# *In silico* study of amyloid $\beta$-protein folding and oligomer formation

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**I. What are key principles giving rise to oligomer formation differences of Aβ40 versus Aβ42?**

**II. What is the 3D structure of Aβ40 versus Aβ42 oligomers?**

# WHAT DO *IN VITRO* STUDIES SHOW?

- **Aβ40: monomers to tetramers, Aβ42: paranuclei (pentamers/ hexamers) and higher order oligomers---multiples of paranuclei (10-12, 15-18)---**

  **Bitan, Kirkitadze, Lomakin, Vollers, Benedek & Teplow, PNAS (2003);**

- **hydrophobic nature of C-terminal Ile41 and Ala42 plays a key role in Aβ42 paranuclei formation---**

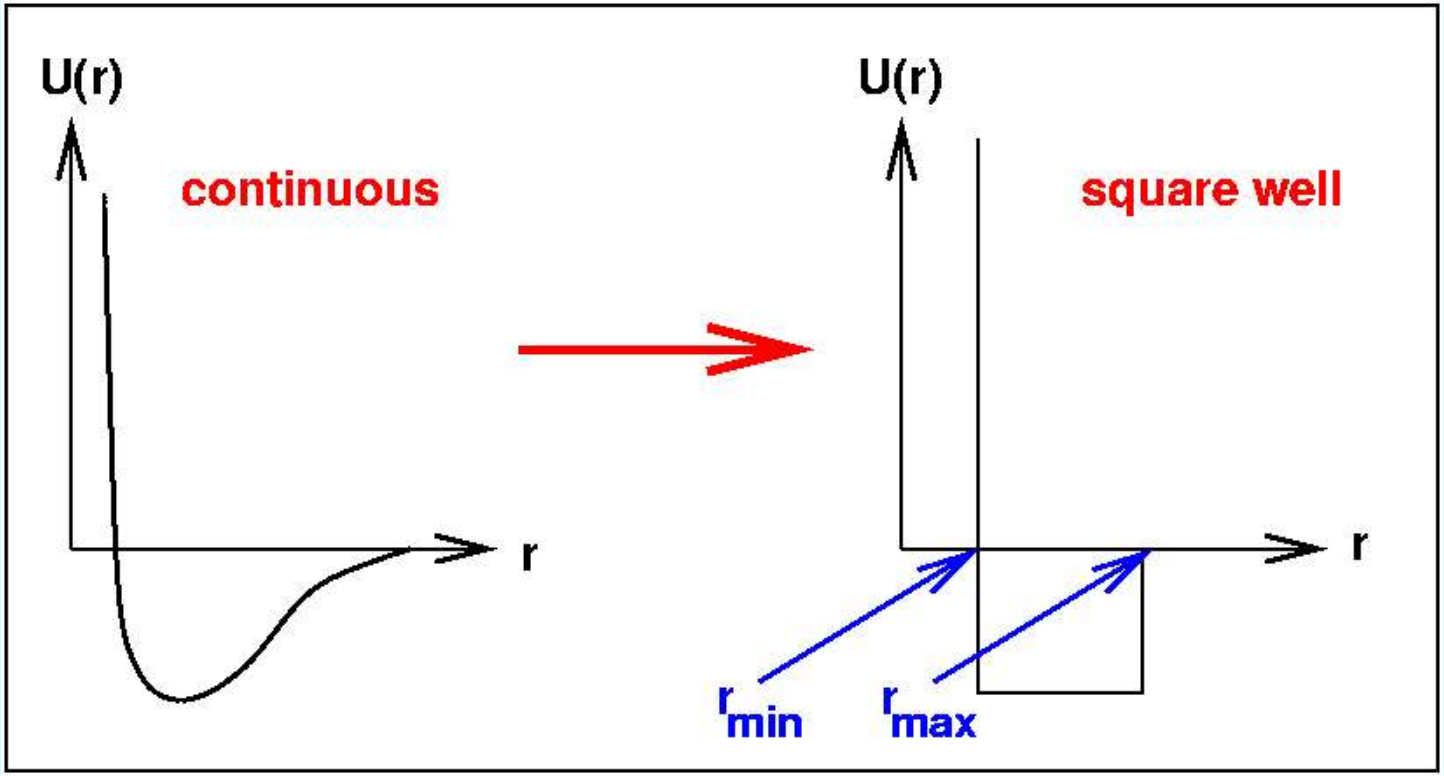
  **Bitan, Vollers & Teplow, JBC (2003);**

- **oxidation of Met35 disruptsAβ42 paranuclei formation---**

  **Bitan, Tarus, Vollers, Lashuel, Condron, Straub & Teplow, JACS (2003).**

# METHOD: discrete molecular dynamics (DMD)

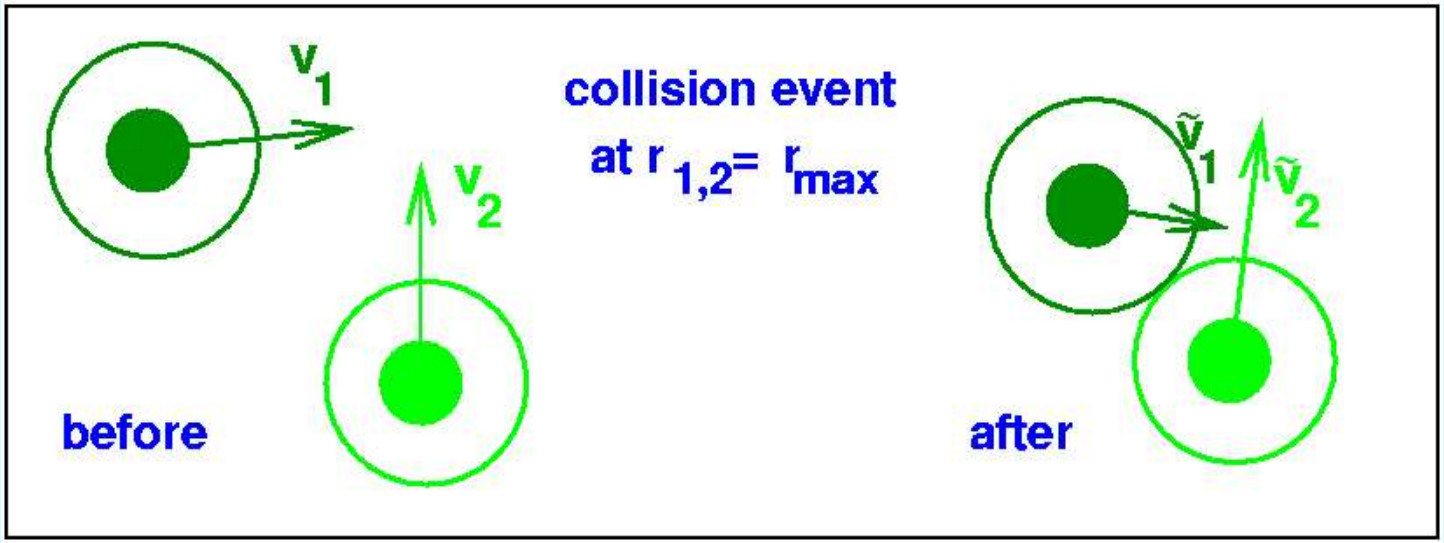
**Interparticle potential**




**Keep track of collision events**

**Collisions conserve:**

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

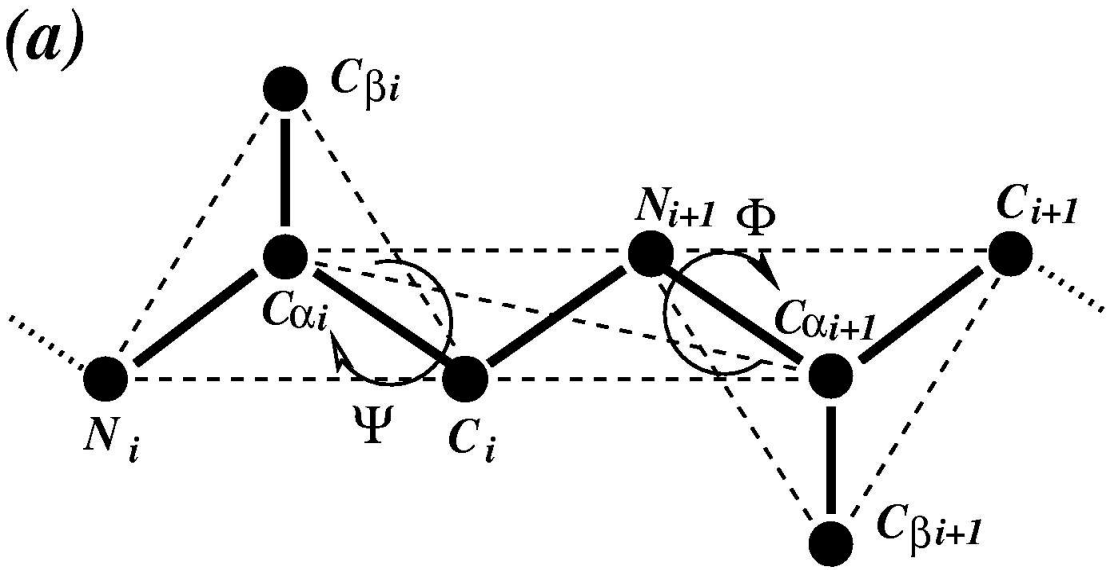



Zhou, Hall & Karplus, PRL, 1996; Zhou & Karplus, PNAS, 1997; Dokholyan, Buldyrev, Stanley & Shakhnovich, Fold. Des., 1998.

