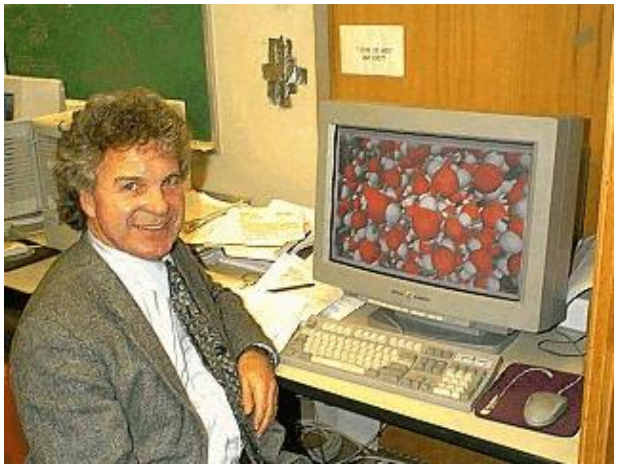
In vitro driven computer simulations of relevance to Alzheimer's disease

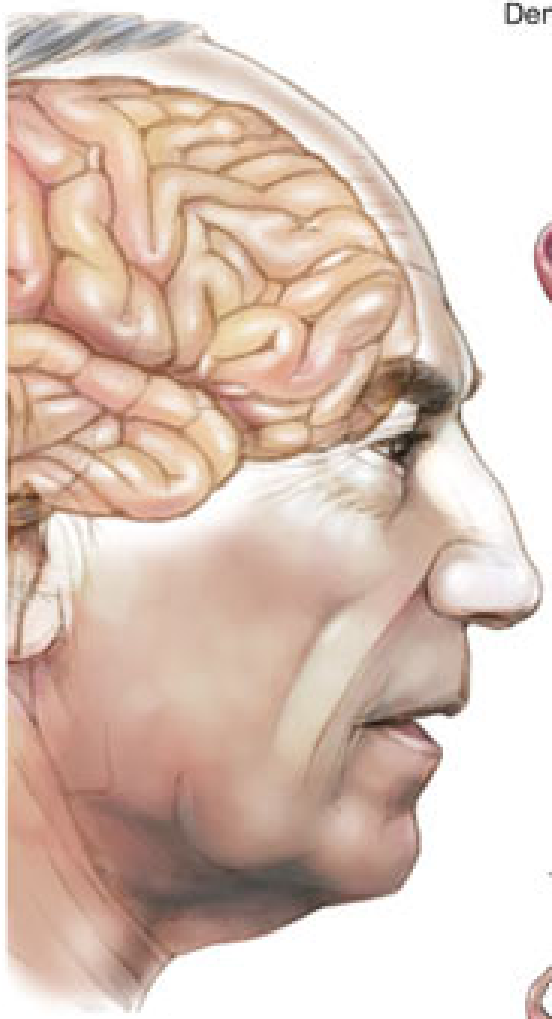
Brigita Urbanc
Boston University
Center for Polymer Studies

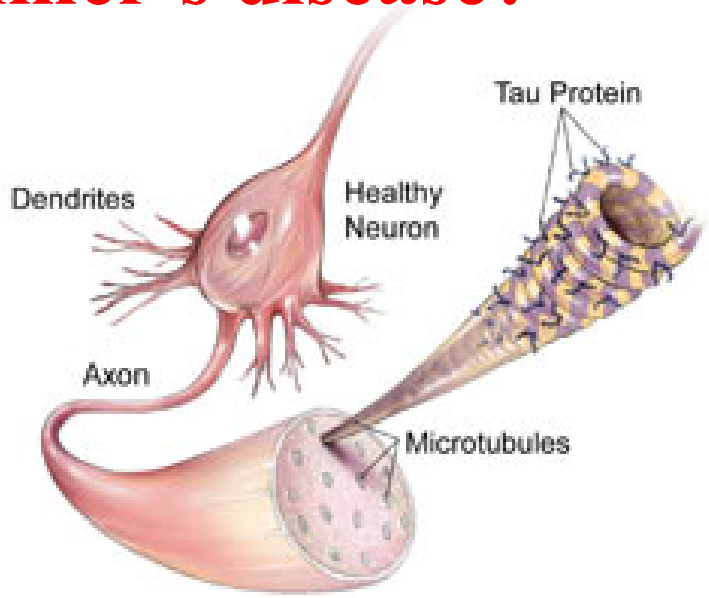


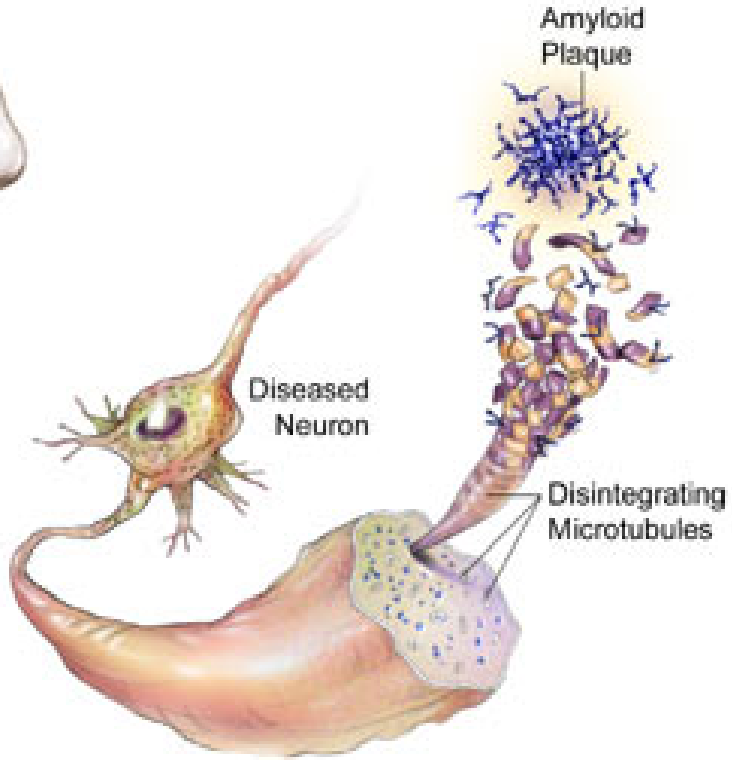
H. Eugene Stanley

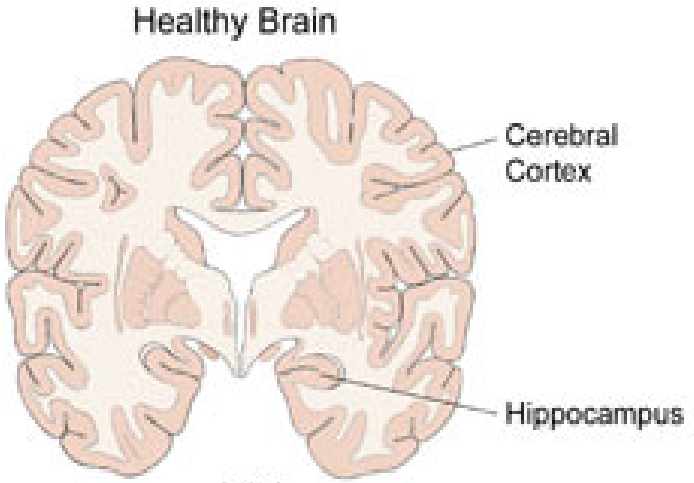
Collaborators:
L. Cruz, A. Lam, S. Peng, S. Yun (BU)
F. Ding & N. V. Dokholyan (UNC)
J.M. Borreguero (BU & Georgia Tech)
G. Bitan, E. Fradinger, N. D. Lazo, D. B. Teplow (UCLA)
S. V. Buldyrev (BU & Yeshiva U)

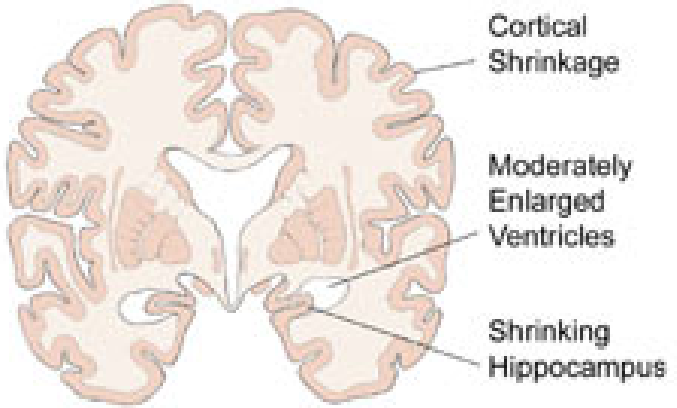
What is Alzheimer's disease?

