Blocking amyloid assembly with chemical denaturants

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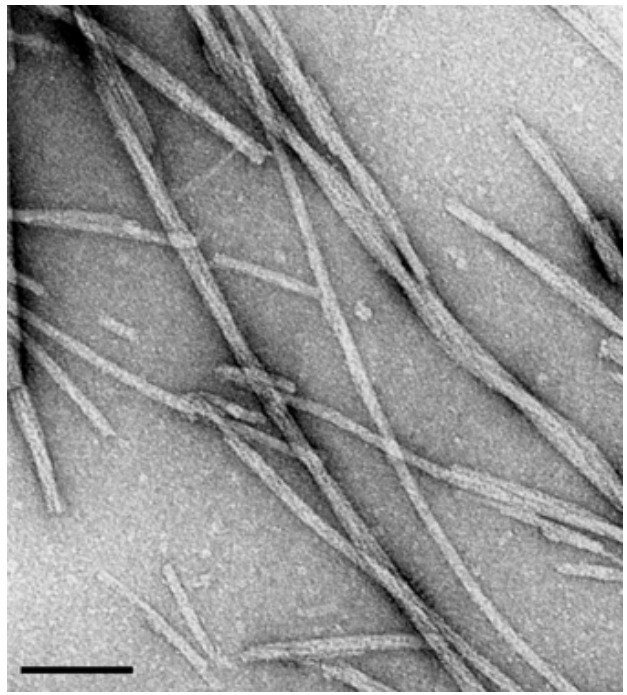
Amyloid fibrils

Protein aggregation is an alternative to monomeric folding

monomers → aggregates → amyloid fibrils

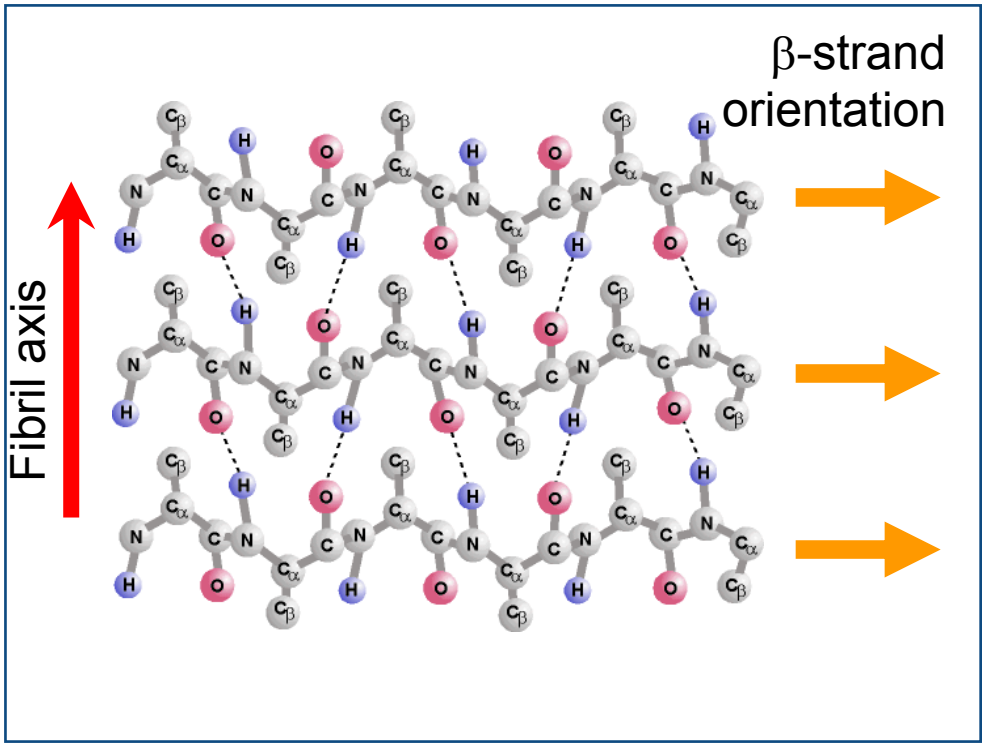


Amyloid fibrils:

1. Long unbranched nanostructures
2. Extremely stable, form irreversibly
3. Assembly timescale >> folding timescale
4. Generic structure available for **any** polypeptide sequence
5. Generally non-functional and cytotoxic, linked to a new class of diseases

Structure of amyloid fibrils

- Universal internal organization based on β-sheet *in registry* structure
- β-sheet is stabilized by hydrogen bond network
- β-sheets are laminated in layers