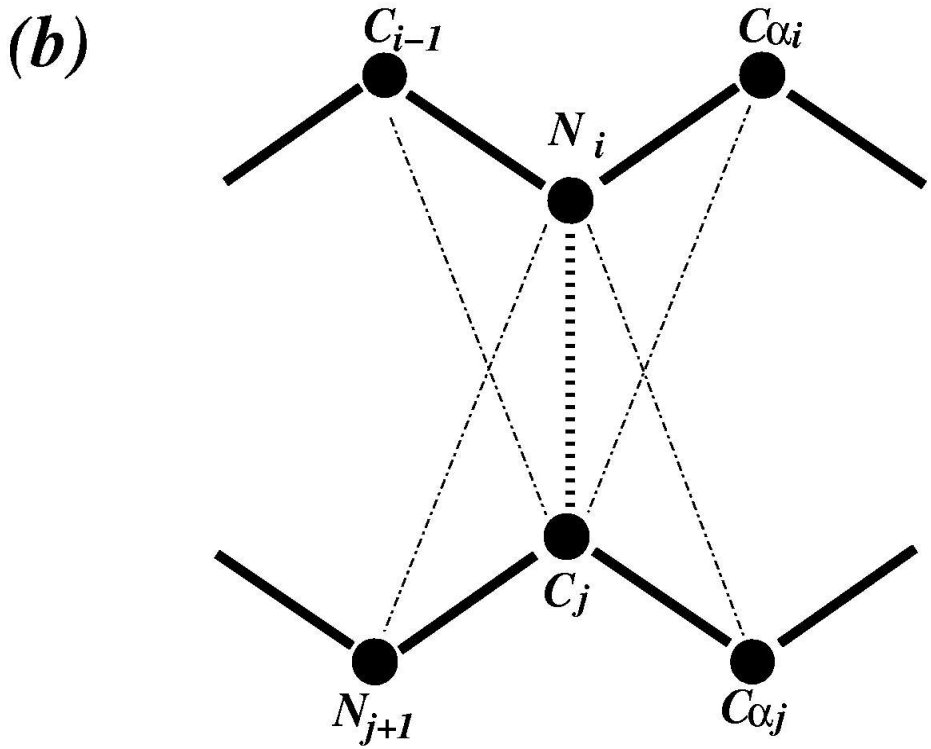
# Four-bead peptide model

Ding, Borreguero, Buldyrev, Stanley & Dokholyan, Proteins, 2003.

**beads, bonds, & constraints**




**hydrogen bond implementation**



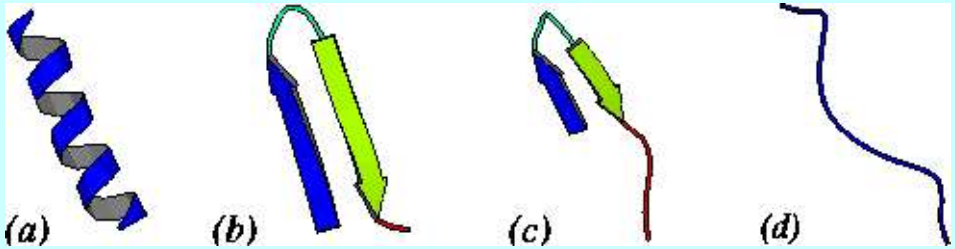

$N_i$ ... amino group

$C_\alpha$ ... α carbon group

$C'_i$ ... carboxyl group

$C_\beta$ ... side chain group

**α-helix to β-hairpin transition in polyalanine**

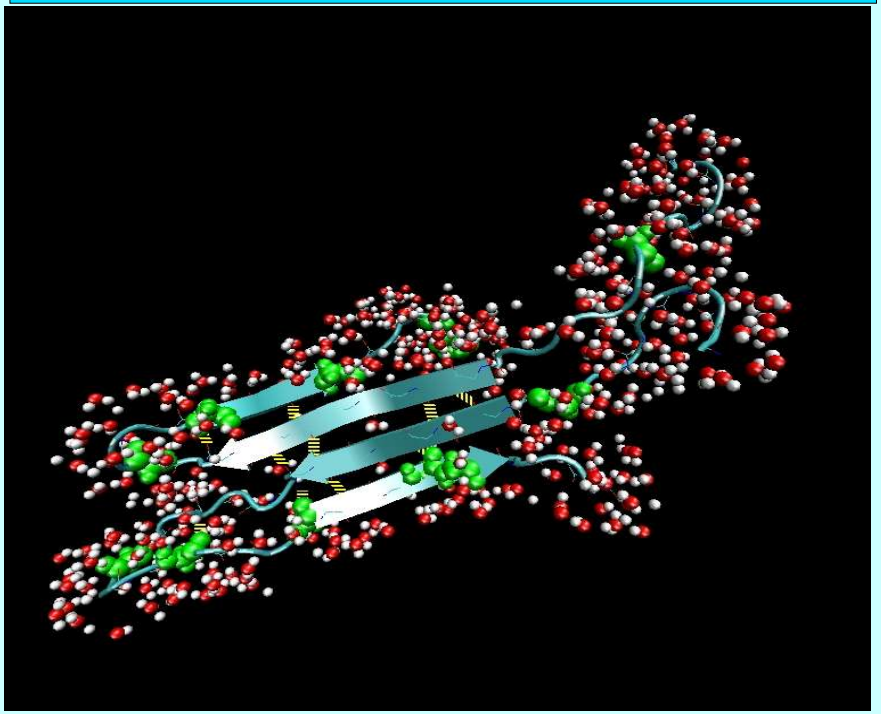



**low temperature ---> high temperature**

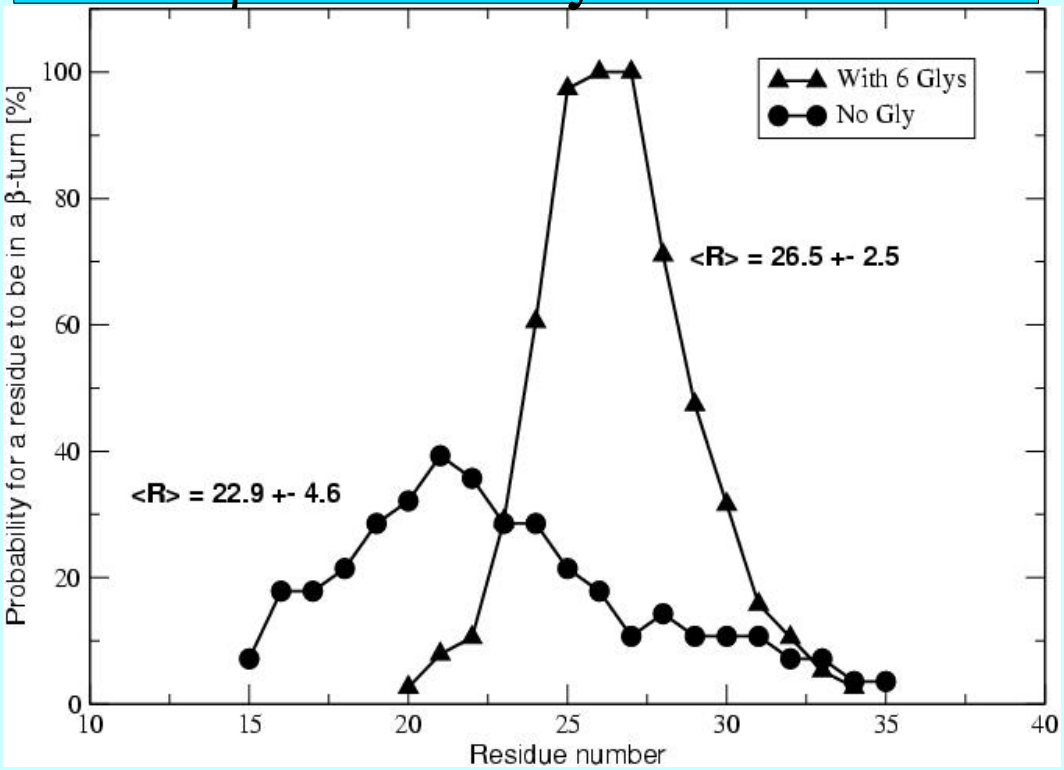
Urbanc, Cruz, Ding, Sammond, Khare, Buldyrev, Stanley & Dokholyan, Biophys. J., 2004.

