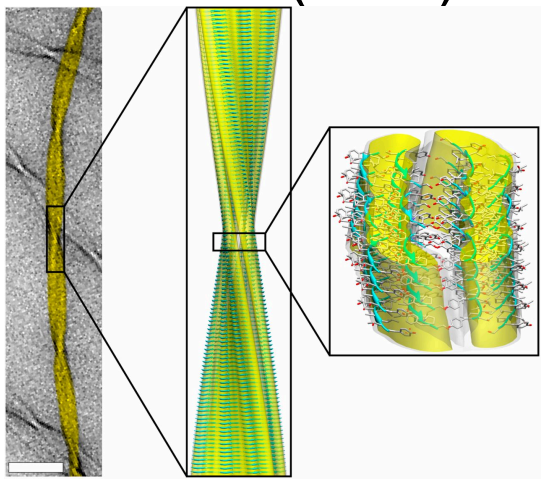
Soluble Aβ Peptides



Misfolded Aβ **Oligomers**

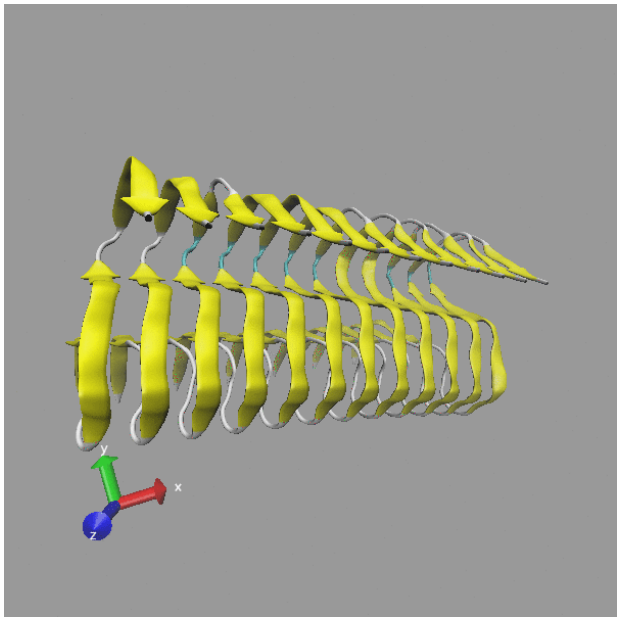


Aβ protofibril

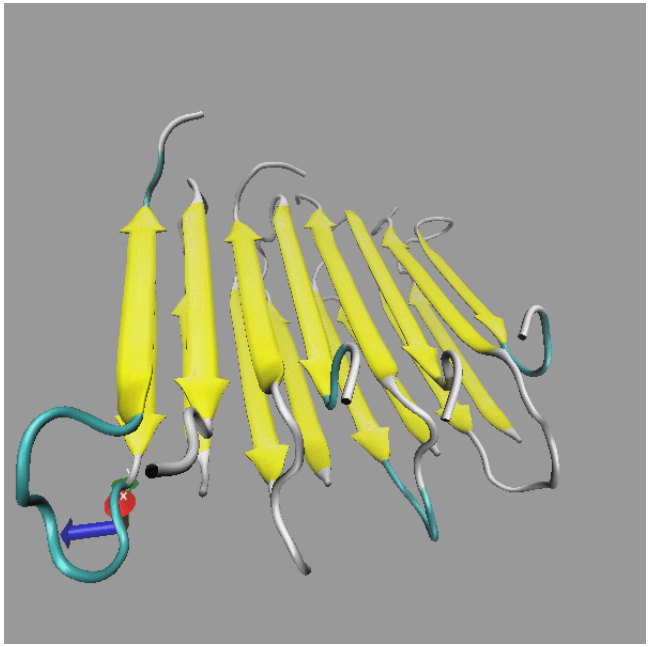


Mature Fibril (visible by MRI)

Model of AD: Parallel vs. Anti-Parallel Fibrils



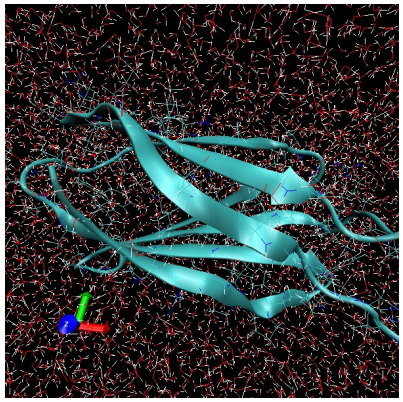
WildType Aβ **Parallel**



Iowa Mutant Aβ **Anti-Parallel**

In **Iowa Mutant** Aβ is associated with **early onset** (**< 60 years**) of **AD**