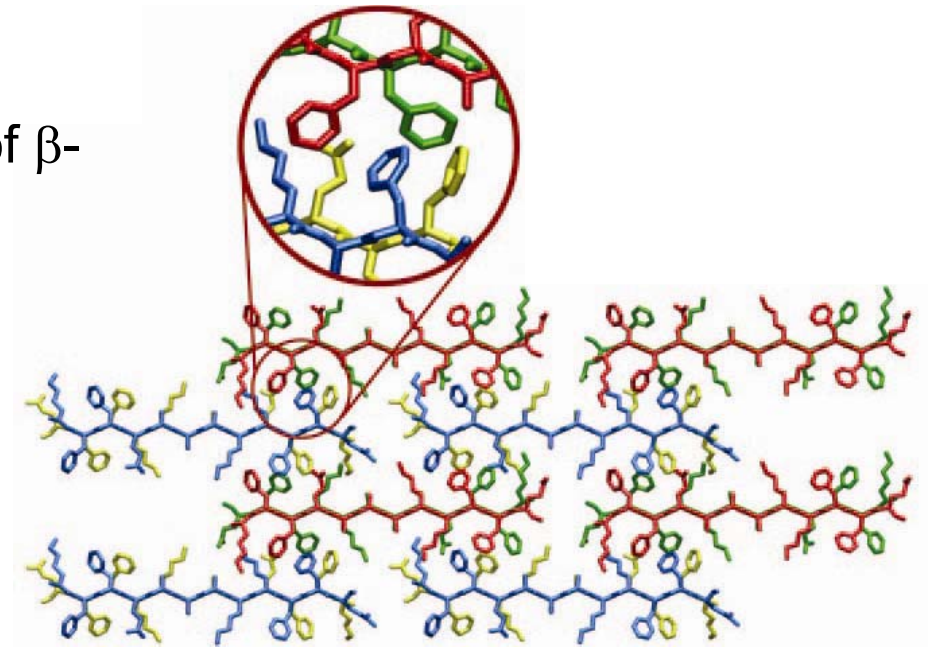


Amyloid fibril structures

First “real” conformation of peptides in fibrils
(Serpell, *PNAS* 2005)

3D structure of the designed **KFFEAAAKKFFE** peptide

- Antiparallel β-sheets
- Layered brick-like arrangement of β-sheets



Aβ peptides and Alzheimer's disease

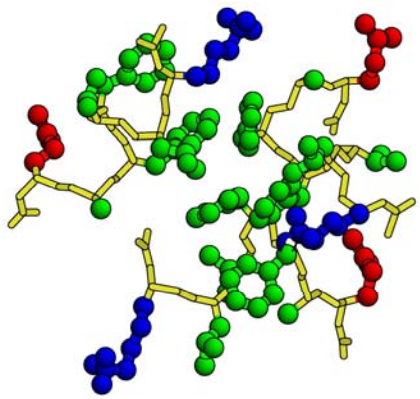
- Aβ peptides:
  - Natural product of cell proteolysis
  - Exist in a variety of lengths (39-42mers)
  - **Form amyloid fibrils**

$A\beta_{1\text{-}42}$ sequence

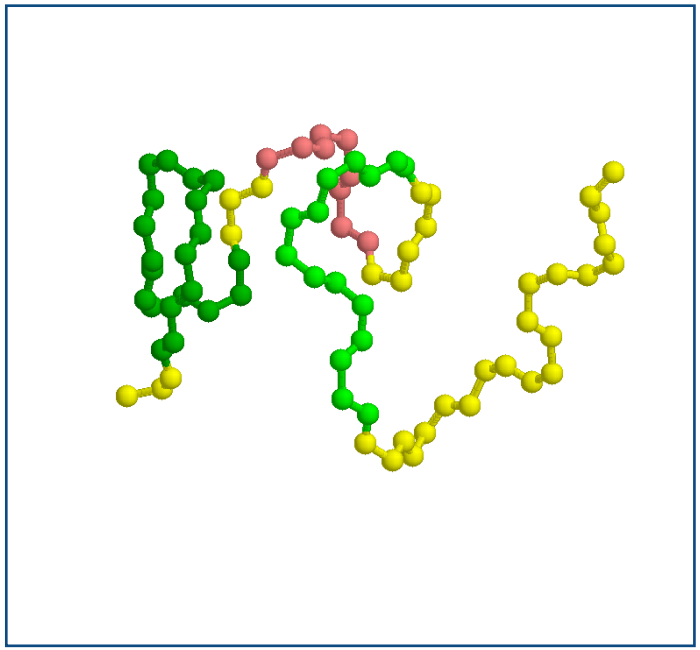
Hydrophilic N-terminal | CHC | Hydrophobic C-terminal

DAEFRHDSGYEVHHQKLVFFAEDVGSNKSAIIGLMVGGVVIA

- Aβ amyloid hypothesis for Alzheimer's disease (AD)
- Aβ oligomers are potent neurotoxins
- AD neurotoxic agent: Aβ fibrils or oligomers?
- Oligomers are possibly involved in other neurogenerative diseases