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

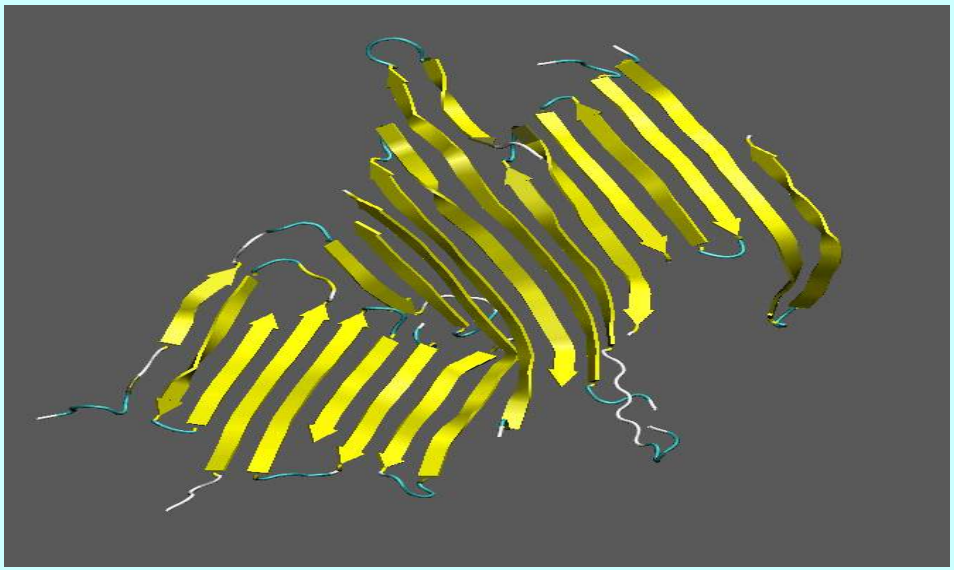
## Planar β-sheet dimer in water



## Glycines at 9, 25, 29, 33, 37, 38 induce a β–turn at Gly25-Asn27




## Single-layer β-sheet oligomer



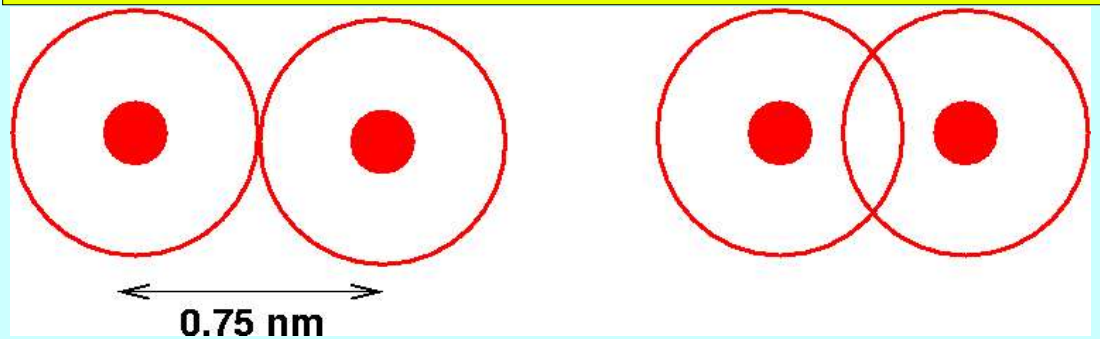
# Amino acid-specific side chain interactions: Effective hydropathy