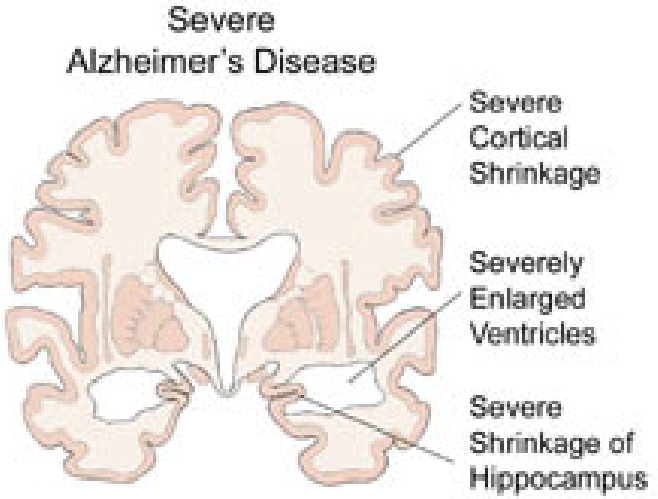










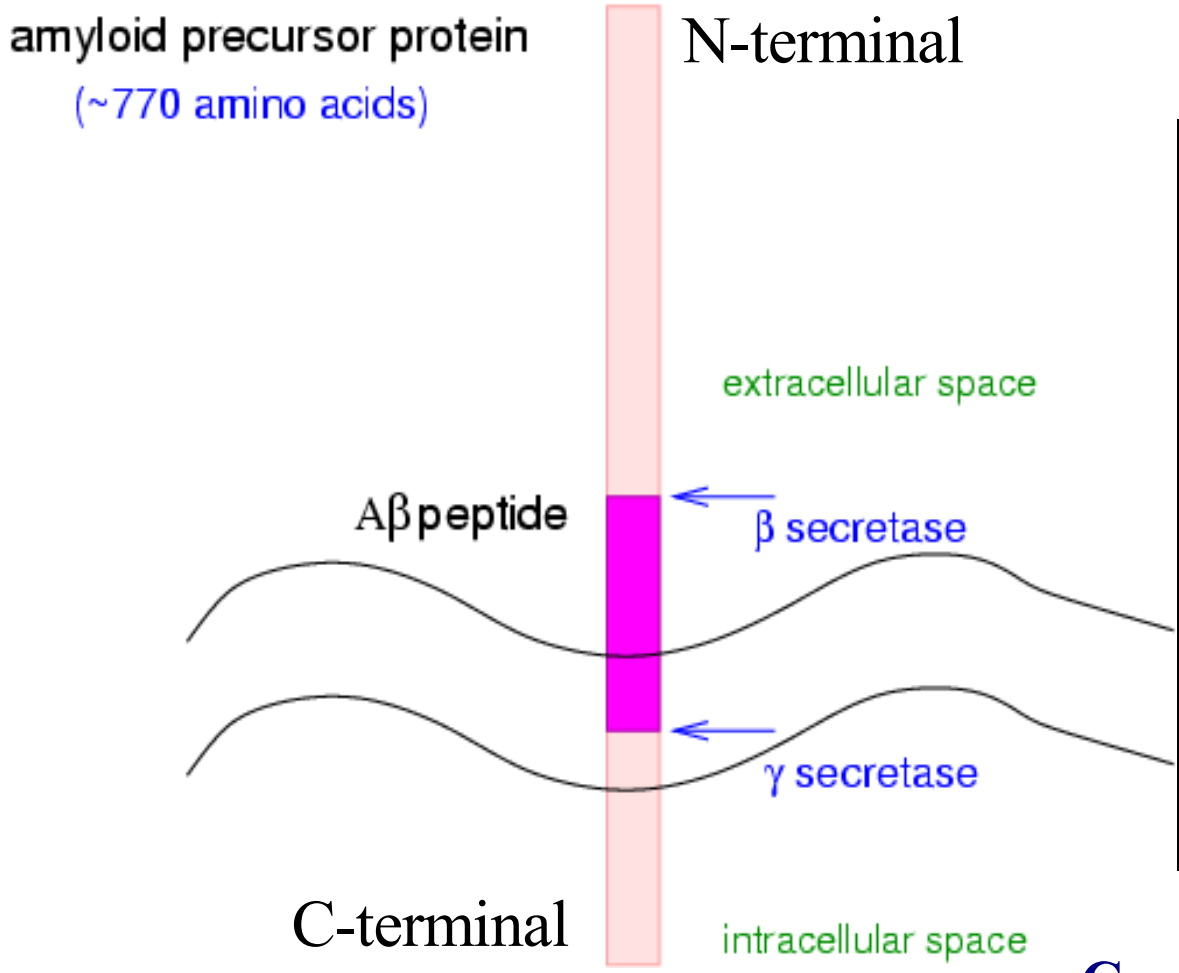


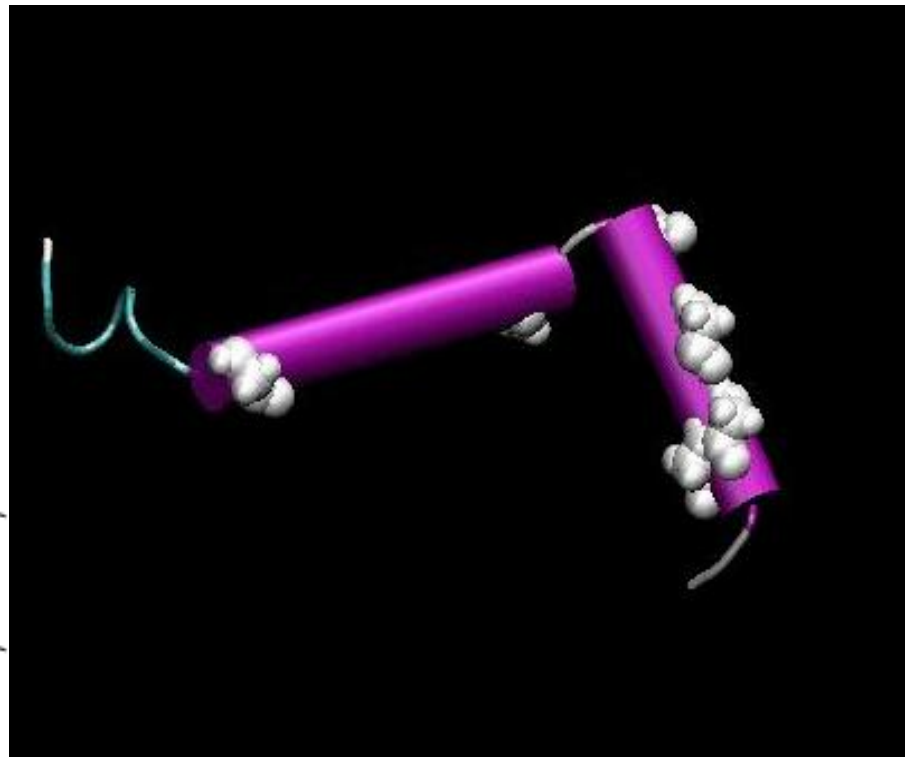
Q: What are plaques made of? How do they form?

A: Amyoid β-protein (Aβ). Aggregation of Aβ.

Aβ40 and Aβ42 amino acid sequence:

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV IA





in apolar microenvironments

Crescenzi et al., Eur. J. Biochem. (2002)

Two most abundant forms of Aβ in human body are Aβ40 and Aβ42:

- **Aβ40 is more abundant in the body (90%), however in amyloid deposits, Aβ42 is the dominant alloform;**
- ***in vitro* Aβ42 aggregates into fibrils faster than Aβ40;**
- ***in vitro* Aβ42 fibrils are considerably more toxic to neurons than Aβ40;**
- **genetic evidence shows that Aβ42 is associated more strongly with a risk for Alzheimer's disease than is Aβ40.**

How can a difference of two amino acids (IA) matter?

Aggregation of Ab: Working Hypothesis

Bitan et al, PNAS (2003); JBC (2003); JACS (2003).

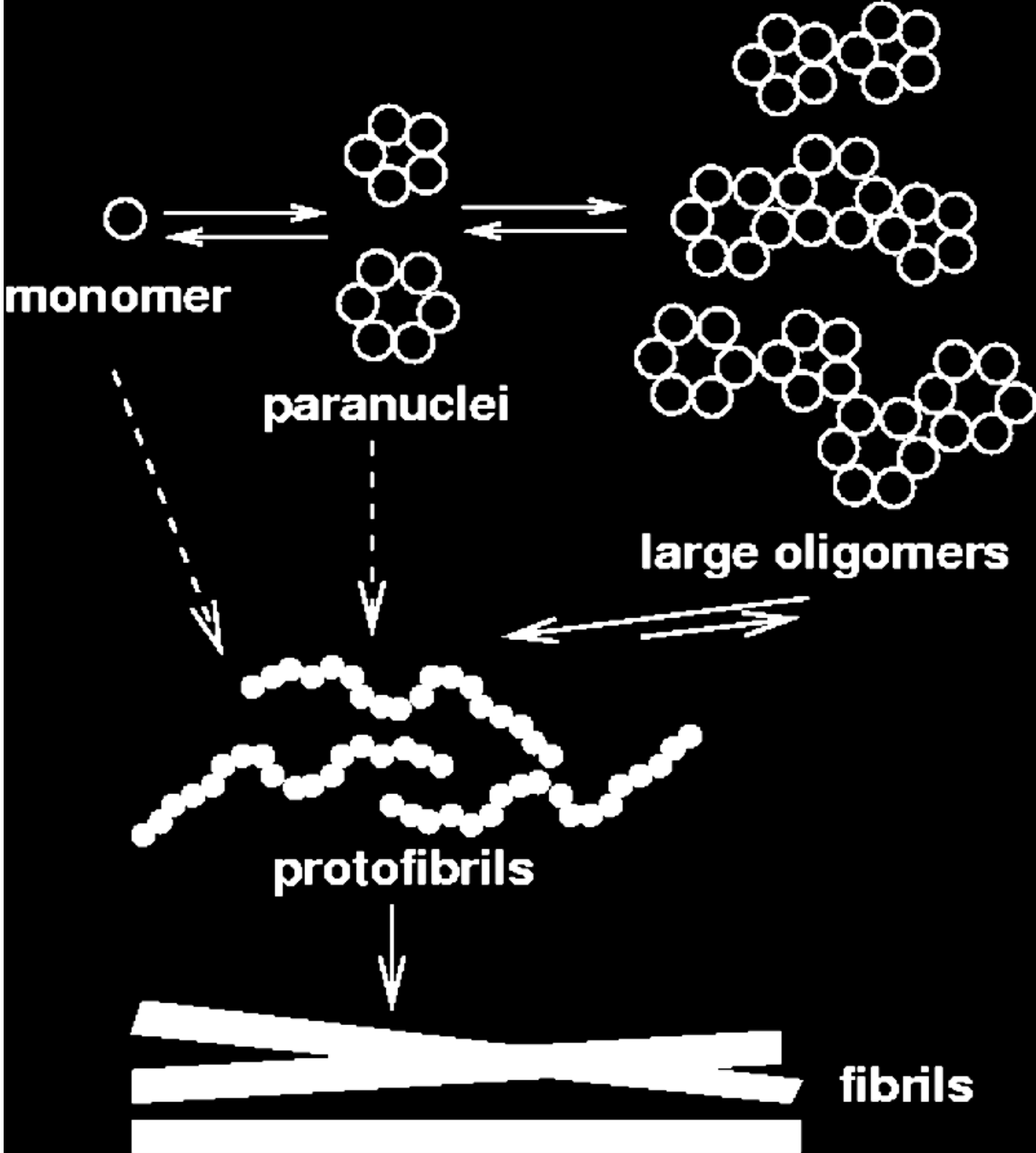
Unstructured:

- monomers
- pentamers/hexamers (paranuclei)
- large oligomers

β-strand rich:

- protofibrils
- fibrils

OLIGOMERS ARE THE MOST TOXIC!