Assembly of Aβ amyloids



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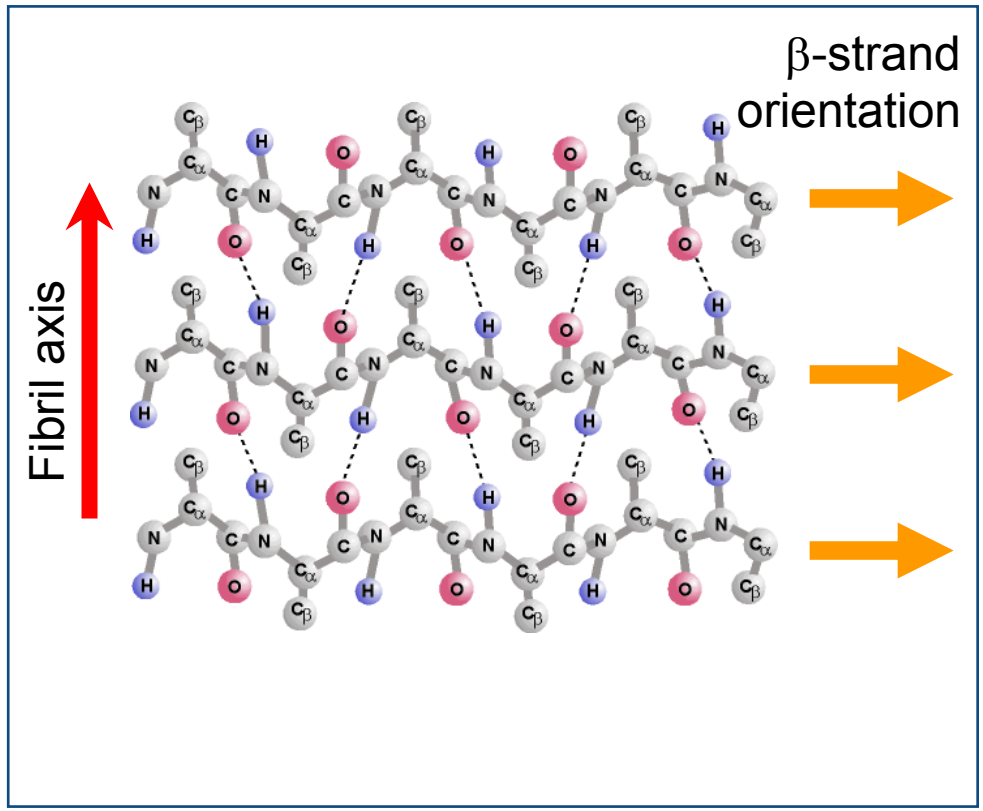
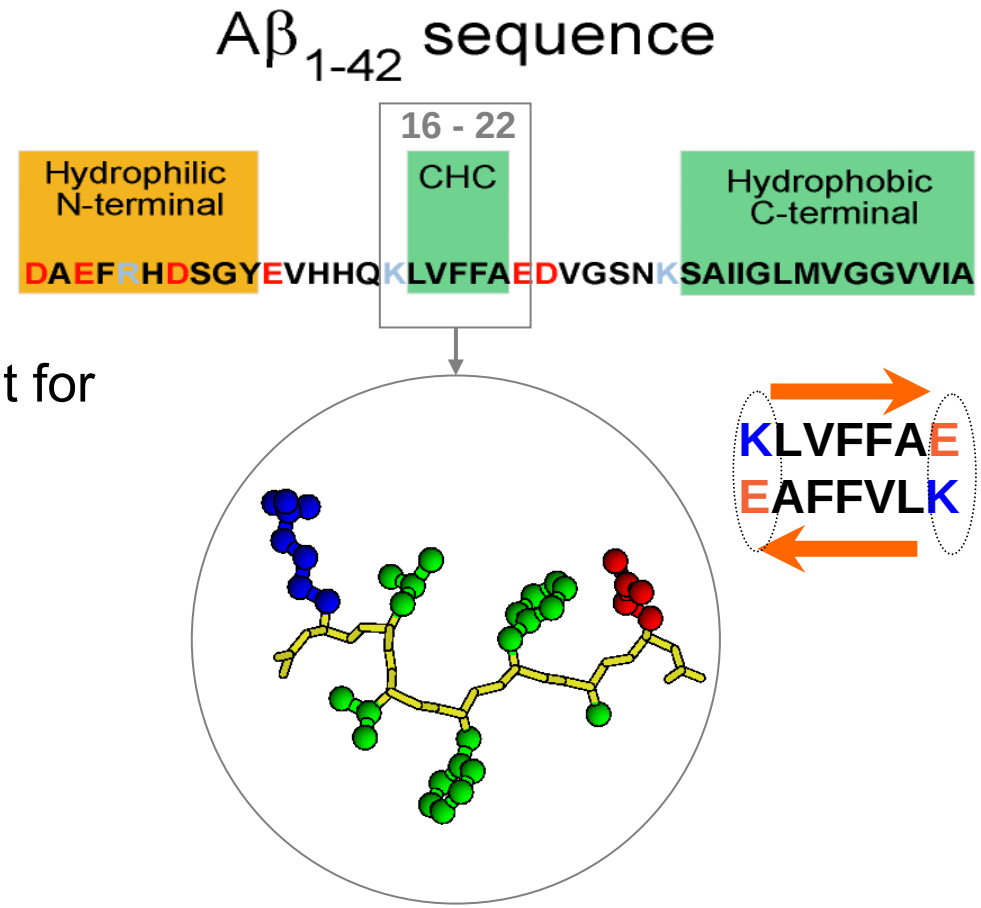