**Phenomenological hydropathy scale---Kyte and Doolittle, JMB, 1982.**

**hydrophobic residues: (from the most to the least hydrophobic)**

**Ile, Val, Leu, Phe, Cys, Met, Ala**

**hydrophobic effect --> minimize the ``solvent''exposed surface --> attraction**

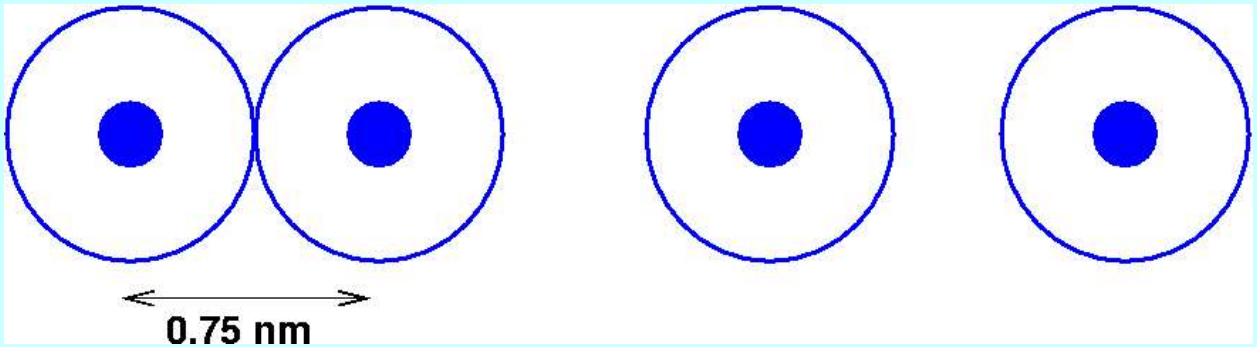



**two hydrophobic side chains attract via attractive square well**

**hydrophilic residues: (from the most to the least hydrophilic)**

**Arg+, Lys+, Asp-, Glu-, Asn, Gln, His**

**hydrophilic effect --> maximize the ``solvent''exposed surface-->repulsion**




**two hydrophilic side chains repel via repulsive square well if:**

- **both non-charged;**
- **one charged and the other non-charged.**

**Purpose: Separate hydropathic from electrostatic effects.**

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

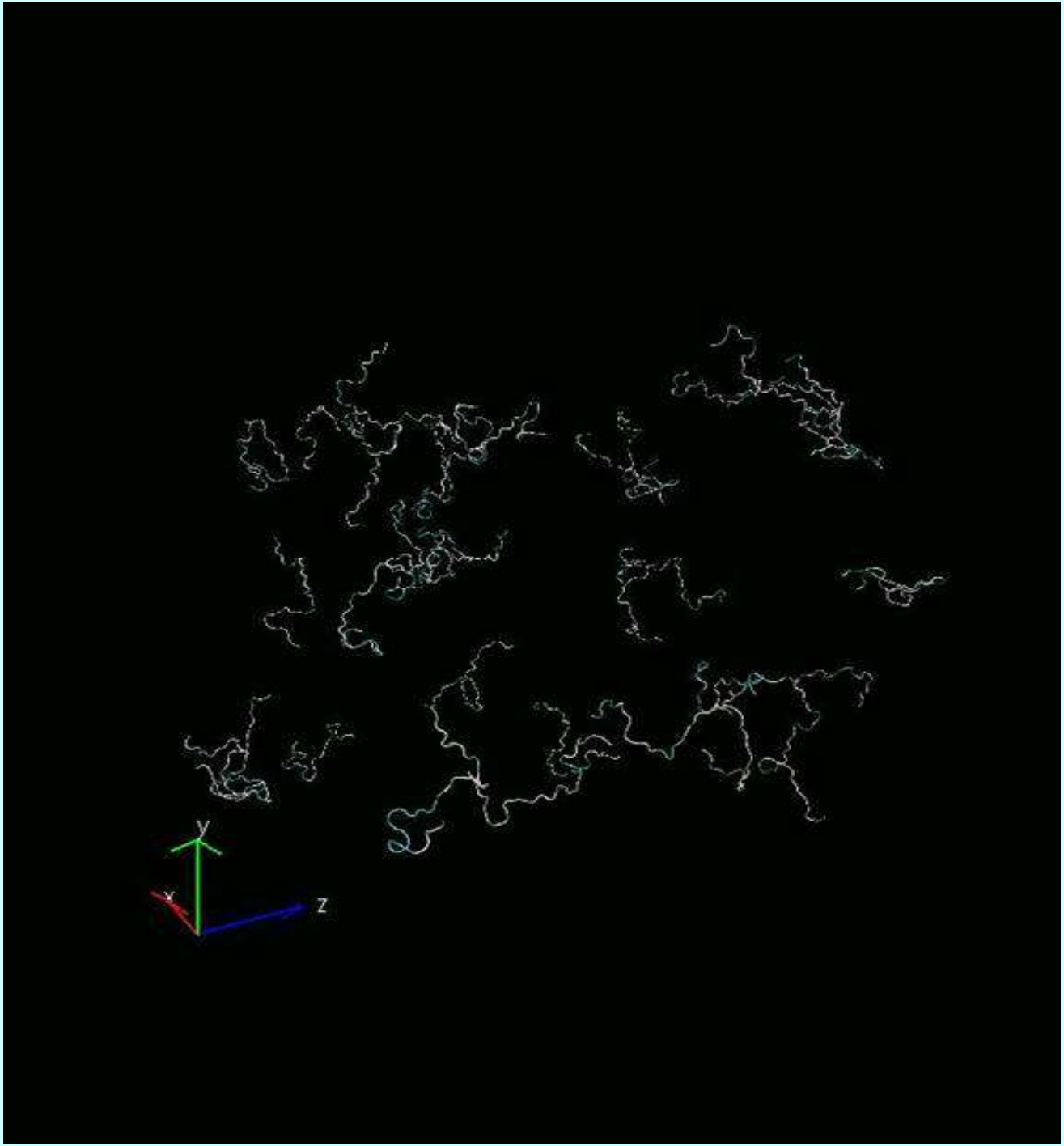
**Parameters:**

$E_{HB} = 1$