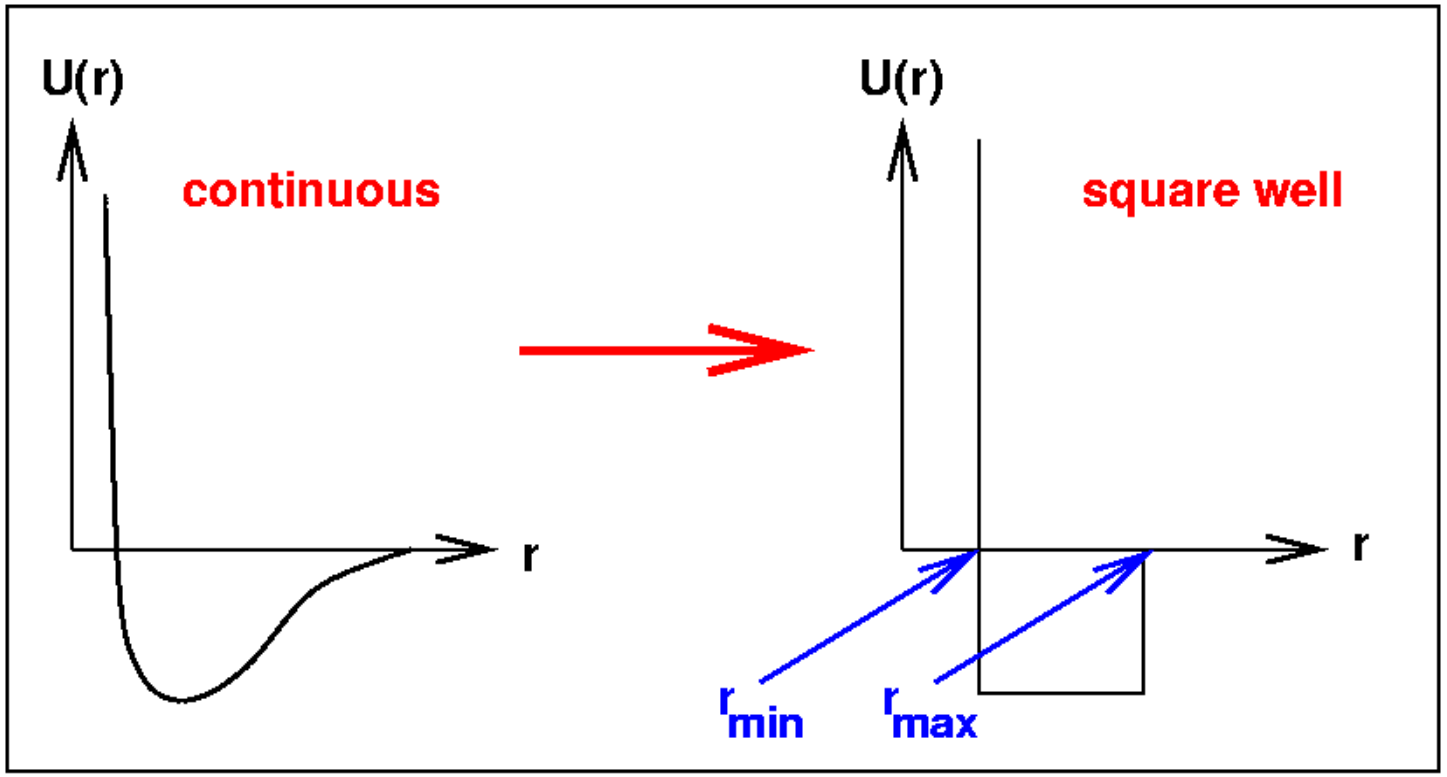


PROBLEM:
Experimental methods (X-ray, NMR, light scattering, etc.) to determine the 3D structure of oligomers are limited and do not give atomic detail.

OUR PROPOSED SOLUTION:
Molecular Dynamics Simulations

METHOD: Discrete Molecular Dynamics (DMD)

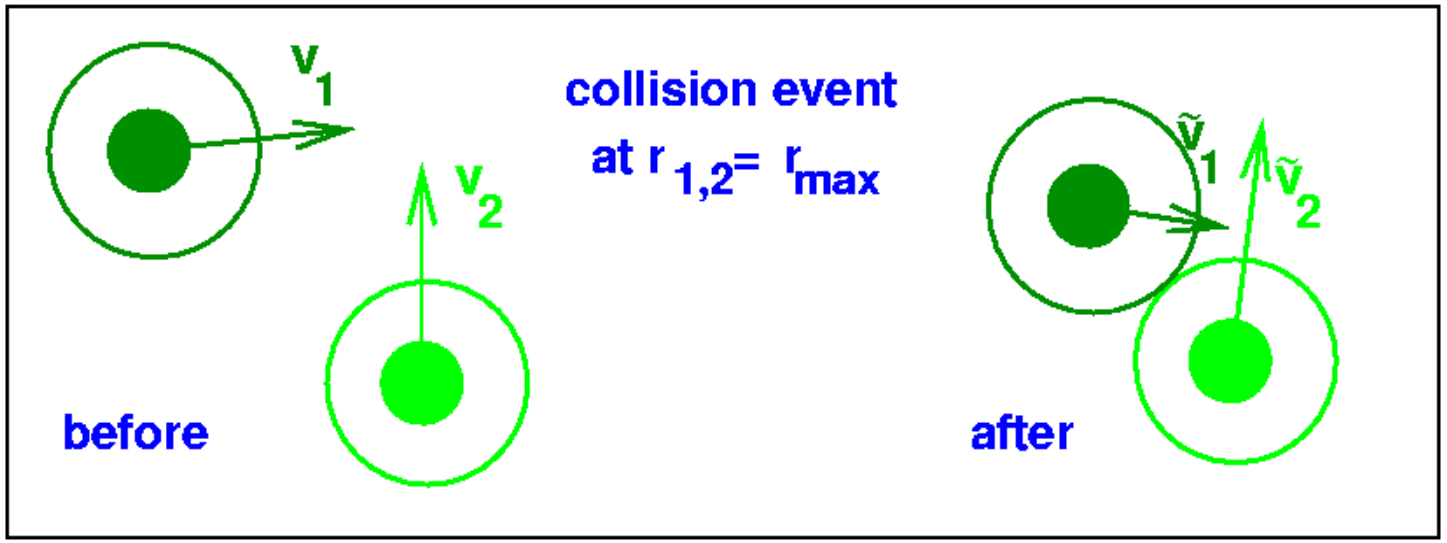
Interparticle potential



Keep track of collision events

Collisions conserve:

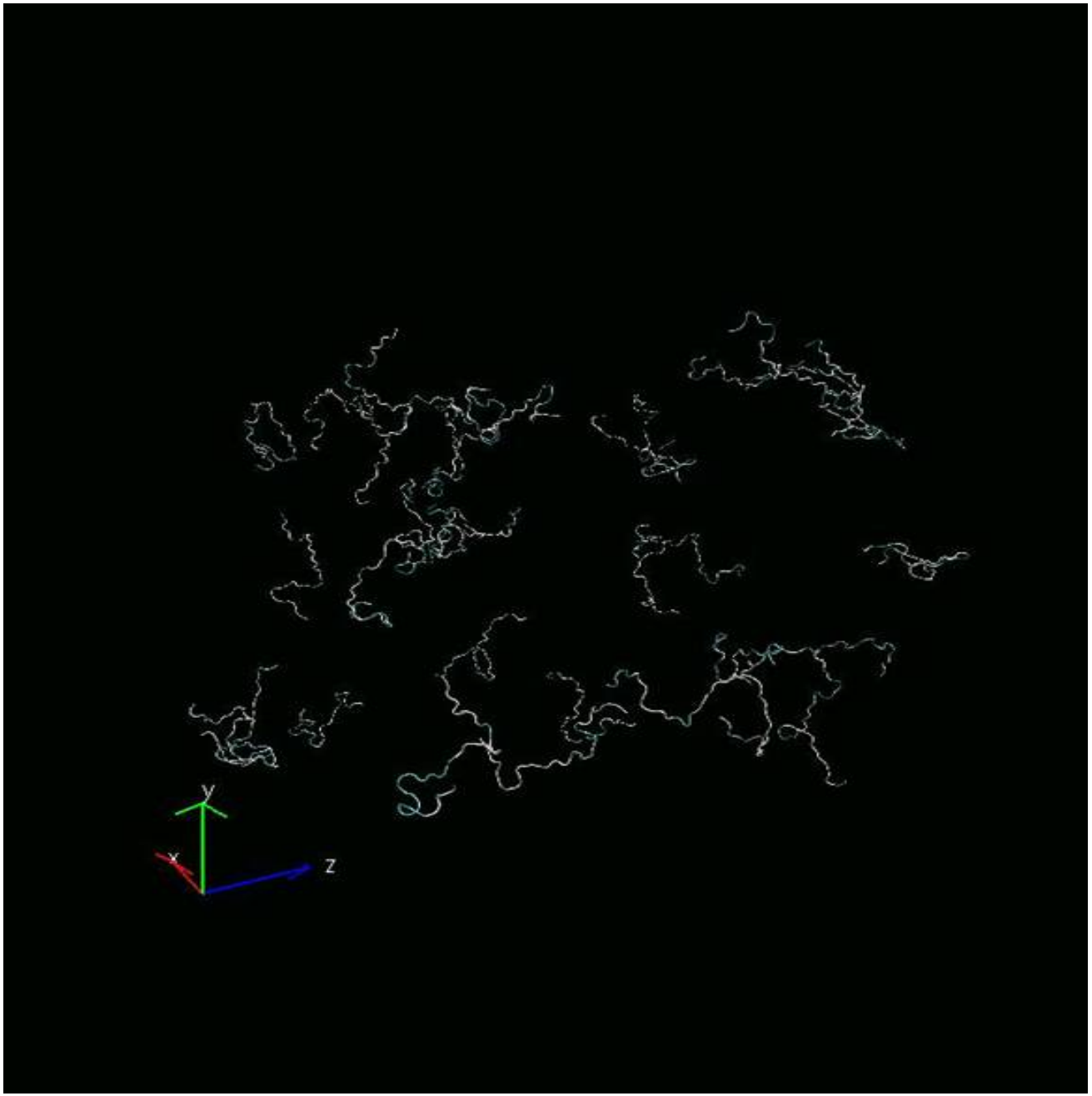
- **energy**
- **momentum**
- **angular momentum**



Zhou et al. PRL, 1996; Zhou & Karplus, PNAS, 1997; Dokholyan et al., Fold. Des., 1998.