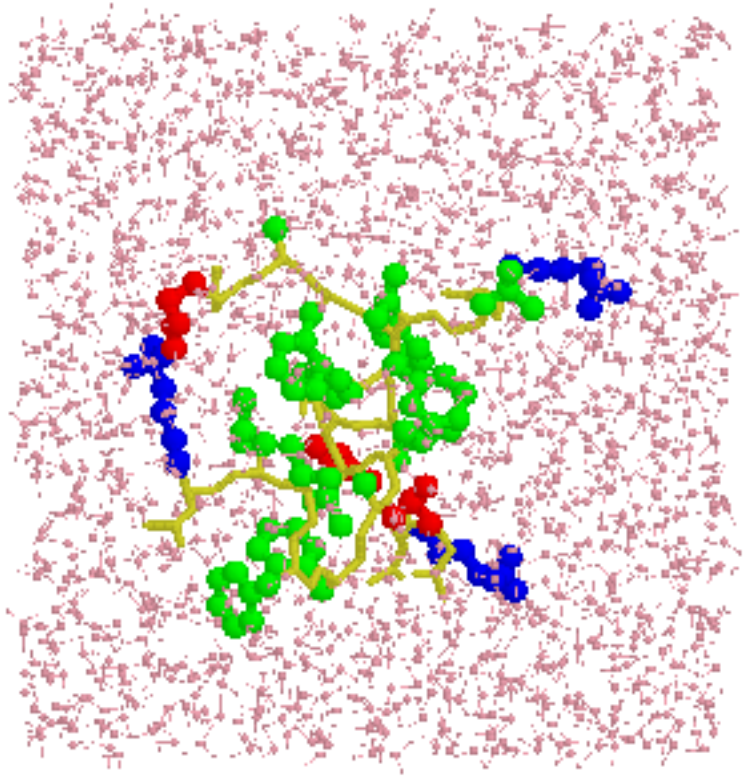
$A\beta_{16-22}$ peptide is a model amyloid system

- Form amyloid fibrils analyzed by solid state NMR (Tycko, NIH)
- Includes CHC, which is crucial for fibrillization of full-length peptides
- Brings in the interactions important for amyloid fibril formation
  - Hydrophobic
  - Electrostatic
  - Hydrogen bonding
- Small system amenable for MD



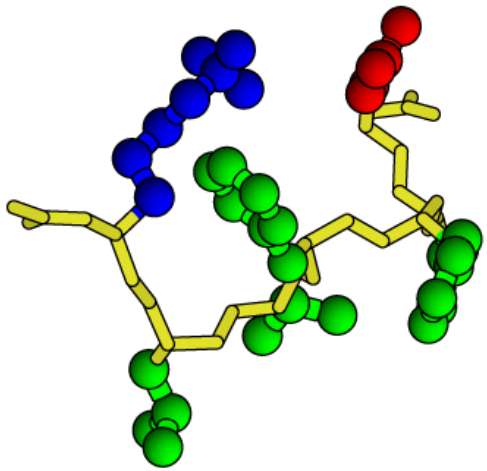
MD simulations of $A\beta_{16\text{-}22}$ oligomers

- OPLS all-atom representation of three $A\beta_{16\text{-}22}$ peptides in water(+urea) (~4,000 atoms in 35Å x 35Å x 35Å unit cell)
- Probe the kinetics of oligomer assembly through multiple NVE MD trajectories starting with *random* initial structures (>150 *ns* simulation time)



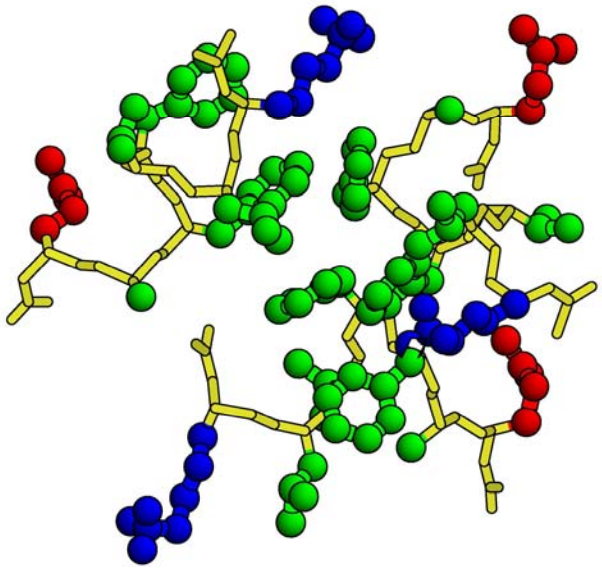
MD simulations of Aβ16-22 peptides in water