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

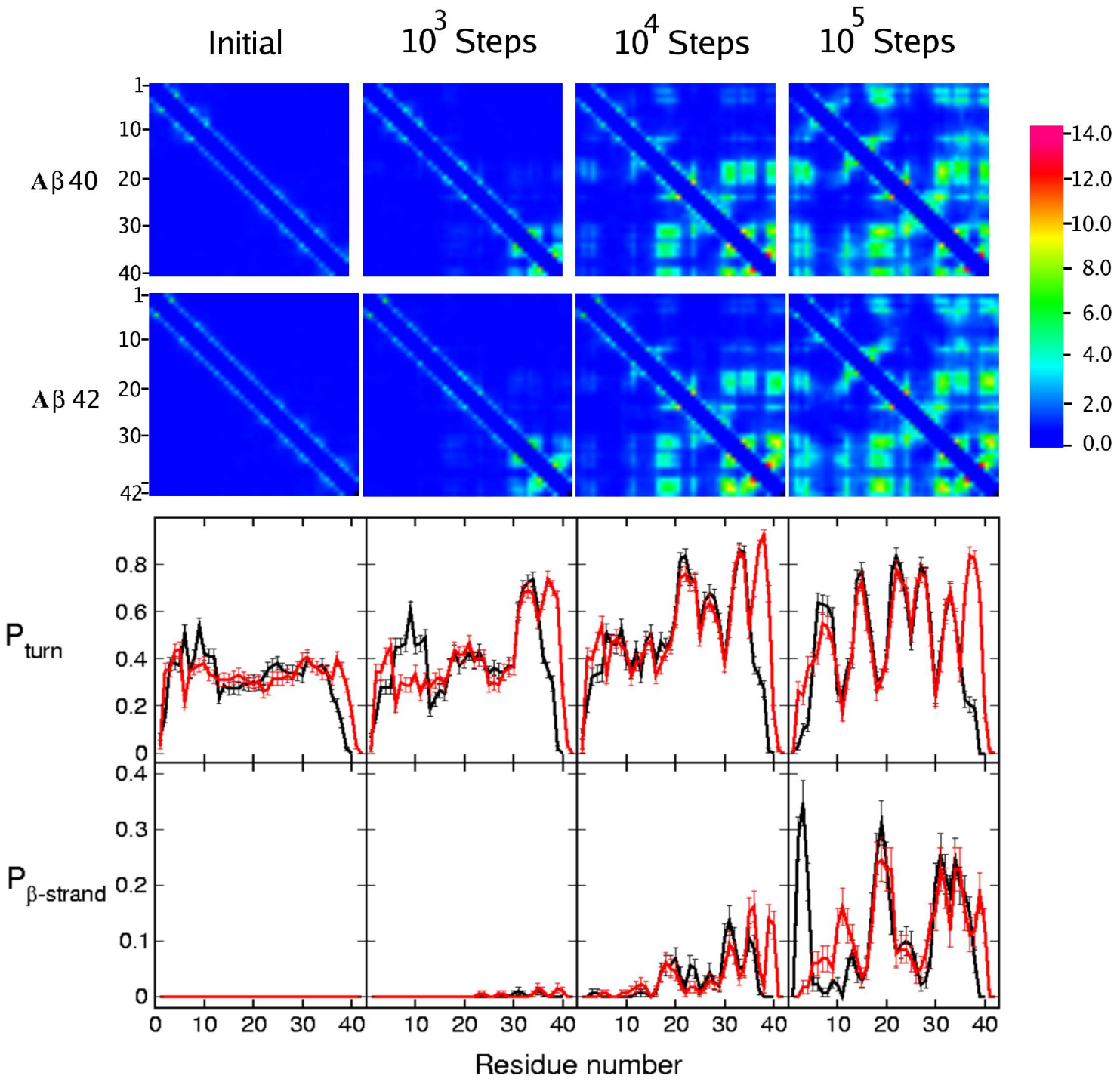
$T = 0.15\ [E_{HB}/k]$

**Initially peptides are in unfolded, zero-energy conformations.**



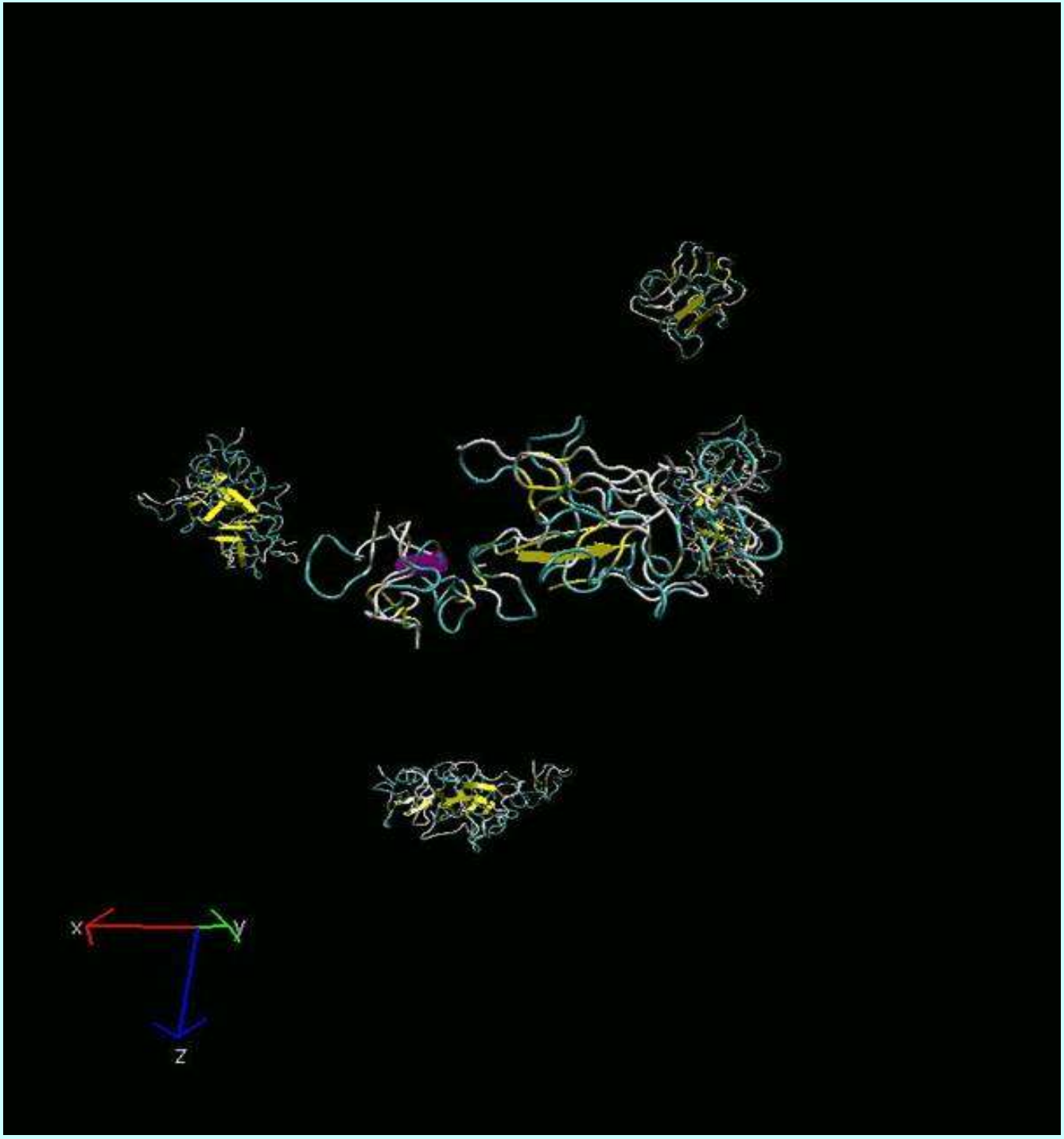
# Aβ40 versus Aβ42 monomer folding

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

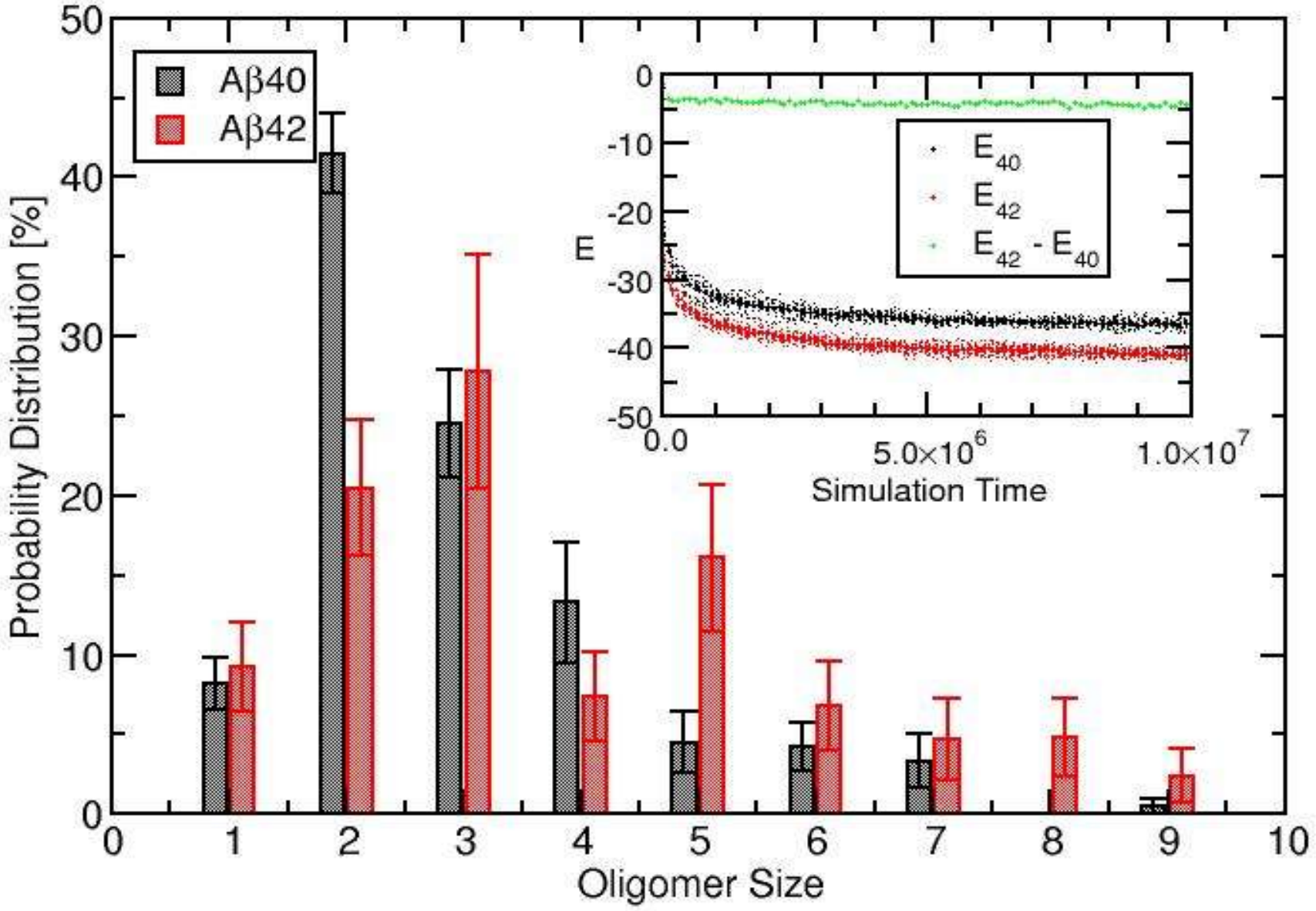


**8 trajectories of each Aβ40 and Aβ42 with 32 peptides in a cubic box of 25 nm**

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



# Aβ40 versus Aβ42 oligomer size distributions

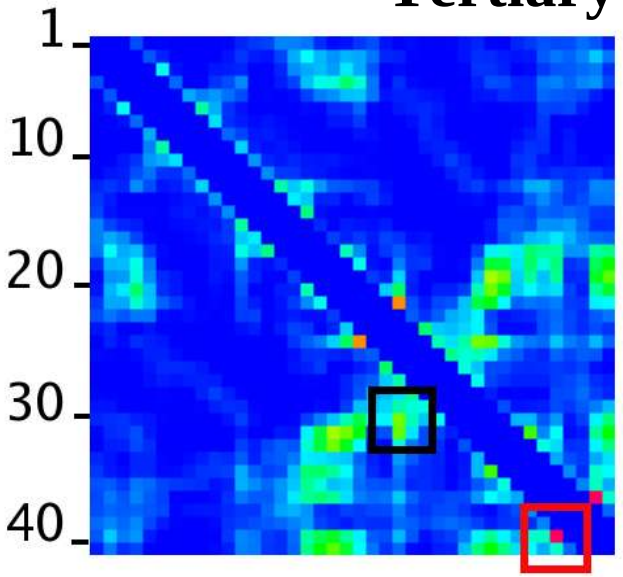


Bitan et al, JACS, 2003.

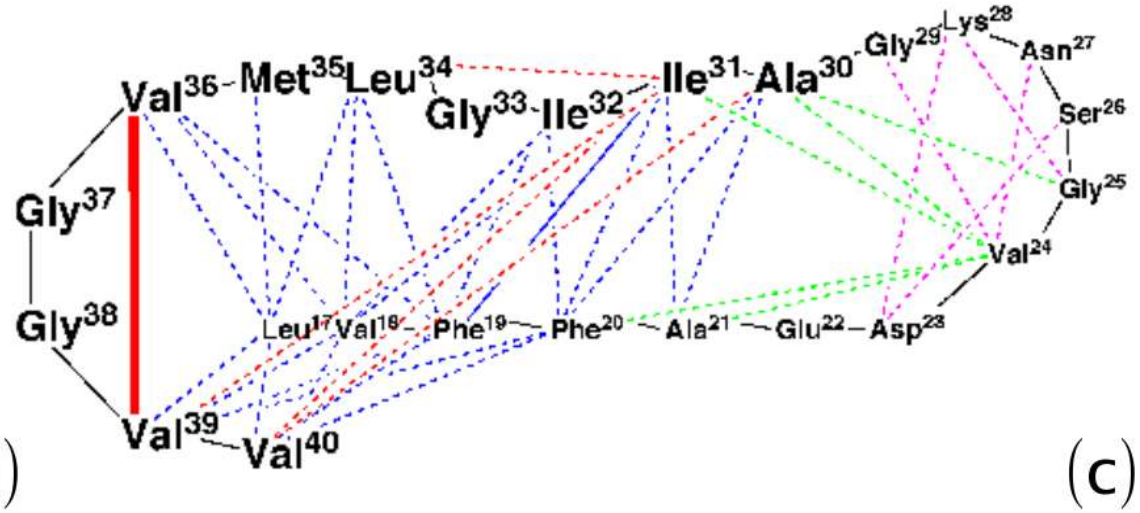
# Oxidation of Met35 blocks Aβ42 paranuclei (pentamer/hexamer) formation

## Tertiary structure of Aβ pentamers

Aβ40

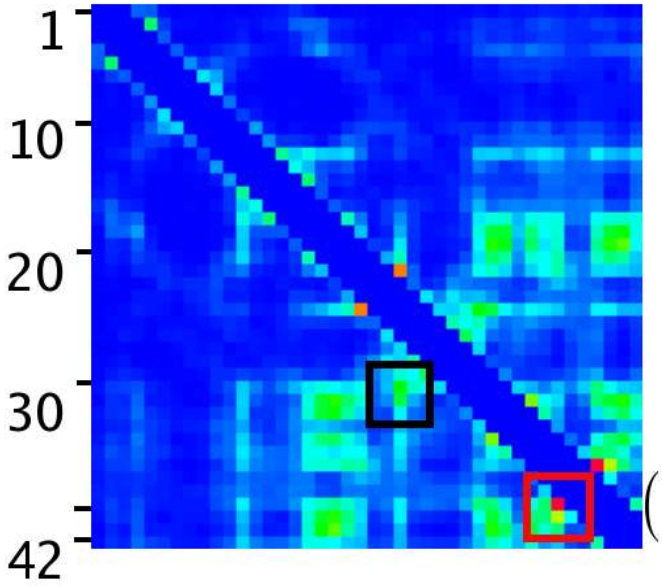



(a)

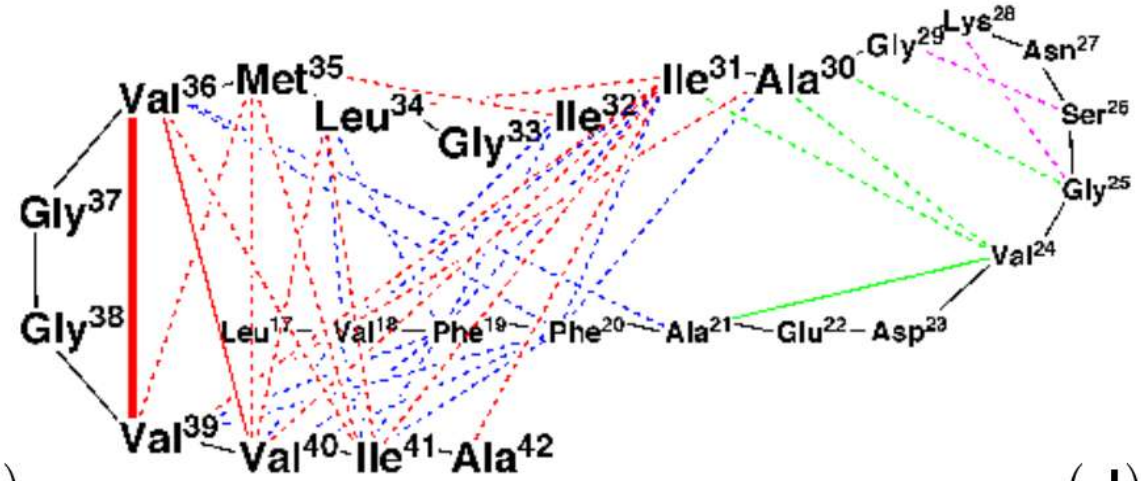



(c)

Aβ42



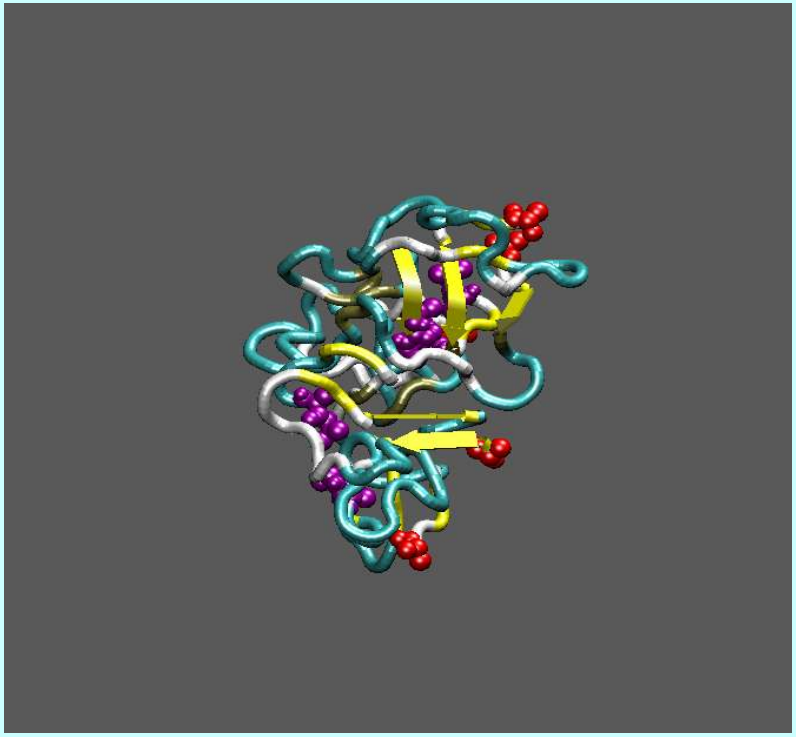

(b)