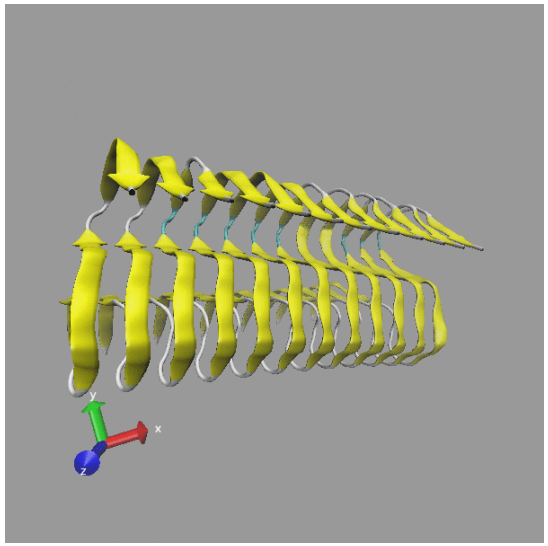
Multi-Scale Considerations



Ehlers-Danlos MD of N ≈ 25 000 atoms

One month CPU time for ≈ 50 ns

CPU time is **real time** used by **computers**



12 $A\beta_{40}$ ≈ 7000 atoms + 50 000 H_2O

N ≈ 57 000

CPU time scales as number of atoms, N

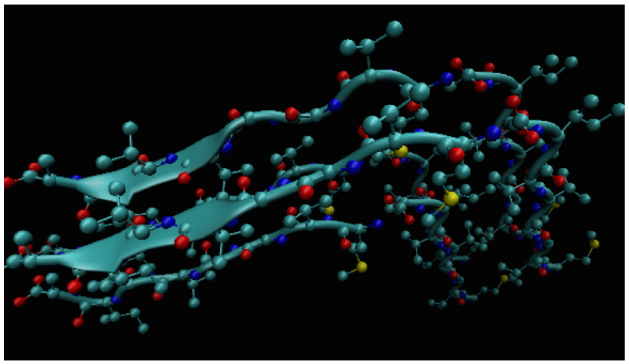
2 months CPU for **≈ 50 ns**

A proto-fibril takes **a few hours** to form

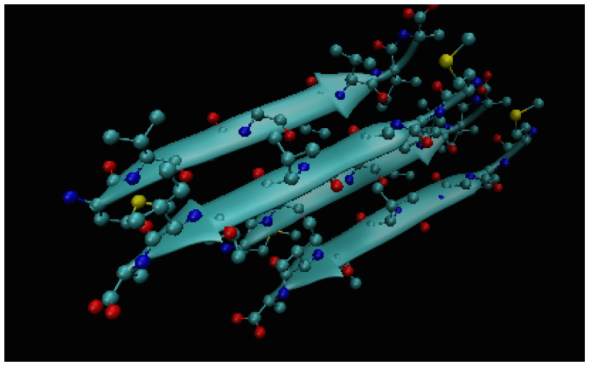
Years or Centuries of CPU !

$A\beta_{35\text{-}42}$ Crystal

- Eisenberg et al (2006), *Nature* 2006; 447: 453-456.
- Fragments of $A\beta_{40}$ readily form stable polymorphic crystals



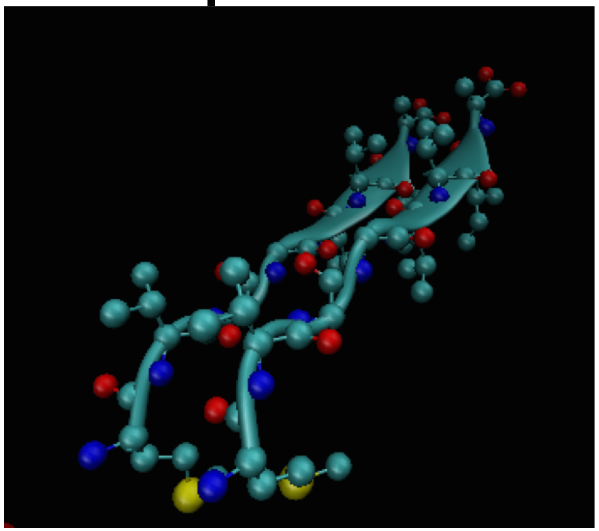
Parallel $A\beta_{35\text{-}42,}$ PDB 2y3k