Aβ16-22 monomers adopt random coil structure in water



Turning on interpeptide interactions…

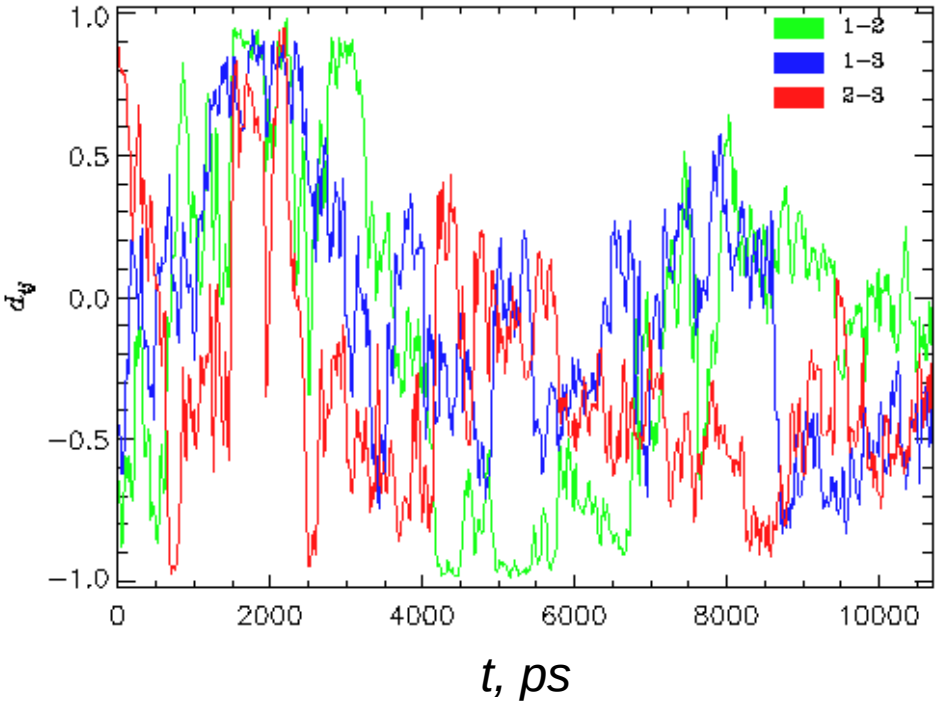
Disordered oligomer is stabilized by hydrophobic interactions