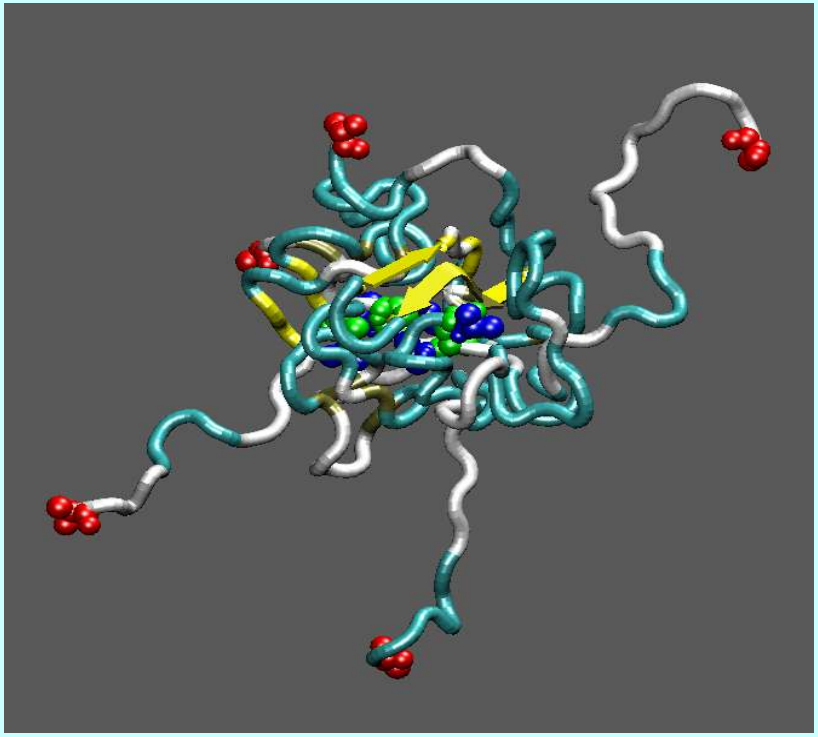



(d)

**Leu34,Met35,Val36 strongly connected to Val40,Ile41,Ala42 in Aβ42, not in Aβ40**

# 3D structure of Aβ40 and Aβ42 pentamers





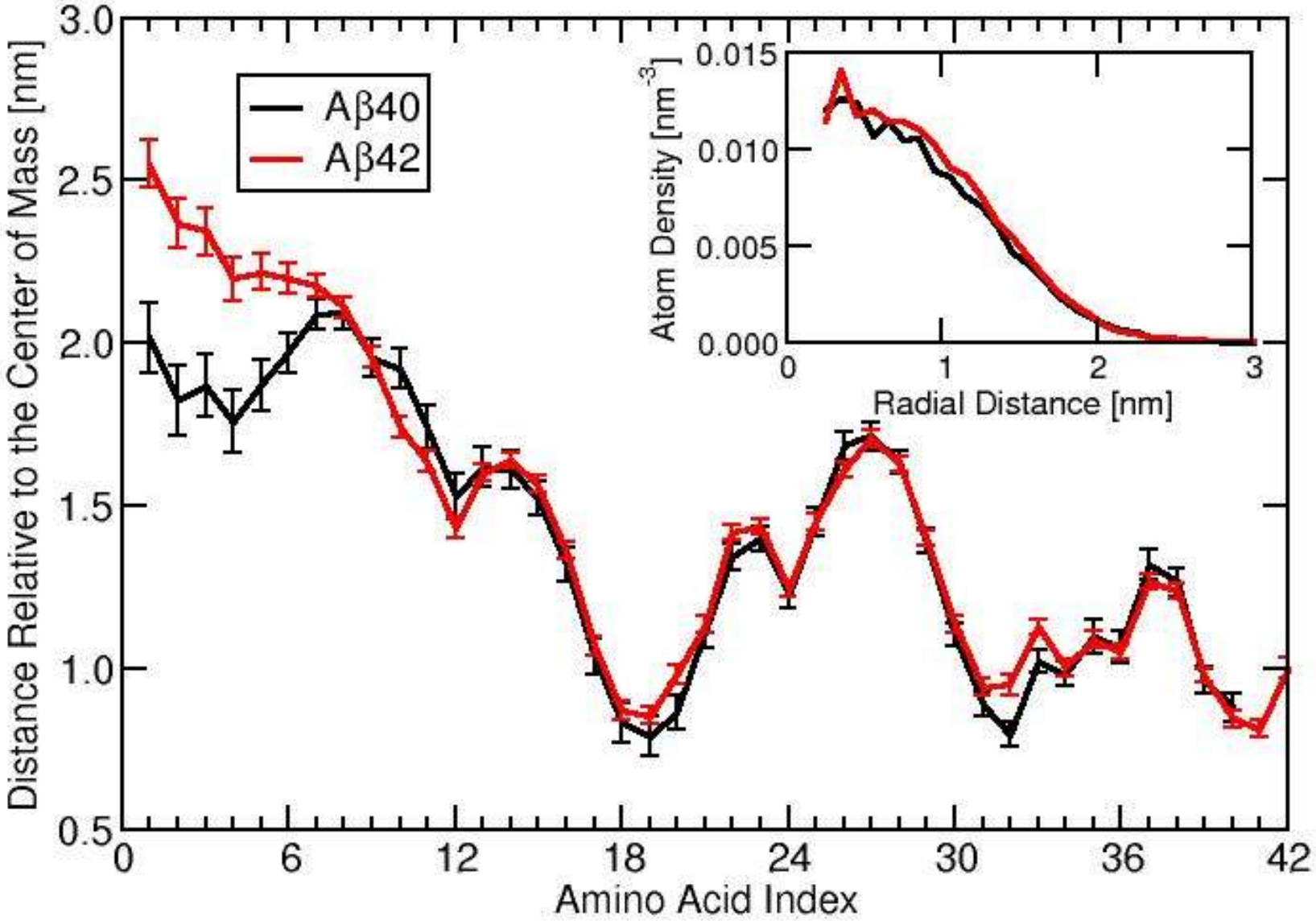
**Aβ40** **Aβ42**

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

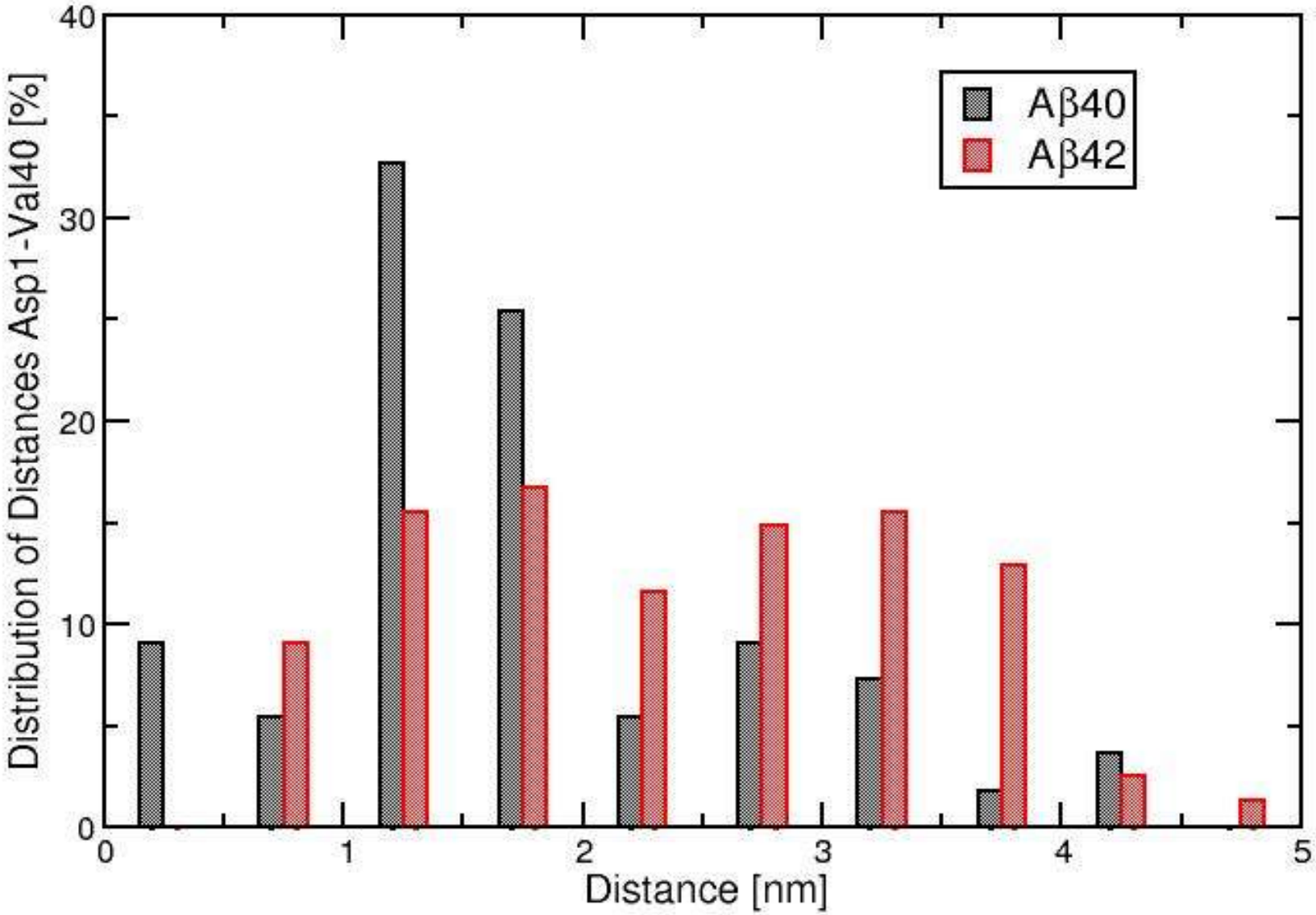
**VMD Software Package (Humphrey et al, JMG, 1996).**

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

# Geometrical characteristics of Aβ40 versus Aβ42 pentamers



# Geometrical characteristics of Aβ40 versus Aβ42 pentamers



## I. What are key principles giving rise to different pathways of Aβ40 versus Aβ42 oligomer formation?

- **hydrophobic/hydrophilic effective interactions are key driving ints in Aβ oligomer formation that underly differences between Aβ40 and Aβ42;**