8 trajectories of each Aβ40 and Aβ42 with 32 peptides in a cubic box of 25 nm

Initially peptides are in unfolded, zero-energy conformations.



Parameters:

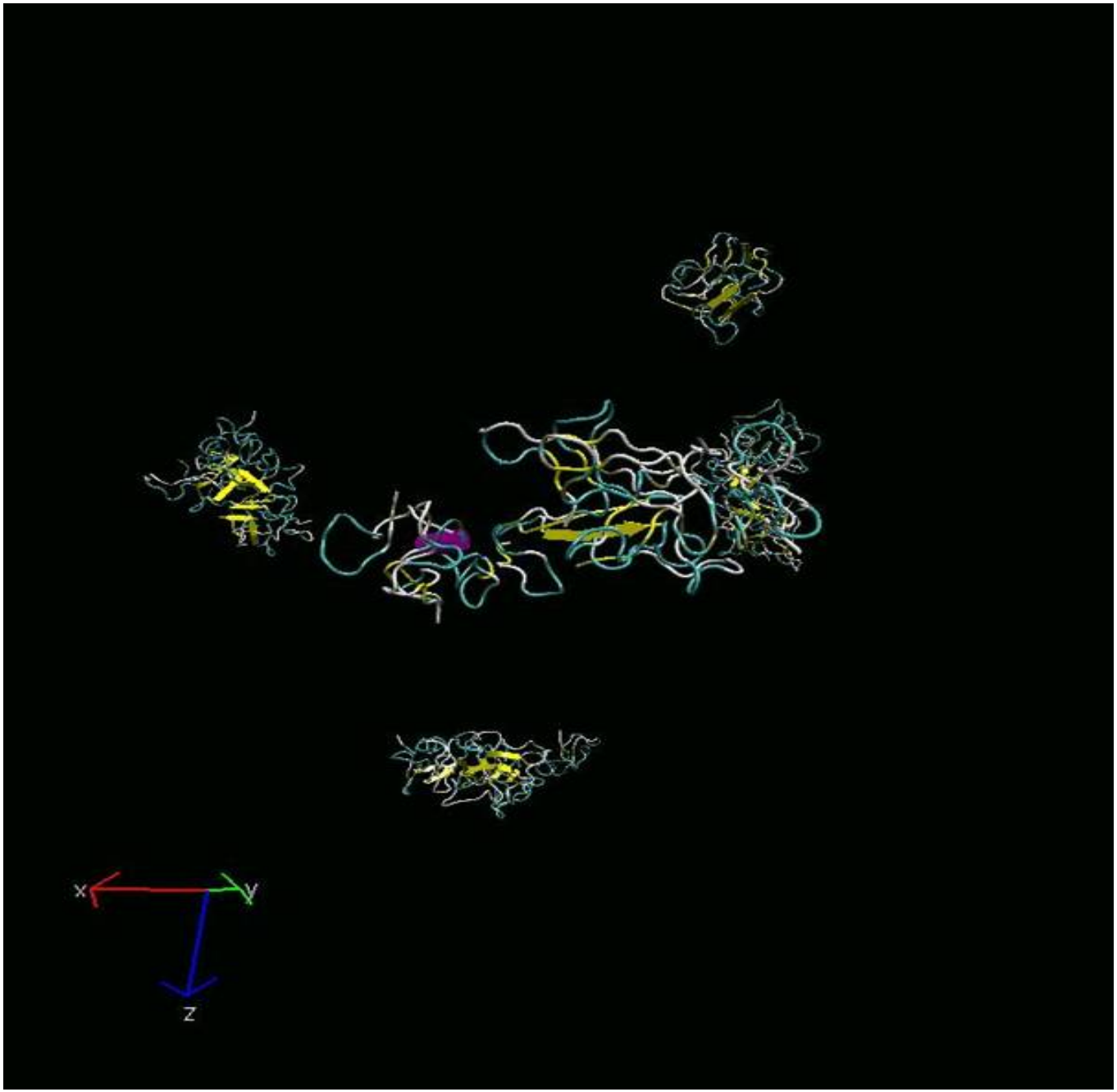
$E_{HB} = 1$

$E_{HP} = 0.3$ (I-I)

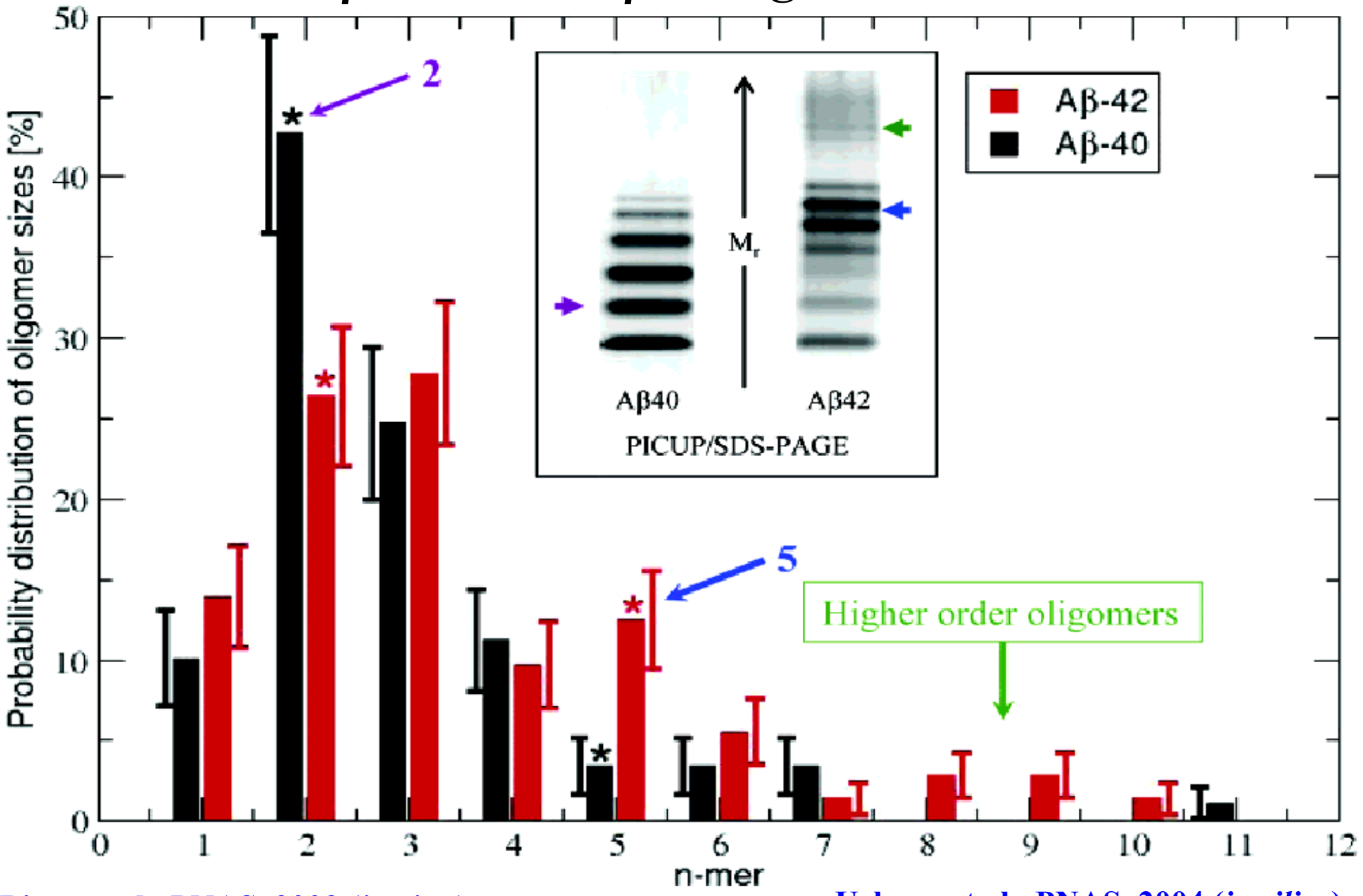
$T = E_{HB} / k_B$

8 trajectories of each Aβ40 and Aβ42 with 32 peptides in a cubic box of 25 nm

After 5-6 million simulation steps, monomers and oligomers are in a quasi-steady state.



Result: Aβ40 versus Aβ42 oligomer size distributions



Bitan et al., PNAS, 2003 (*in vitro*).

Urbanc et al., PNAS, 2004 (*in silico*).

In silico predictions of Aβ40 versus Aβ42 folding and assembly

- folding and assembly is driven primarily by effective hydrophobic attraction among hydrophobic regions of Aβ;
- Aβ42 and Aβ40 folded monomer structures DIFFERENT (Aβ42 has an additional turn at G37-G38);
- Aβ40: assembly is dominated by intermolecular attraction between pairs of central hydrophobic clusters (L17-A21);
- Aβ42: assembly is dominated by intermolecular attraction among pairs of C-terminal regions (V39-A42) as well as mid-hydrophobic regions (I31-M35);
- electrostatic interactions promote formation of larger oligomers.