Interpeptide interactions drive an accumulation of β-structure

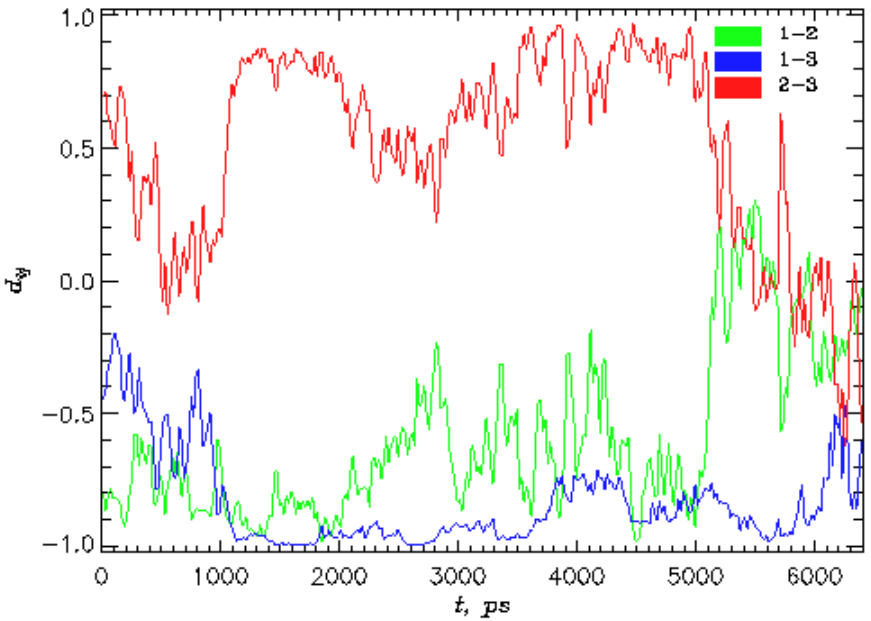
No order in peptides' orientations

$$d_{ij} = (\vec{r}_{1N,i}\vec{r}_{1N,j})$$

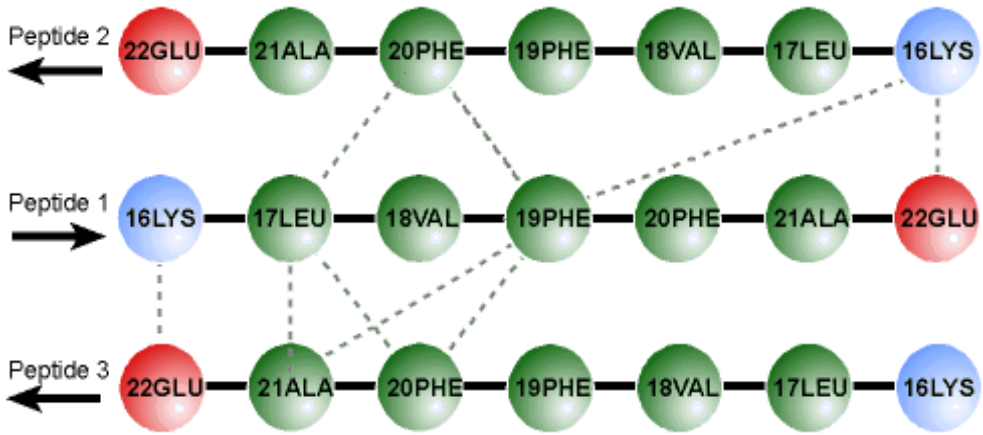


Assembly of ordered $A\beta_{16-22}$ oligomers in water

Antiparallel orientation of peptides in ***ordered oligomers***



Ordered oligomers are stabilized by *hydrophobic+electrostatic* side chain contacts



Structural ordering in Aβ16-22 oligomers resembles fibril organization within ~ 10 ns

Question and motivation

Question:

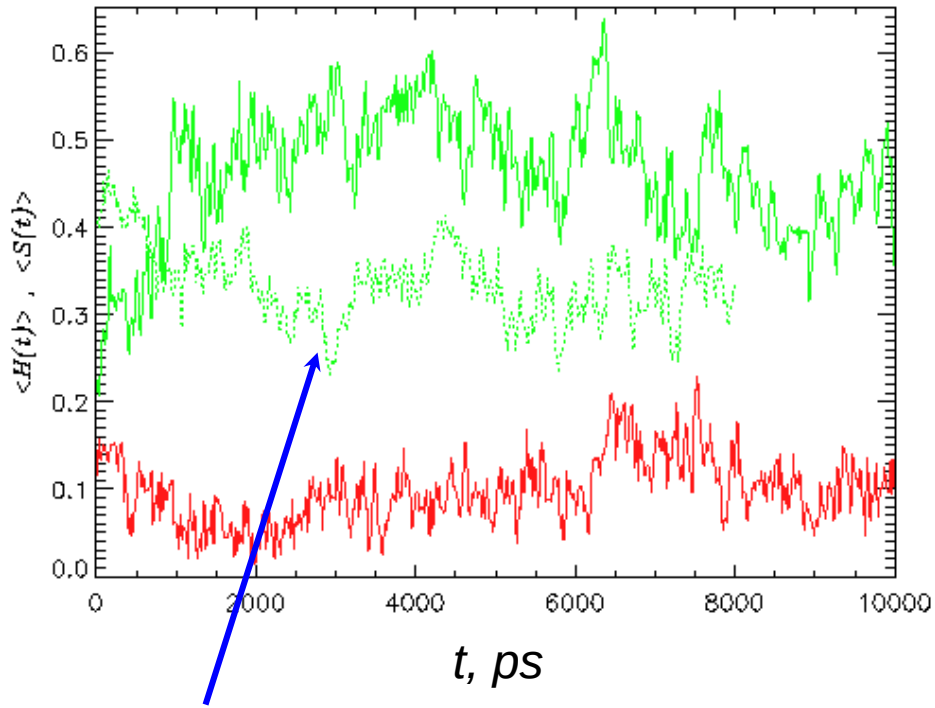
how do chemical denaturants affect oligomer assembly?