## II. What is the 3D structure of Aβ40 versus Aβ42 oligomers?

- **a turn-like element at Gly37-Gly38 is present in folded Aβ42 monomer but not Aβ40 monomer, and associated with the first contacts that form during folding;**

- **oligomers have globular structure with C-termini within the core and N-termini at the surface;**

- **N-termini of Aβ40 oligomers are more spatially restricted than in Aβ42 and form a β-strand structure at Ala2-Phe4 ---> hydrophobic core of Aβ42 oligomers is more exposed ---> Aβ42 more prone to aggregate further.**

**Urbanc, Cruz, Yun, Buldyrev, Bitan, Teplow & Stanley, PNAS, 2004.**

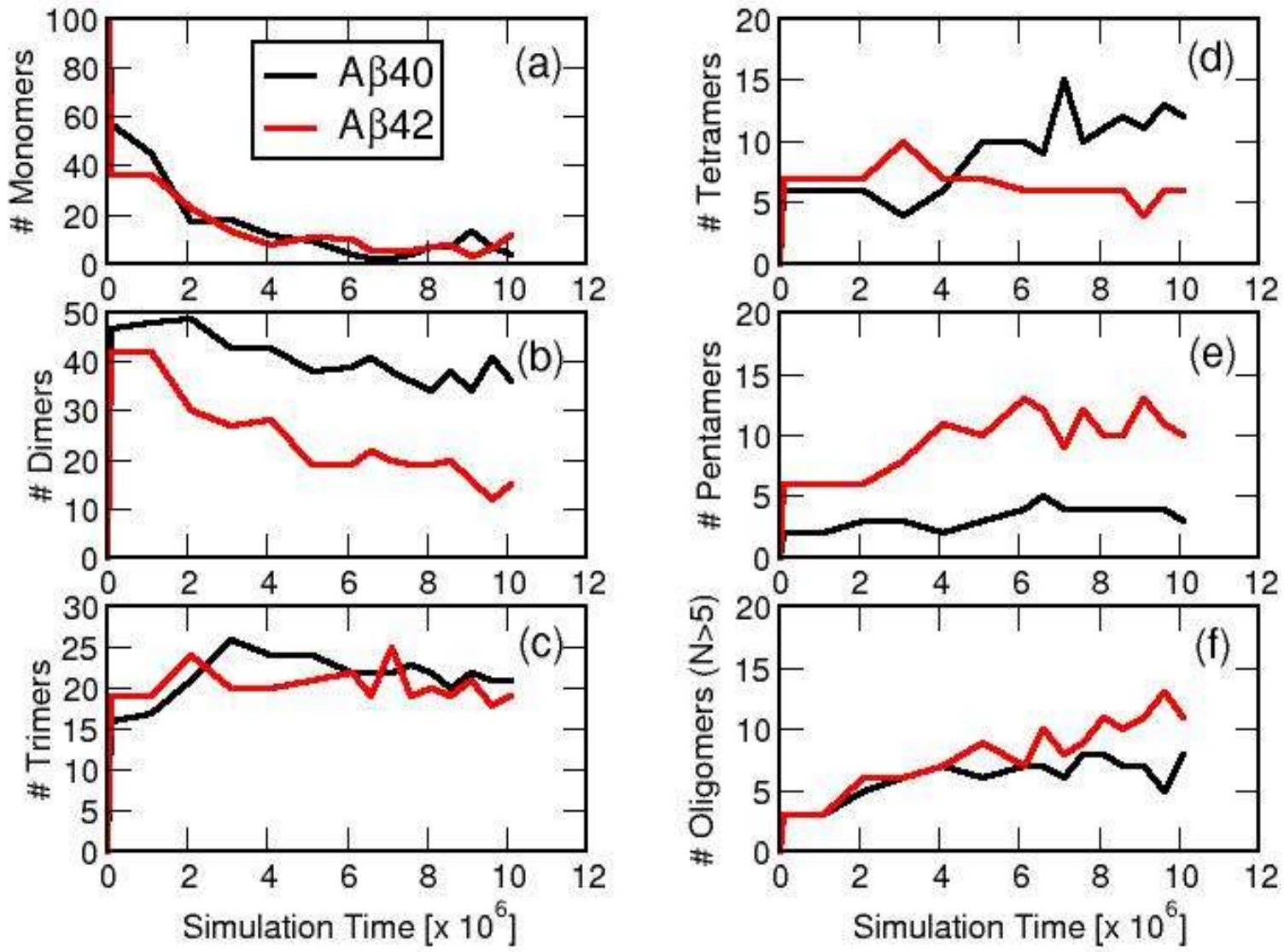
# FUTURE WORK

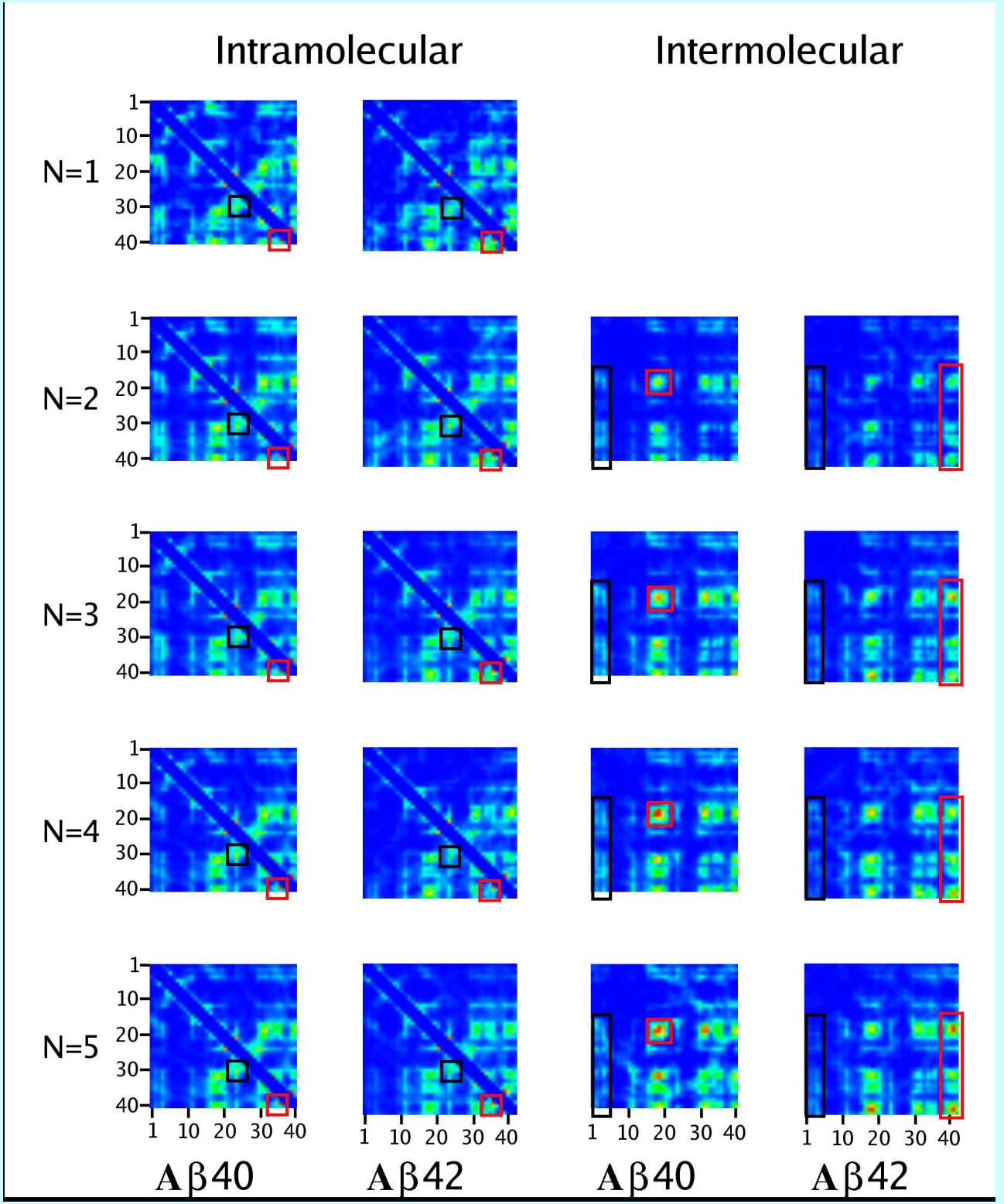
- **systematically study the effects of interaction between charged amino acids on Aβ monomer folding and oligomerization;**