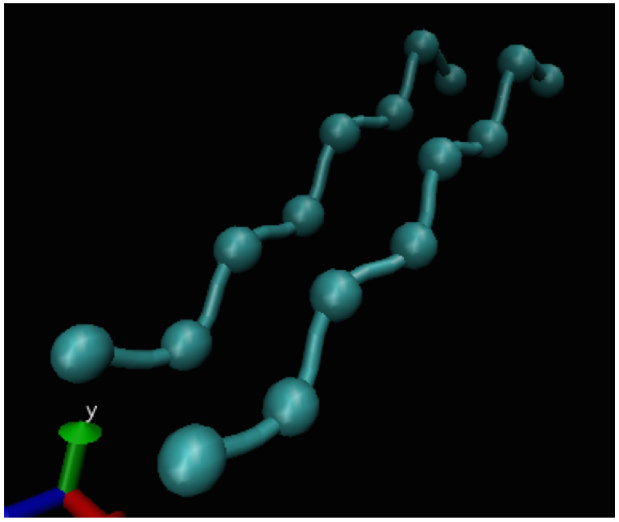
Anti-Parallel $A\beta_{35\text{-}42,}$ PDB 2y3k

Coarse-Grained Cα Model

- Only central Cα carbon of $A\beta_{35\text{-}42}$ is represented in the model

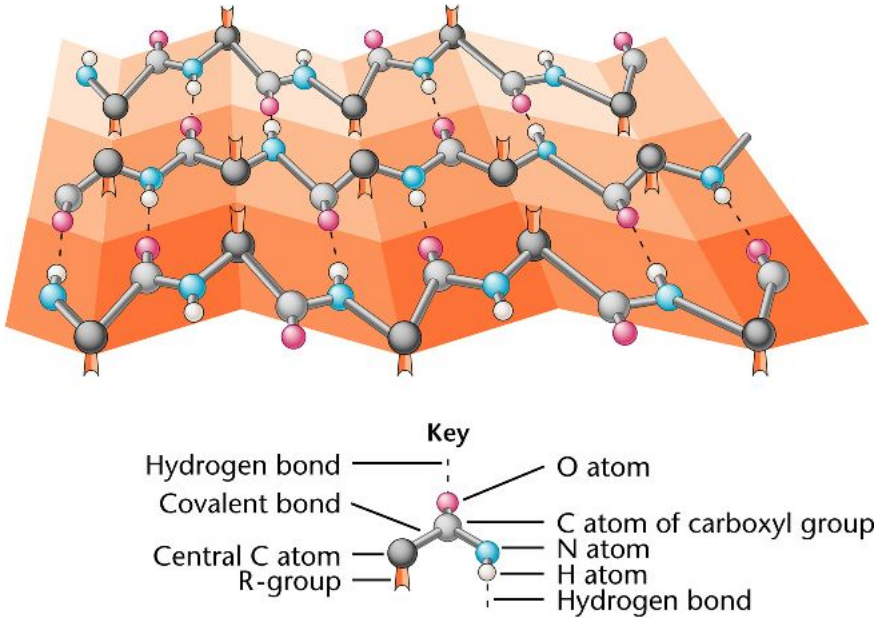


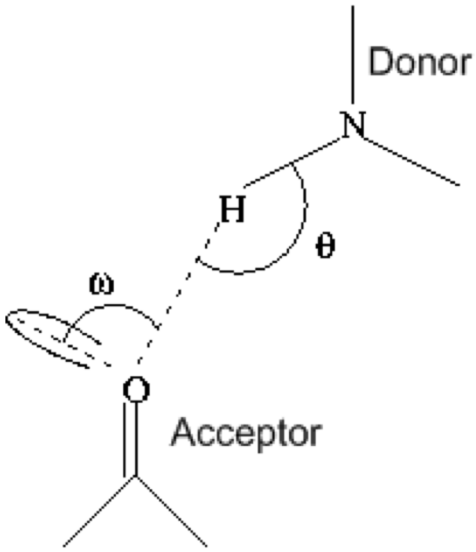
All atom from PDB



Coarse-Grained Cα Model

Beta(β)-Sheet Stabilized by Hydrogen Bonds (HB)