Motivation:

1. Tool for probing the mechanism of amyloid formation
2. Some amyloids (Ig light chain) are formed in the presence of urea
3. Implications for protein unfolding

$A\beta_{16-22}$ monomers in 8M urea

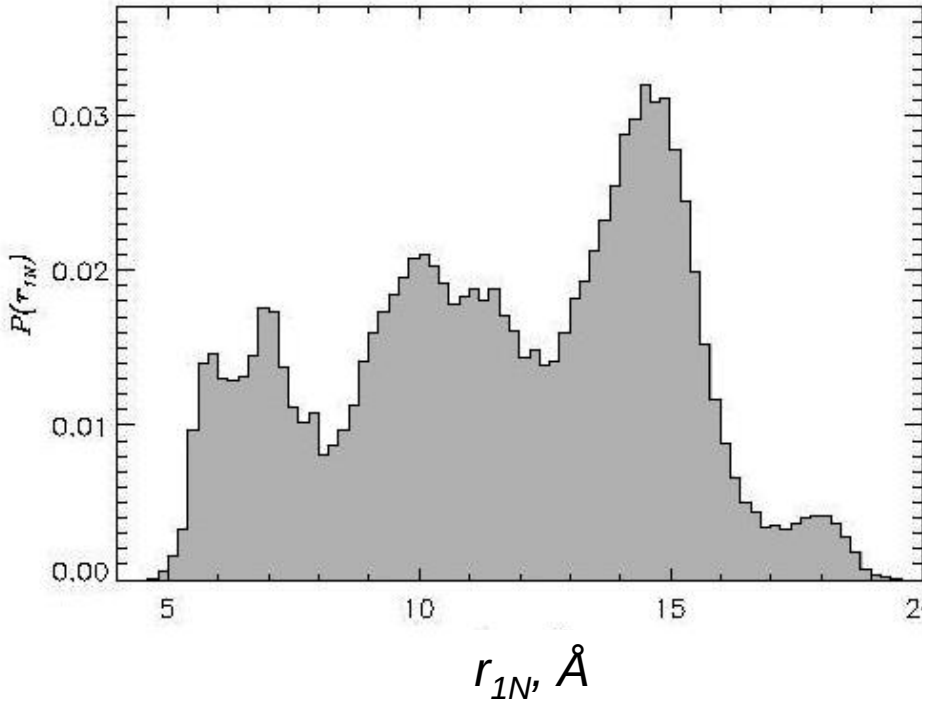
- Urea enhances β-propensity in $A\beta_{16-22}$ peptides
- Distribution of monomer conformational states in urea (water)
  - 46% random coil (68%)
  - 53% β-strand (29%)
  - negligible amount of α-helix
- Conformational properties are static on a timescale of 10 ns



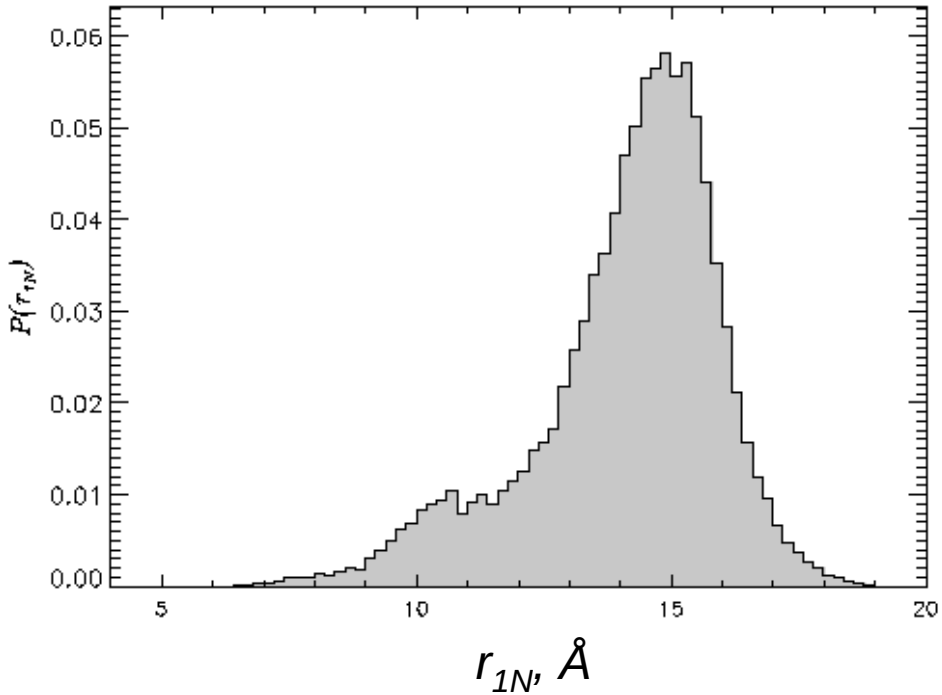
$A\beta_{16\text{-}22}$ monomers in 8M urea