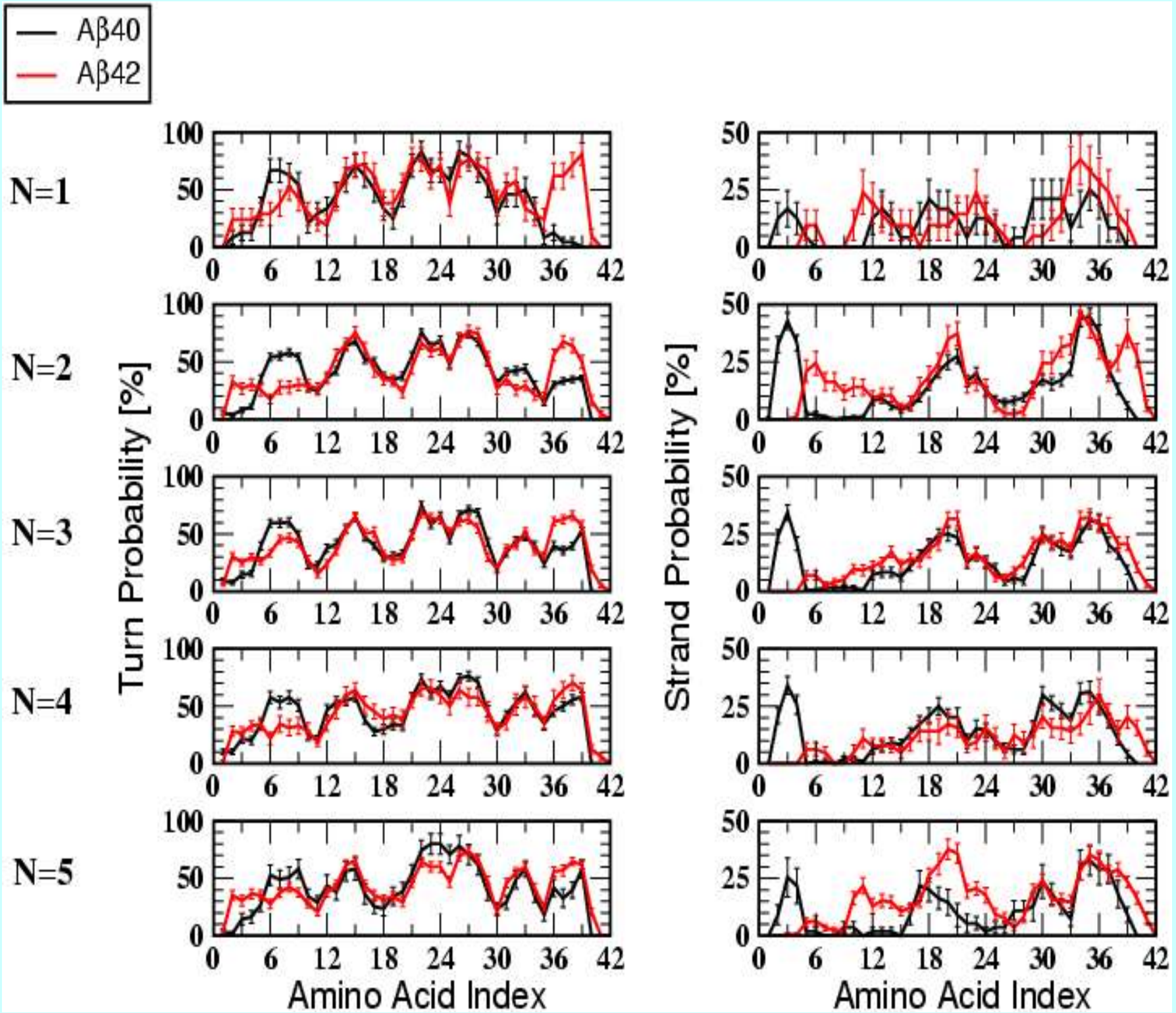
- **expand the four-bead model to a united-atom model to studies details of side chain-side chain interaction (Borreguero et al, submitted to PNAS).**

**Our approach provides experimentally testable hypotheses about Aβ folding and oligomerization and provides an *in silico* method for testing therapeutic compounds by inclusion of these compounds with Aβ monomers during simulations and study the effects on folding and aggregation.**

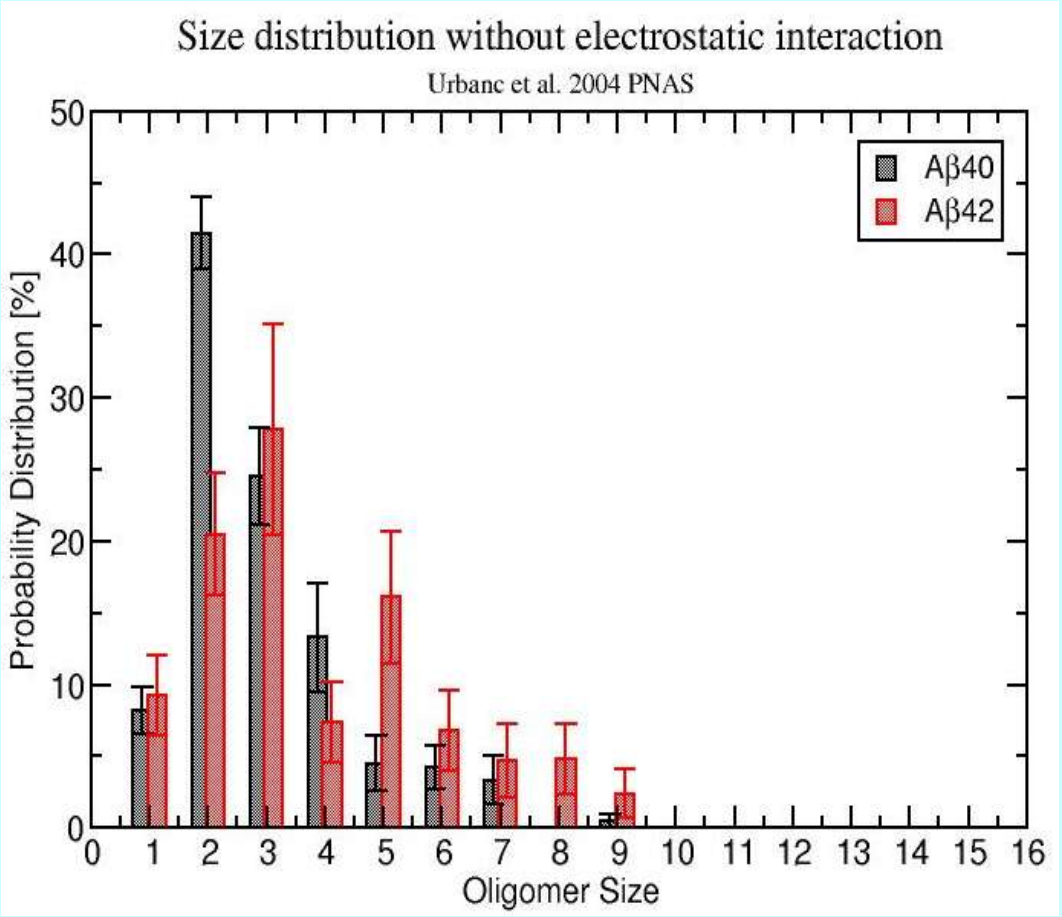
# Number of monomers and oligomers versus simulation time

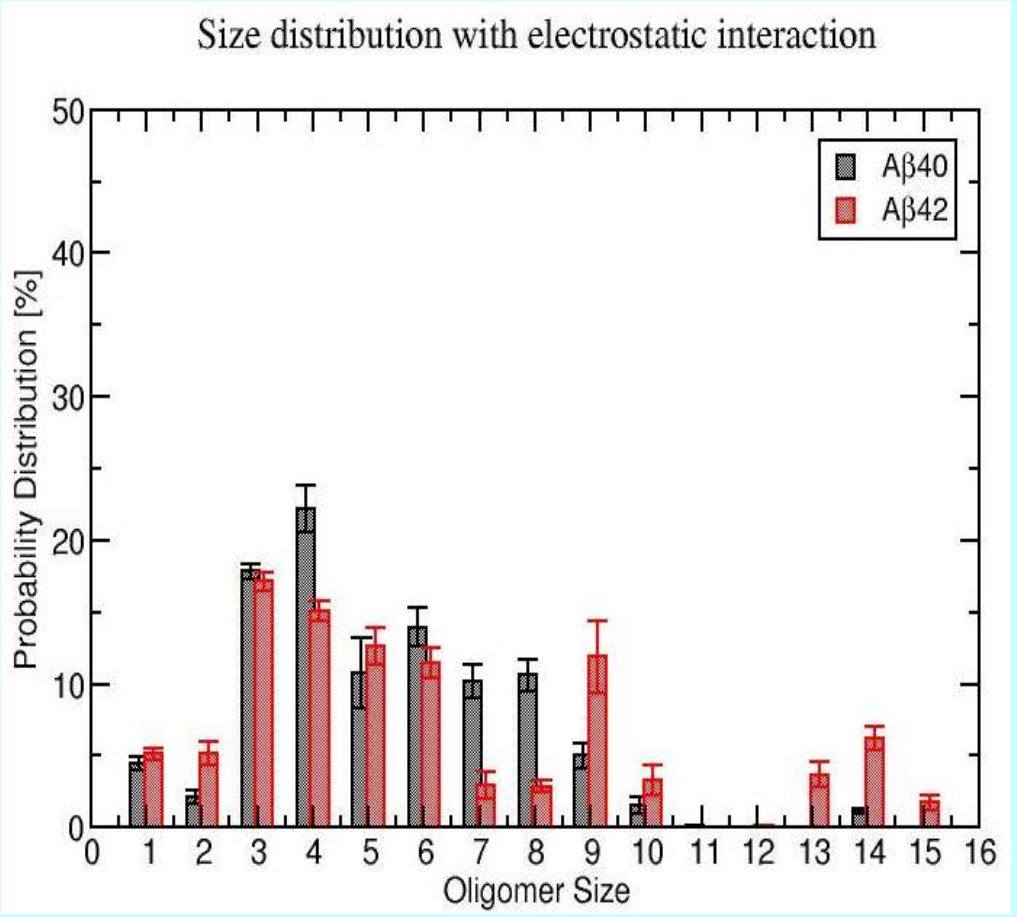



Intramolecular
Intermolecular
N=1
N=2
N=3
N=4
N=5
1
10
20
30
40
Aβ40
Aβ42
Aβ40
Aβ42


Aβ40
Aβ42
N=1
N=2
N=3
N=4
N=5
Turn Probability [%]
Strand Probability [%]
Amino Acid Index
Amino Acid Index

# Effects of the interaction between two charged side chains on oligomer size distribution




**Interactions between charged side chains speed up oligomerization, but do not affect the degree of the difference between Aβ40 and Aβ42.**

# Time evolution of monomer folding: Hydropathy and Charge