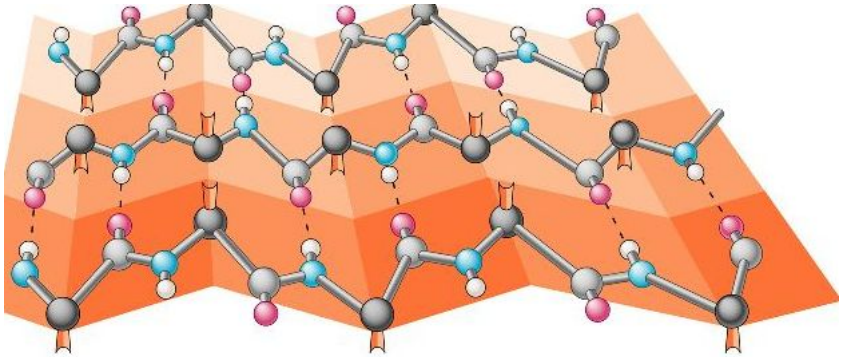


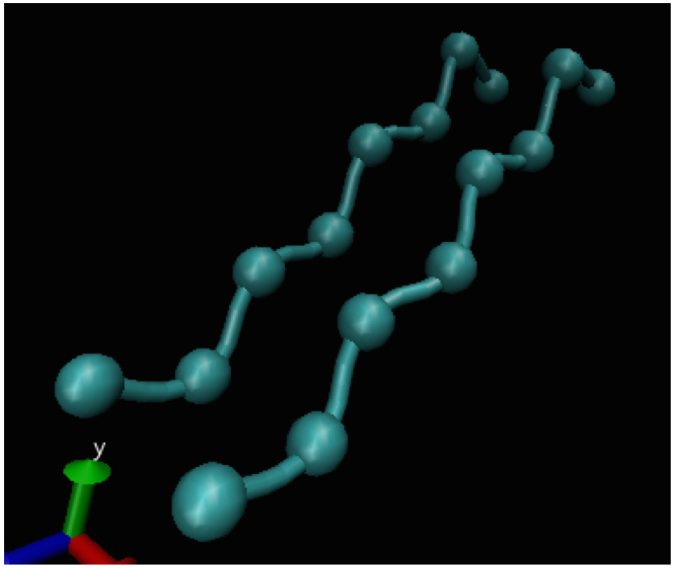


HB stabilized β-sheets

Needs **all atoms** to represent HB

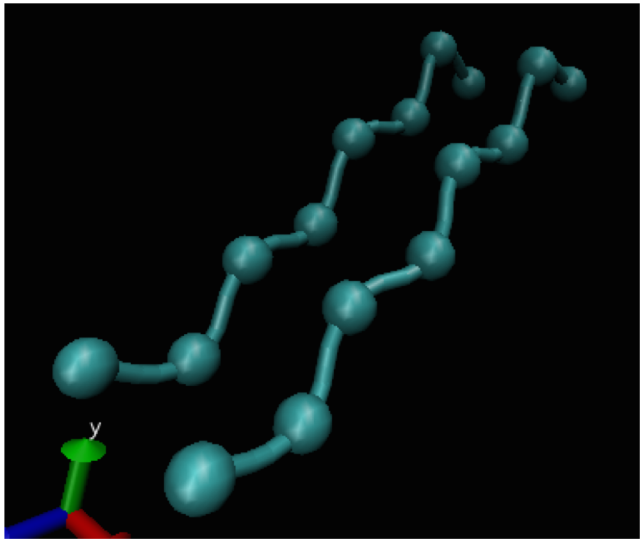
Pseudo Hydrogen Bond Potential





- Length between 2 adjacent Cα ≈ 3.8 A
- Angle between 2 adjacent Cα ≈ 104˚
- **Pseudo Hydrogen Bond Potential** will make Cα model of $A\beta_{35\text{-}42}$ prefers to align side-by-side