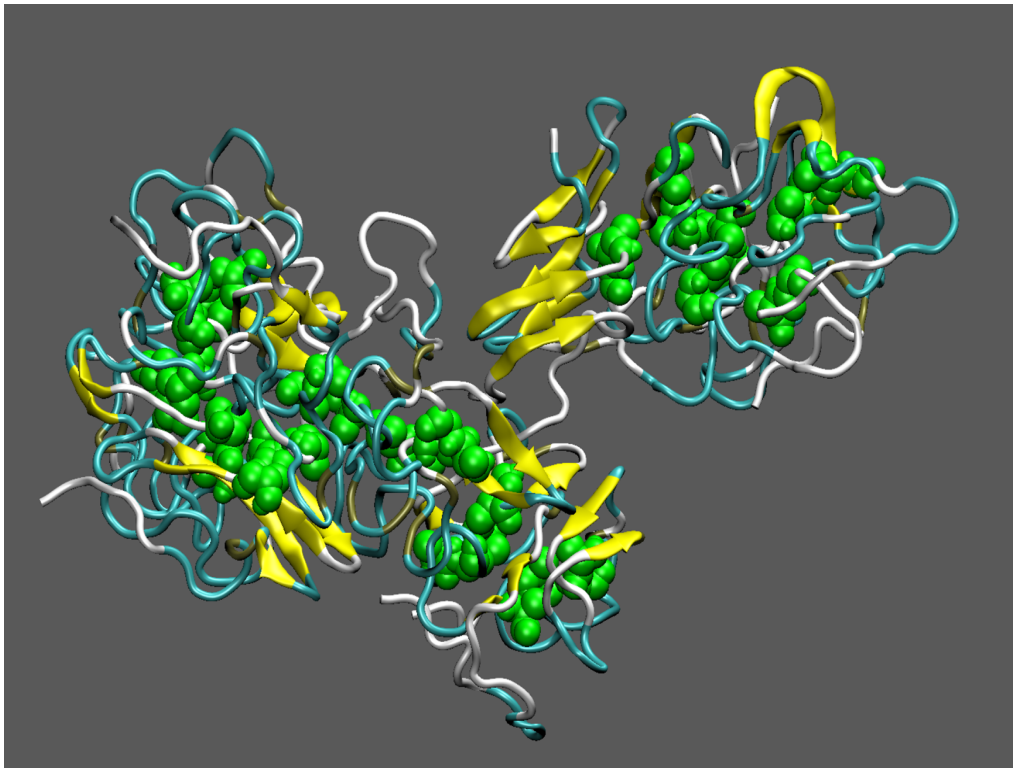
Urbanc et al., PNAS (2004); Yun et al., Biophys. J. (2007).

This work was supported by grants from the National Institutes of Health, Alzheimer's Association Zenith Fellows award, and the Petroleum Research Fund.

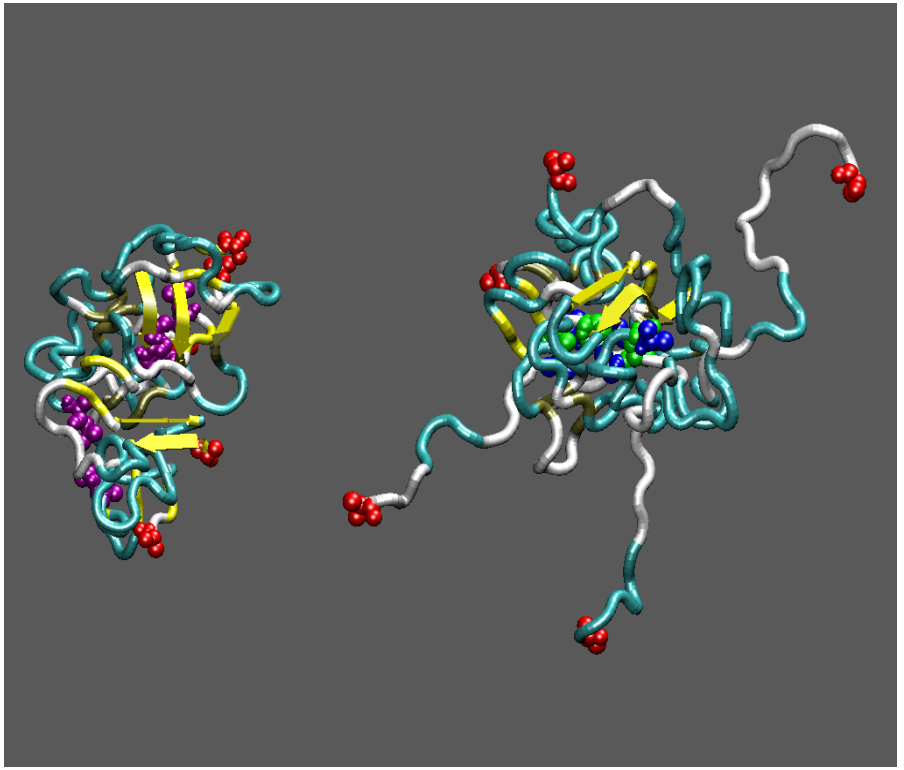
HIERARCHY OF MODELS: LEVEL 1 APPROACH

- Discrete Molecular Dynamics (DMD)
- No explicit solvent
- Simplified protein model (four beads per amino acid)

Aggregate of 16 Aβ42



Aβ40 and Aβ42 pentamers



Urbanc et al., Biophys. J. (2004); PNAS (2004).

HIERARCHY OF MODELS: LEVEL 2 APPROACH

- DMD
- No explicit solvent
- United atom protein model (all atoms except hydrogens)

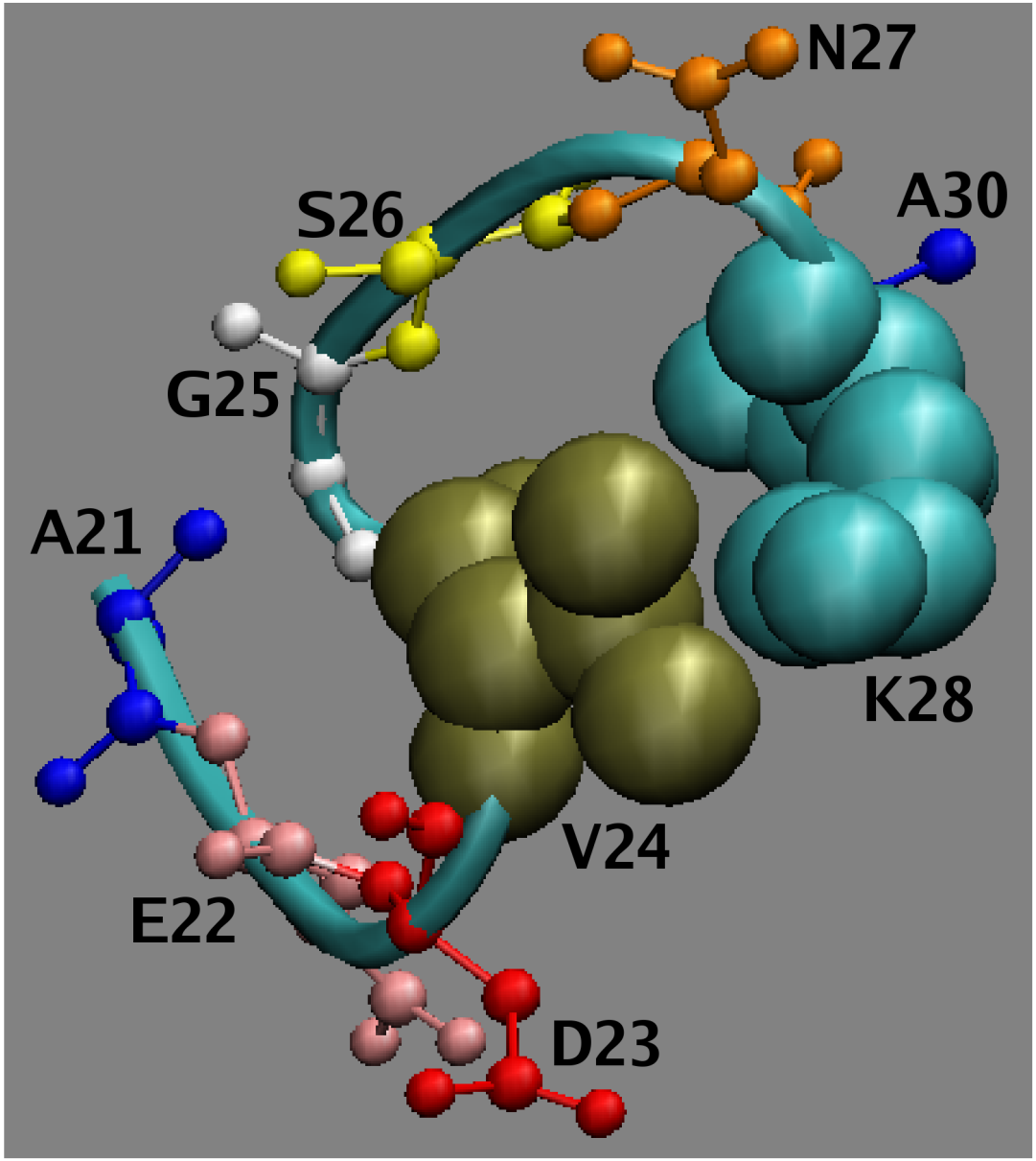
Borreguero et al., PNAS (2005).

MOTIVATION:

In vitro **studies of Aβ(21-30) suggest that this Aβ fragment nucleates folding of full-length Aβ40 and Aβ42.**

Lazo et al., Proteins (2005).