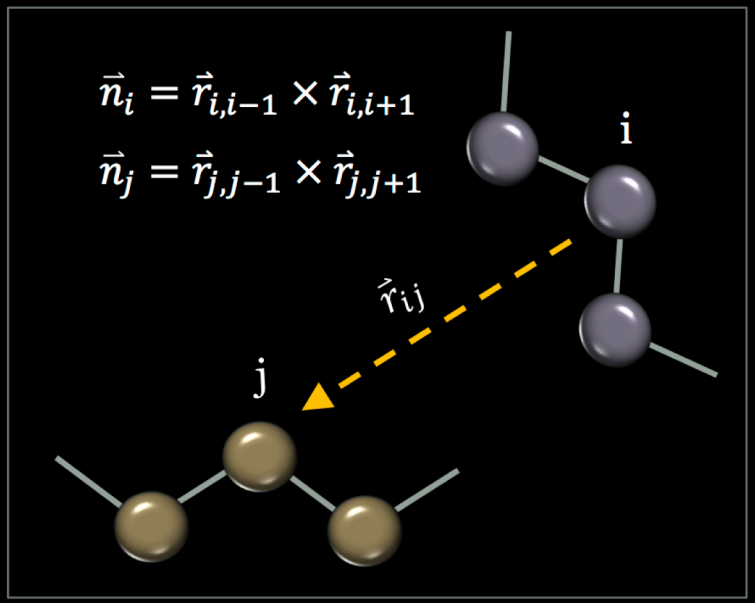
Pseudo-Hydrogen Bond Minimizes Potential Energy for Side-by-Side Conformations



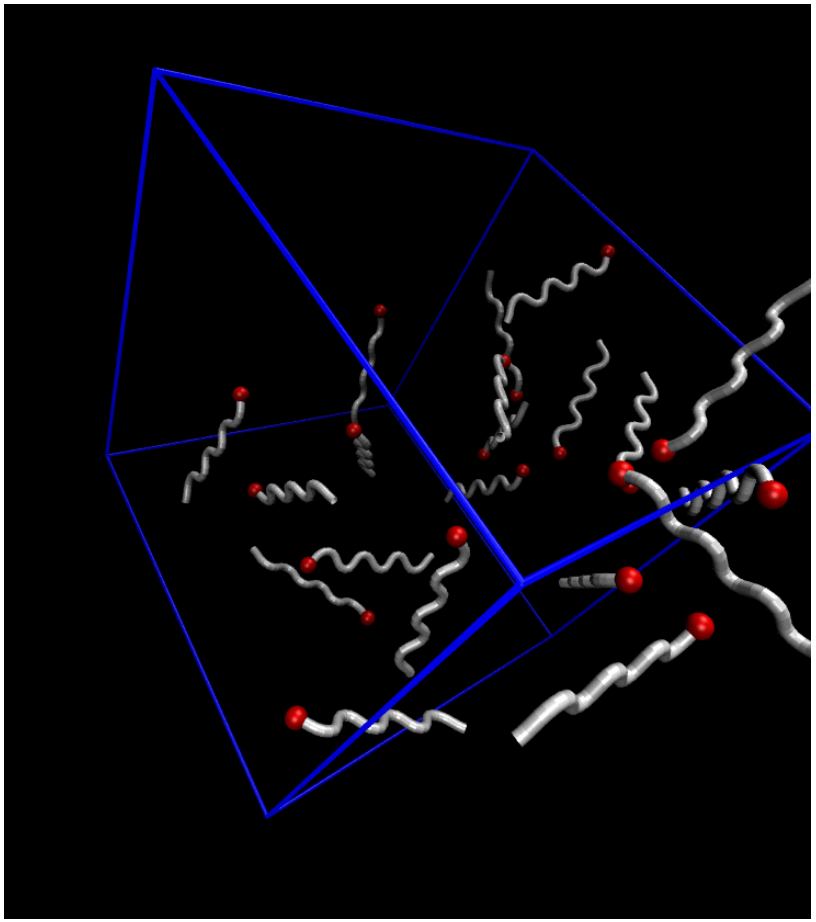
$$U_{HB} \sum_{\substack{i,j \geq i+3 \ intrachain \\ i,j \ interchain}} \varepsilon_{HB} e^{\frac{-(r_{ij}-r_{HB})^2}{\sigma^2_{HBdist}}} e^{\frac{(|\hat{n}_i \cdot \hat{r}_{ij}|-1)}{\sigma^2_{HB}}} e^{\frac{(|\hat{n}_j \cdot \hat{r}_{ij}|-1)}{\sigma^2_{HB}}}$$