Folded conformation of a decapeptide Aβ(21-30)



HIERARCHY OF MODELS: LEVEL 3 APPROACH

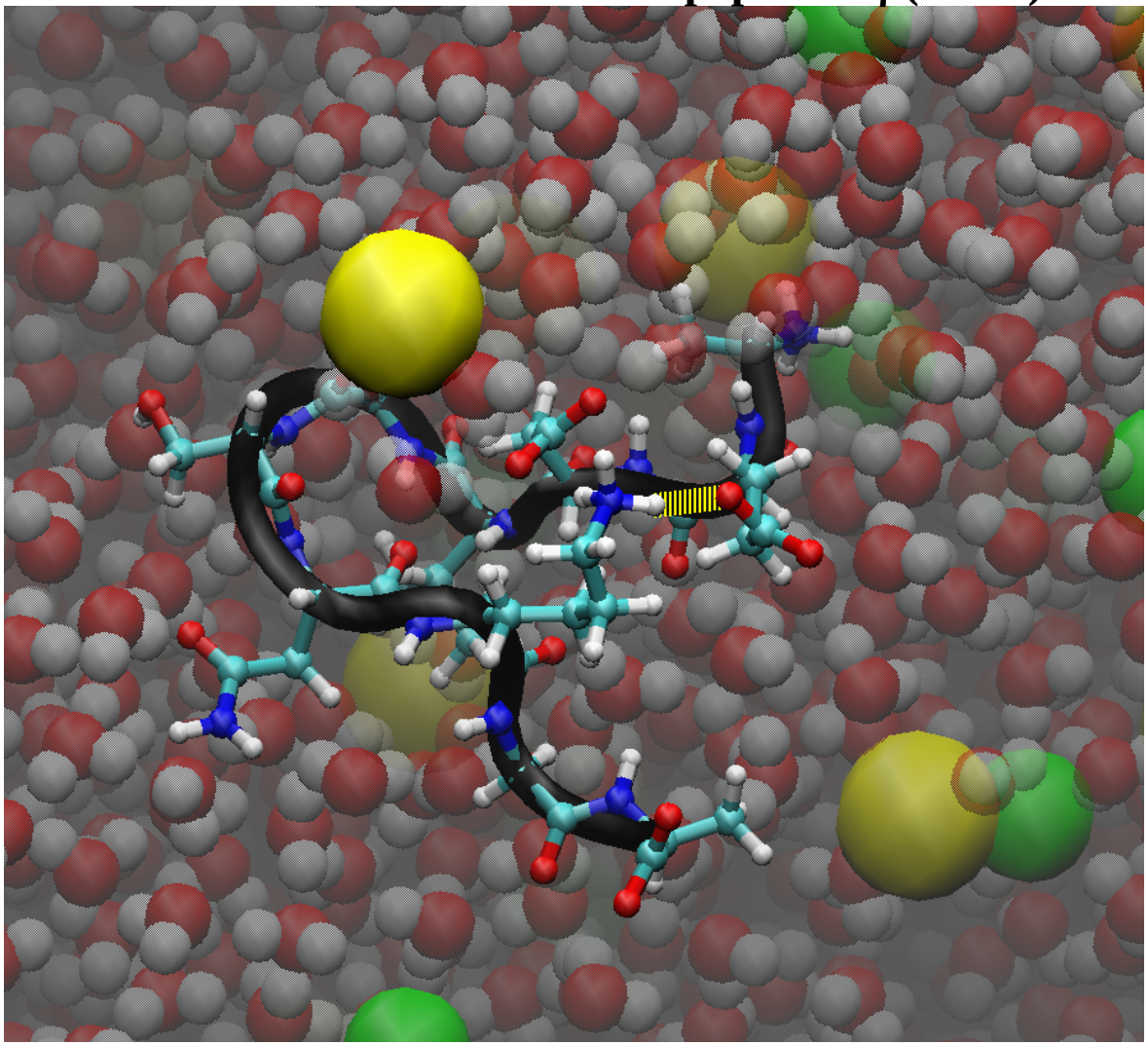
- all-atom MD
- explicit solvents:
  - -water
  - -low-density water
  - -water with salt ions

Cruz et al., PNAS (2005).

RESULTS:

Aβ(21-30) folding sensitive to small changes in solvent. Decapeptide with the Dutch mutation (E22Q) has rare folding events.

Folded conformation of a decapeptide Aβ(21-30)



Legend: backbone (black), O (red), H (white), Na+ (green), Cl- (yellow), E22-K28 salt bridge (yellow hashed)

WHAT DO *IN VITRO* STUDIES SHOW?

- **Aβ42 but not Aβ40 forms paranuclei (pentamers/hexamers) and multiples of paranuclei (~12, ~18-mers)-**

  Bitan et al., PNAS (2003);

- **C-terminal's Ile41 and Ala42: a key role in Aβ42 paranuclei formation-**

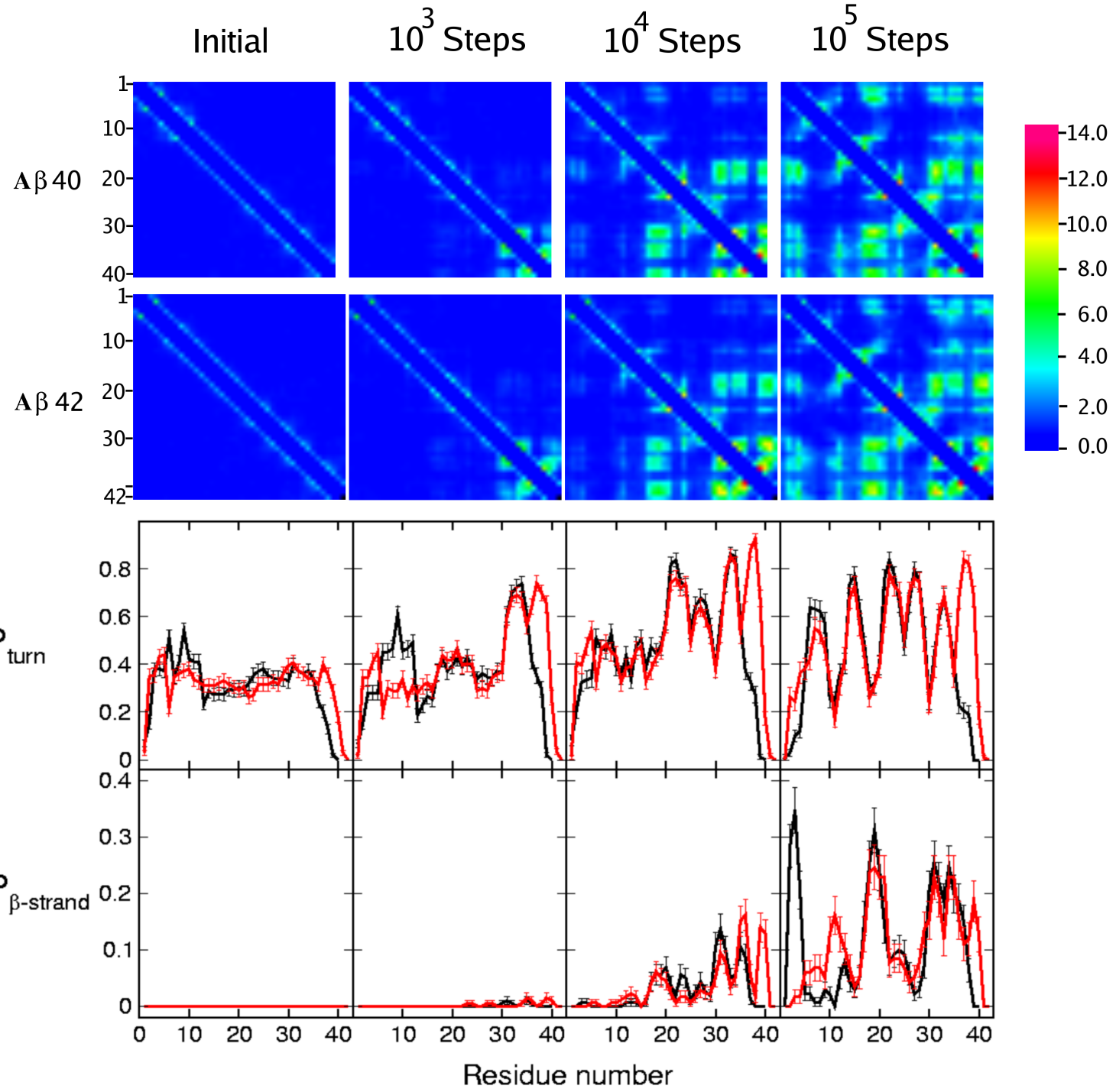
  Bitan et al., JBC (2003);

- **Oxidation of Met35: Aβ42 paranuclei formation abolished-**

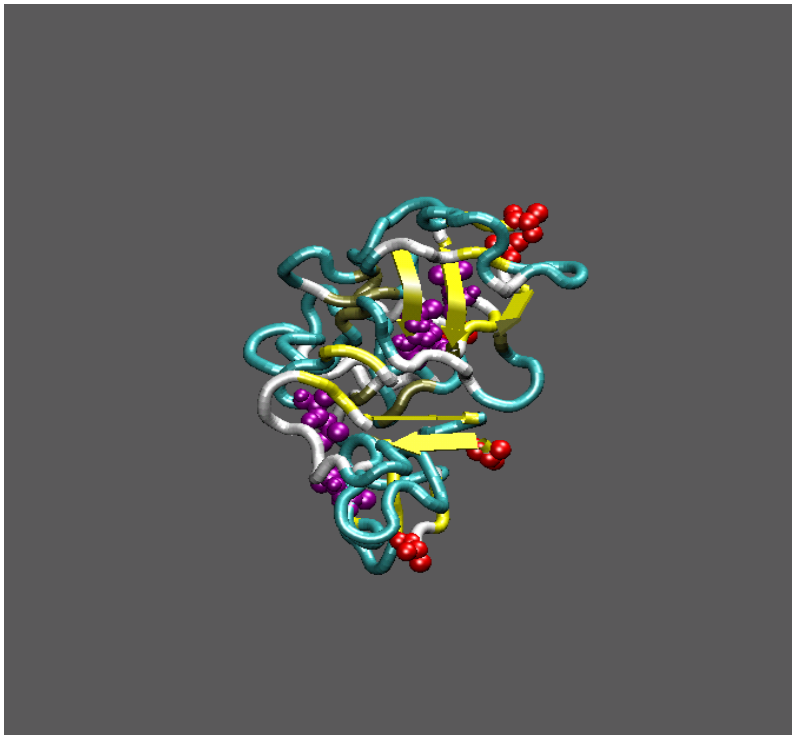
  Bitan et al., JACS (2003).

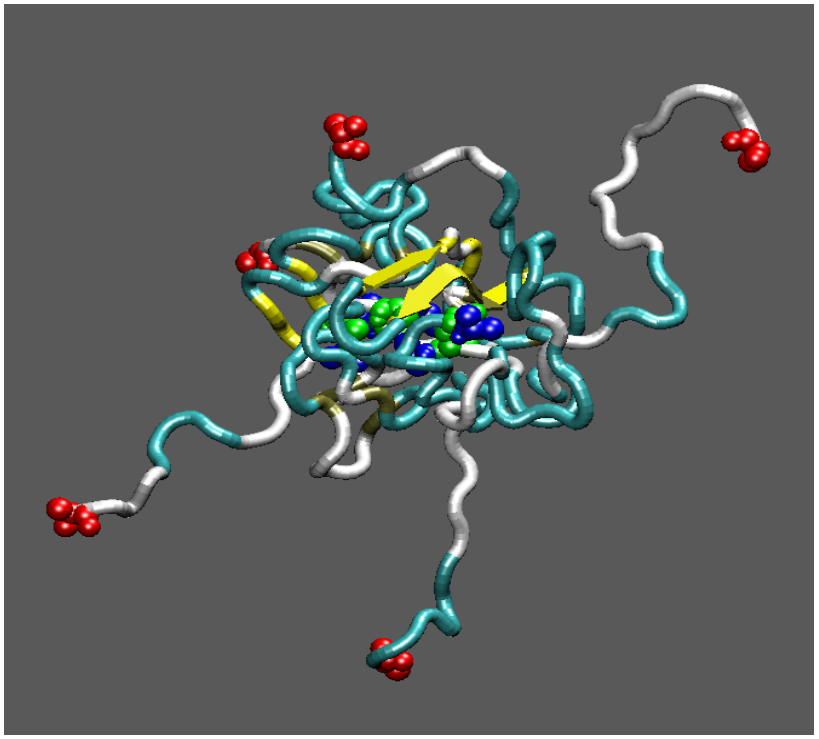
Aβ40 versus Aβ42 monomer folding

- contacts form first at the C-termini
- turn at Gly37-Gly38 forms in Aβ42 only
- β-strand at Ala2-Phe4 forms in Aβ40 only



3D structure of Aβ40 and Aβ42 pentamers





Aβ40 **Aβ42**

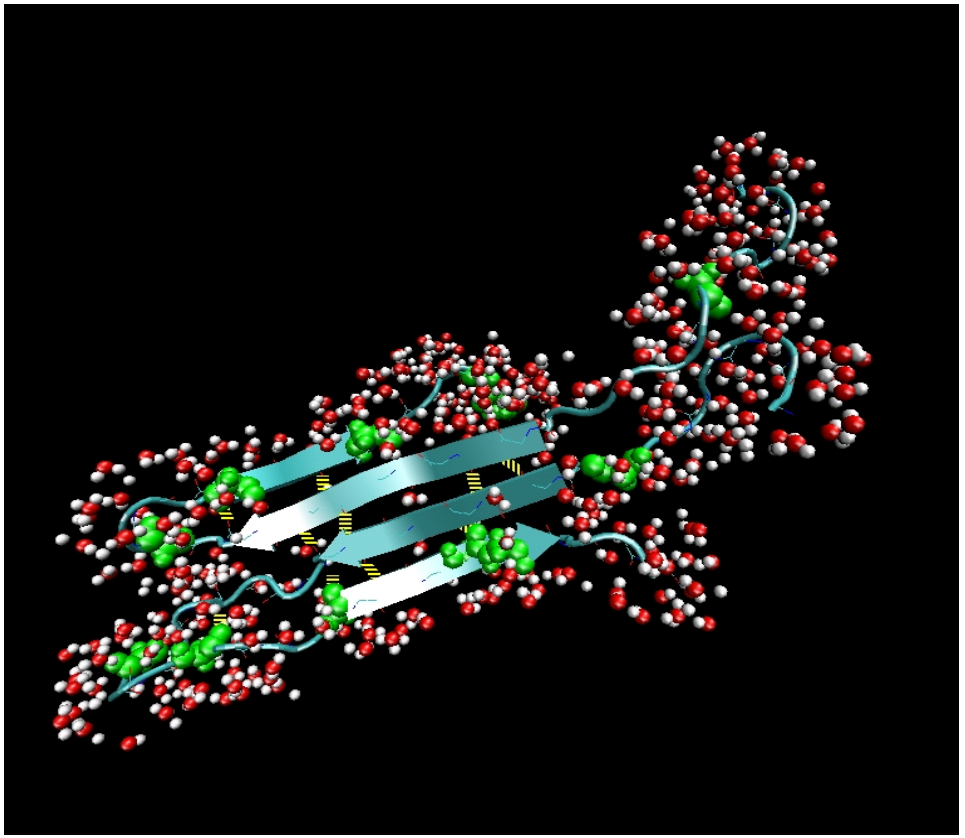
red spheres	... Asp1		
purple spheres	... Val40	**yellow** ribbon	... β-strand
green spheres	... Ile 41	**turquoise** tube	... turn
blue spheres	... Ala42	**silver** tube	... no s.s.

VMD Software Package (Humphrey et al, JMG, 1996).

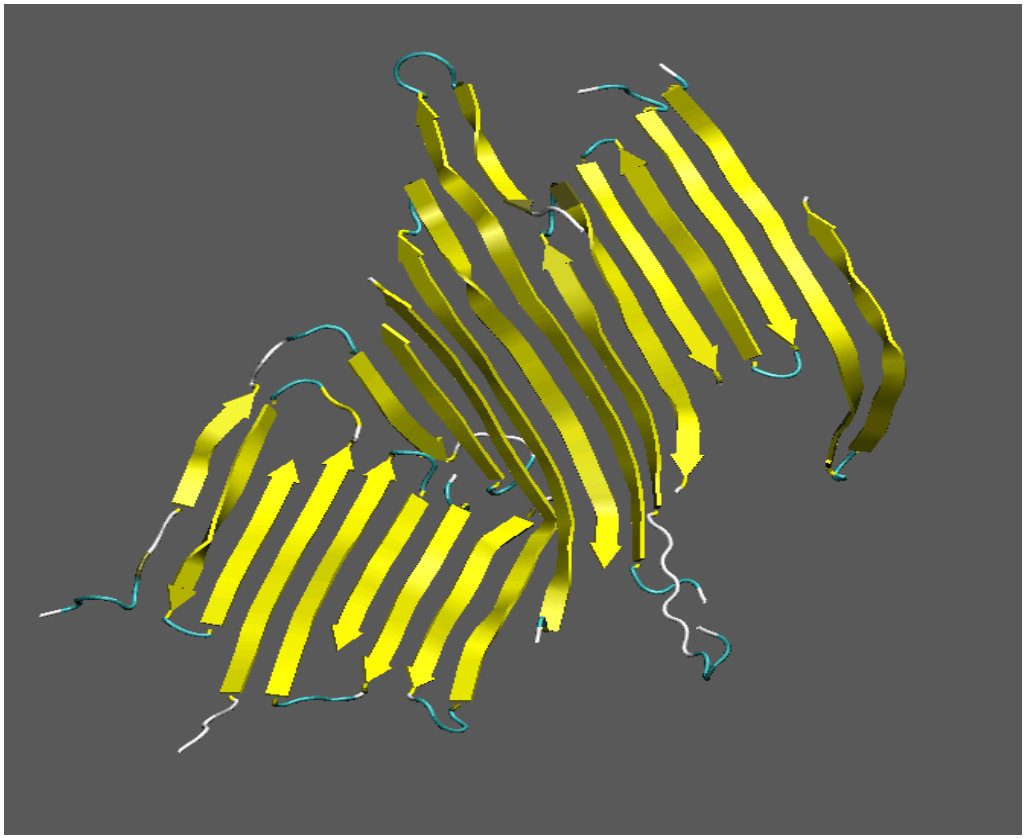
STRIDE program for s.s. calculation (Heinig and Frishman, 2004).

Four-bead model with hydrogen bonds: planar β-sheet dimers and single-layer β-sheet oligomer structure

Planar β-sheet dimer in water



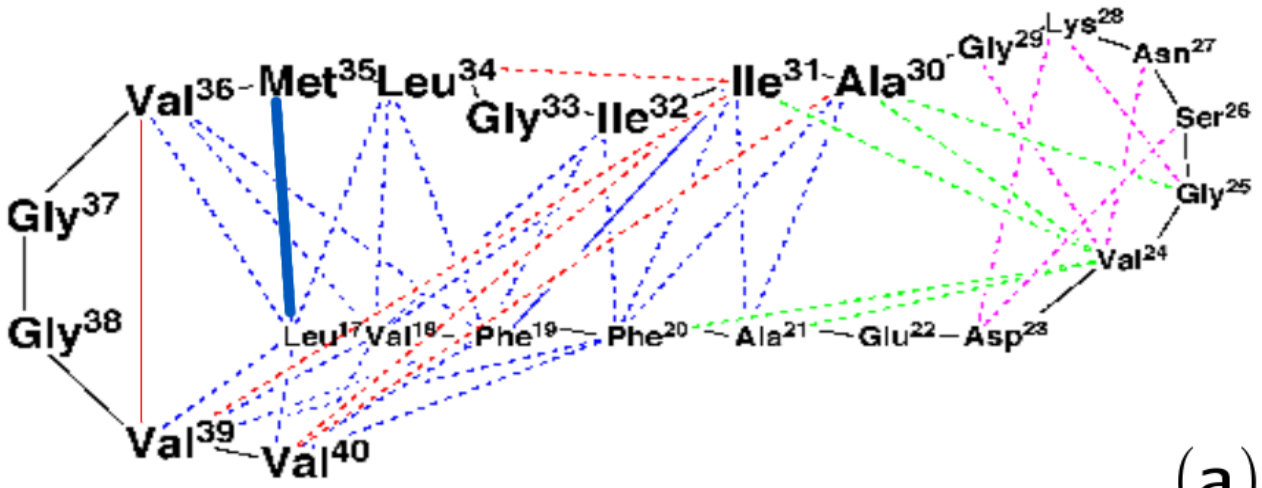
Single-layer β-sheet oligomer



Urbanc et al., Biophys. J., 2004.