$$\vec{n}_i = \vec{r}_{i,i-1} \times \vec{r}_{i,i+1}$$

$$\vec{n}_j = \vec{r}_{j,j-1} \times \vec{r}_{j,j+1}$$

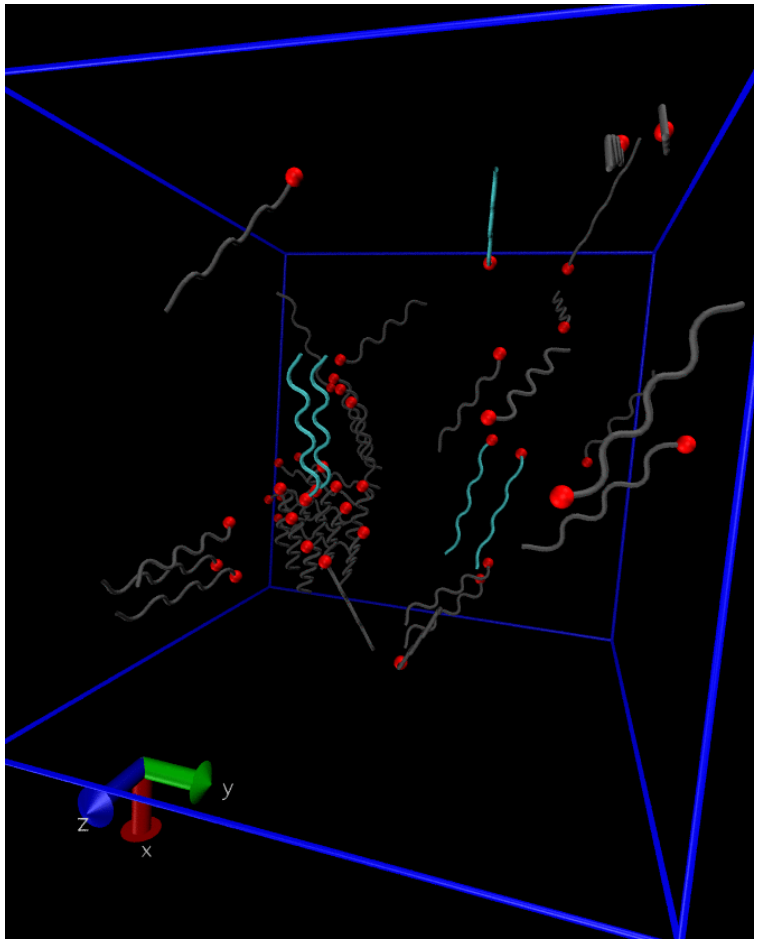


40 $A\beta_{35\text{-}42}$ in 50AX50AX50A MD simulation: first 16 ns



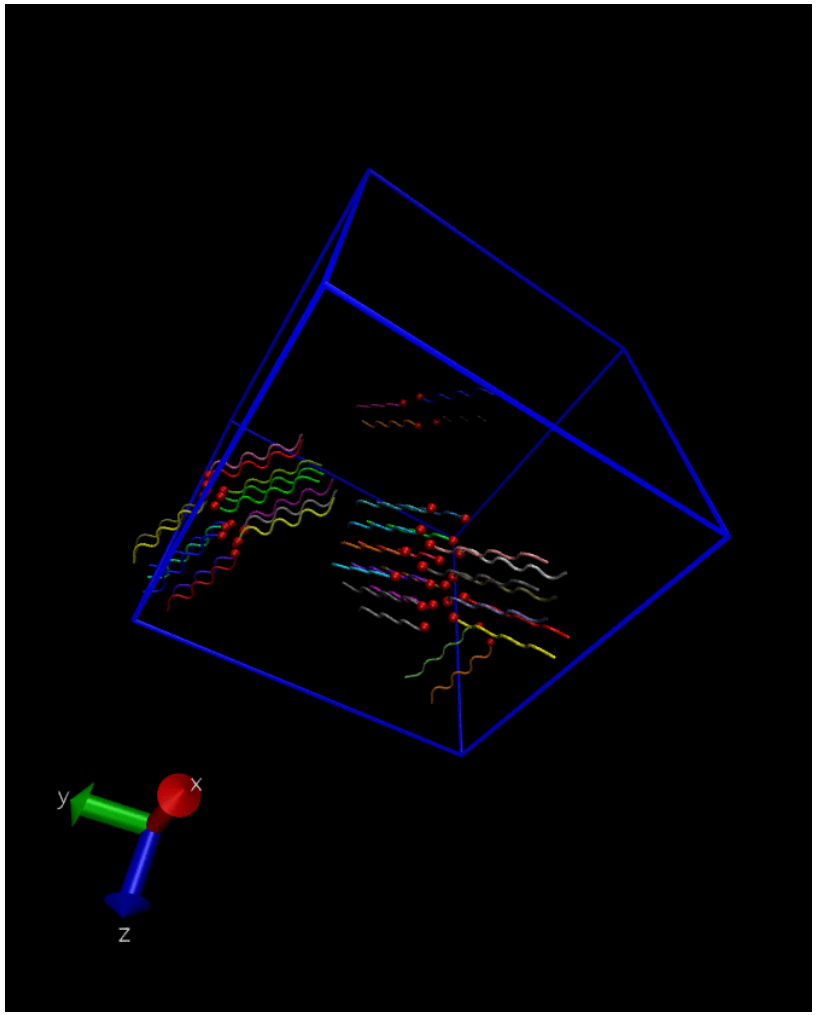
- Concentration ≈ 150 mM, typical of AD
- Unstable Oligomers