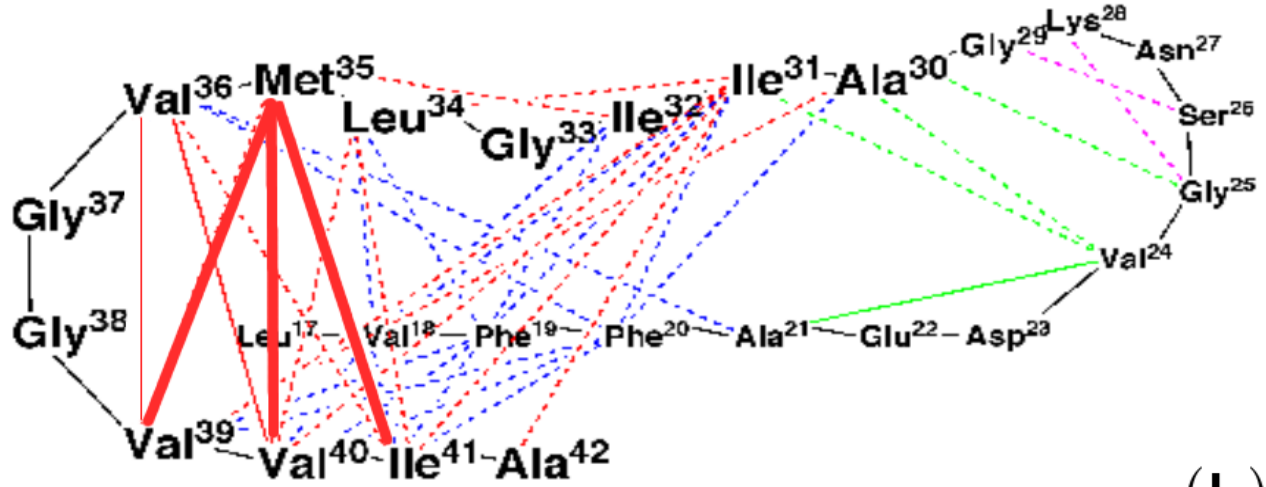
Result: Tertiary structure of Aβ pentamers

Aβ40



(a)

Aβ42



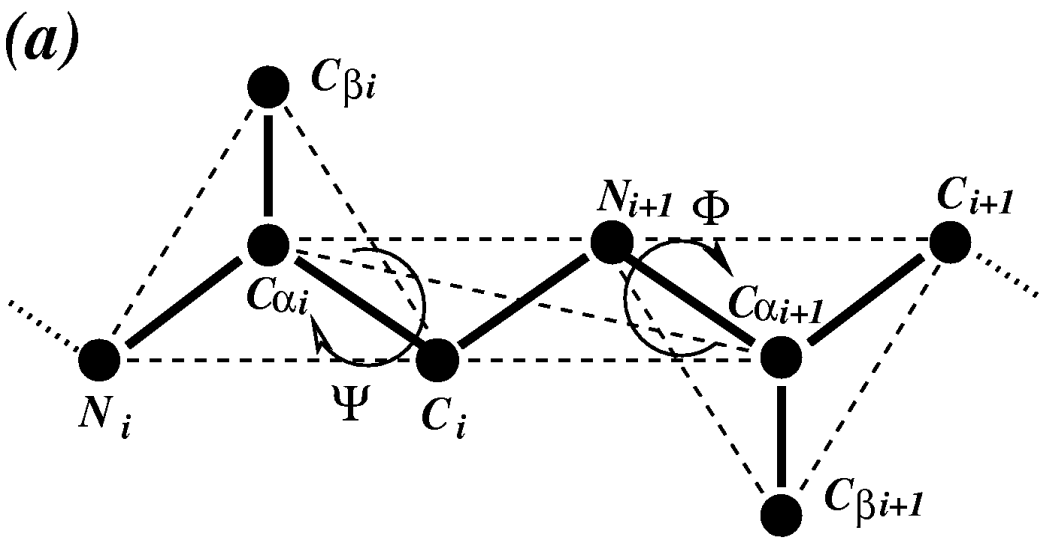
(b)

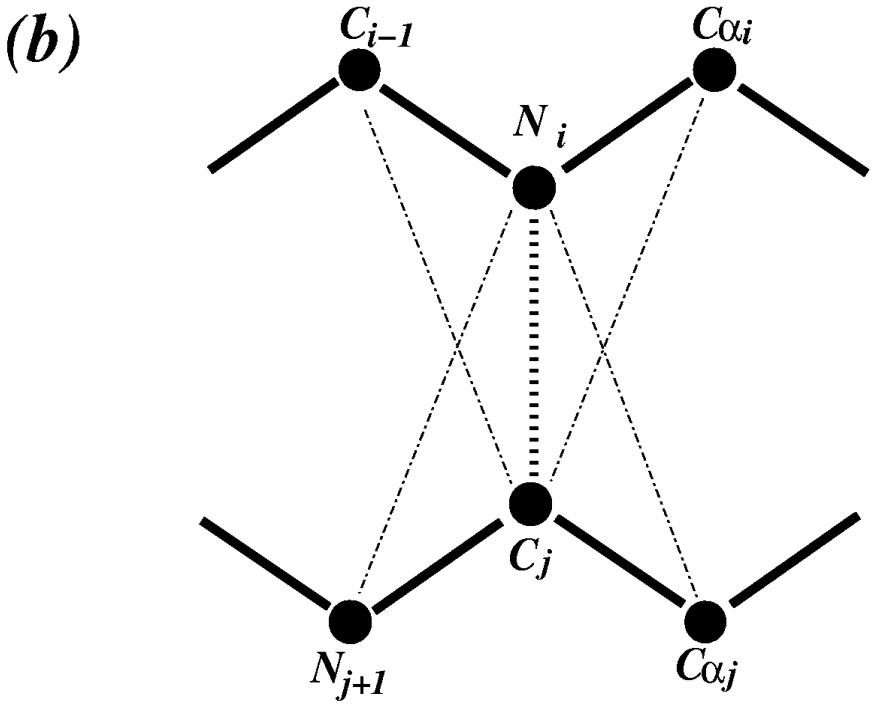
In silico **result (L34,M35,V36 strongly connected to V40,I41,A42 in Aβ42, not in Aβ40) provides an explanation of *in vitro* finding (oxidation of M35 blocks Aβ42 paranuclei formation—Bitan et al., JACS, 2003).**

Four-bead protein model

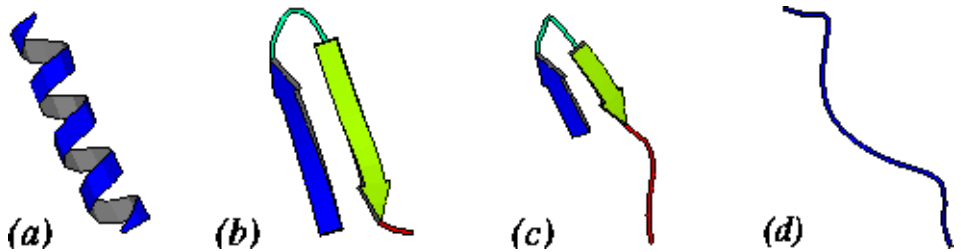
four beads per amino acid

hydrogen bond implementation





α-helix to β-hairpin transition in polyalanine

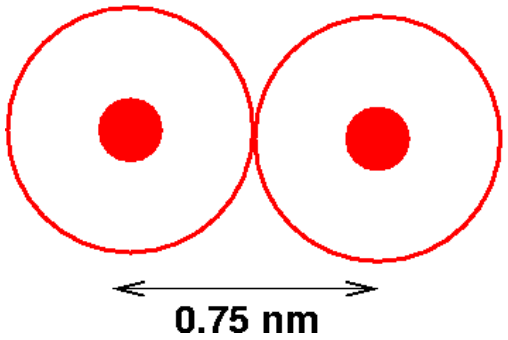


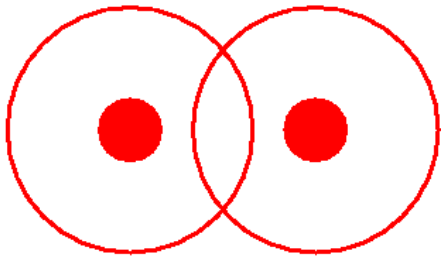
low temperature ---> high temperature

Ding et al., Proteins, 2003.

Amino acid-specific side chain interactions

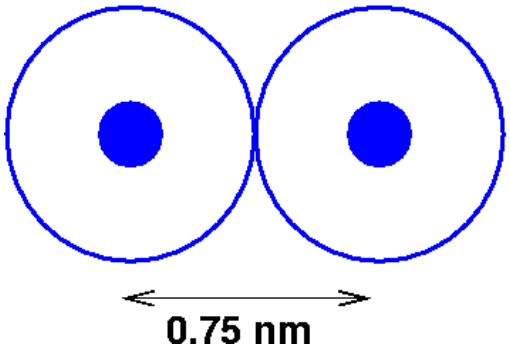
hydrophobic residues: Ile, Val, Leu, Phe, Cys, Met, Ala
hydrophobic effect --> minimize the ``solvent''exposed surface --> attraction

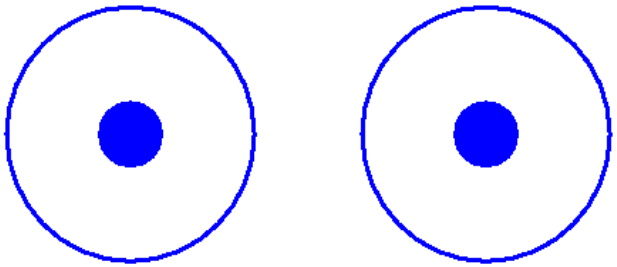




two hydrophobic side chains attract via attractive square well

hydrophilic residues: Arg+, Lys+, Asp-, Glu-, Asn, Gln, His
hydrophilic effect --> maximize the ``solvent''exposed surface-->repulsion





two hydrophilic amino acid: repulsive square well interaction

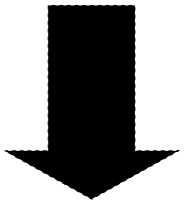
What is the Appropriate Molecular Dynamics (MD) Method?

- **A need for an *in vitro*◄►*in silico* approach to reveal mechanisms of Aβ folding, aggregation, and different pathways of assembly:**

  Teplow et al. "Elucidating Amyloid β-Protein Folding and Assembly: A MultidisciplinaryApproach", *Accounts of Chemical Research* (2006).

- **Current all-atom simulations with explicit solvent are limited to study intial folding events of full-length Aβ on time scales < 1 μs:**

  Urbanc et al. "Computer Simulations of Alzheimer's Amyloid β-Protein Folding and Assembly", *Current Alzheimer Research* (2006).



SIMPLIFY!

- **Develop simplified protein models and use faster and more efficient algorithms, such as discrete molecular dynamics:**

  Urbanc et al. "*Ab initio* Discrete Molecular Dynamics Approach to Protein Folding and Aggregation", *Methods in Enzymology* (2006).