40 $A\beta_{35\text{-}42}$ in 50AX50AX50A
MD: 63 - 70 ns

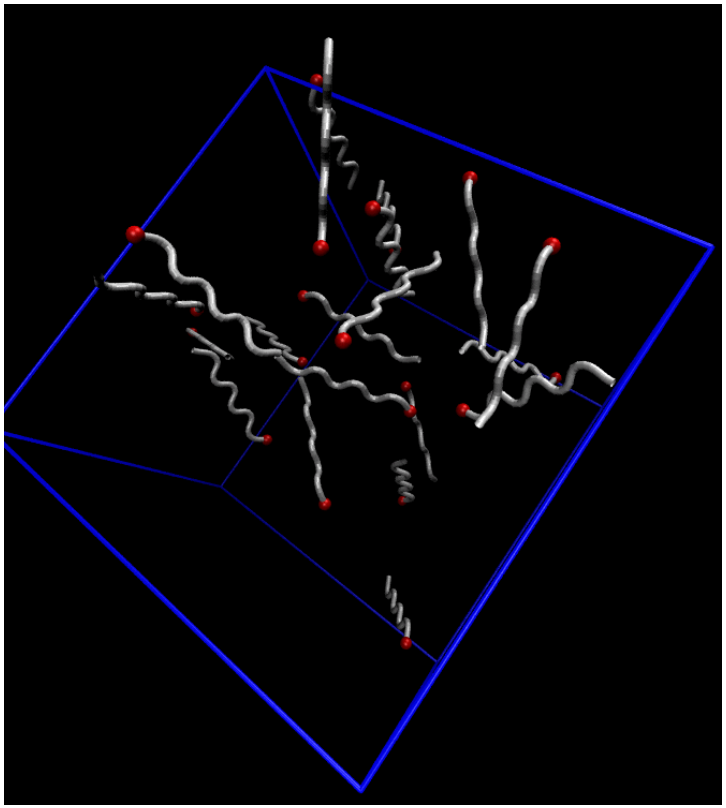


- Growing Proto-fibril

40 $A\beta_{35\text{-}42}$ in 50AX50AX50, 200 ns, Mature Fibril Crystal



Anti-Parallel Sheets



Conclusion on AD

- Cα model with pseudo-hydrogen bond stabilizes Eisenberg's parallel crystal phase of $A\beta_{35\text{-}42}$
- Interaction with lipid membranes
- Aβ are known to be anti-bacterial agent
- Rob Kalisky will present DMD on competition between protein folding and aggregation

Acknowledgement

- Collaborators: Gautam Das, Alla Reznik, Steve Plotkin, Cristiano Dias, Hongbin Li, Shulin Zhuang
- STUDENTS: **Rob Girardin, Rob Kalisky, Carl Fletcher**, Gabrielle Gaultier, Carl Fletcher, MacKenzie Demchuk, Greg Bates, Ian MacKay, Tim Miao, Gianluca Amadei, Casey Howard
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  - NSERC/CREATE
  - Thunder Bay Regional Research Institute(TBRRI)
  - NORTHERN ONTARIO HERITAGE FUND CORPORATION
- COMPUTATIONAL RESOURSES
  - Compute Canada SHARCNET