Effect of urea on the distribution of end-to-end distance $P(r_{1N})$

water : $<r_{1N}>$=12 Å

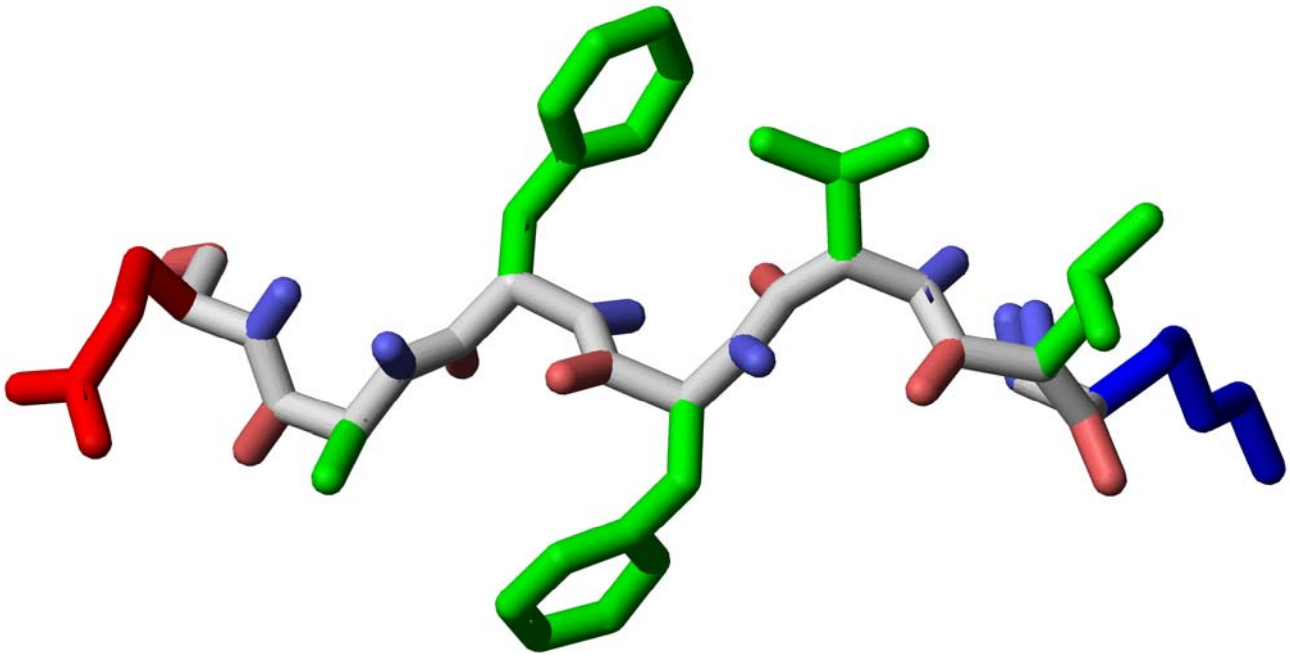


aqueous 8M urea : $<r_{1N}>$=14 Å



Aβ16-22 monomers in 8M urea

What are the interactions that might explain enhanced β-propensity?

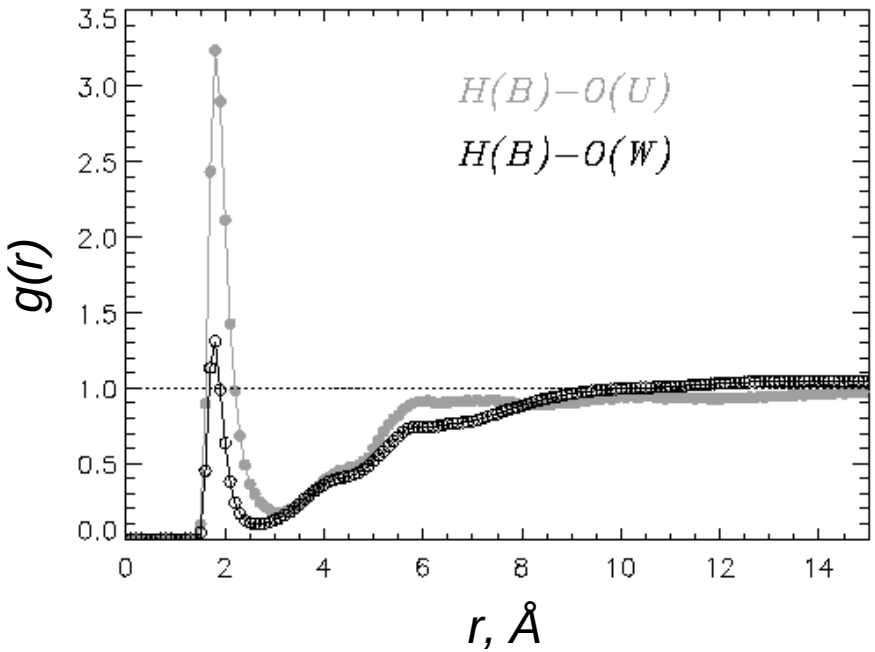


$A\beta_{16\text{-}22}$ monomers in 8M urea

Enhanced β-propensity is due to urea-backbone hydrogen bonding

Urea solvates backbone better than water

- Relative gain factor in [U] over [W] is 3.3
- Average residence times in FSS indicate rapid exchange of solvent molecules
  - $\langle\tau_U\rangle$ = 14.1 ps
  - $\langle\tau_w\rangle$ = 8.9 ps



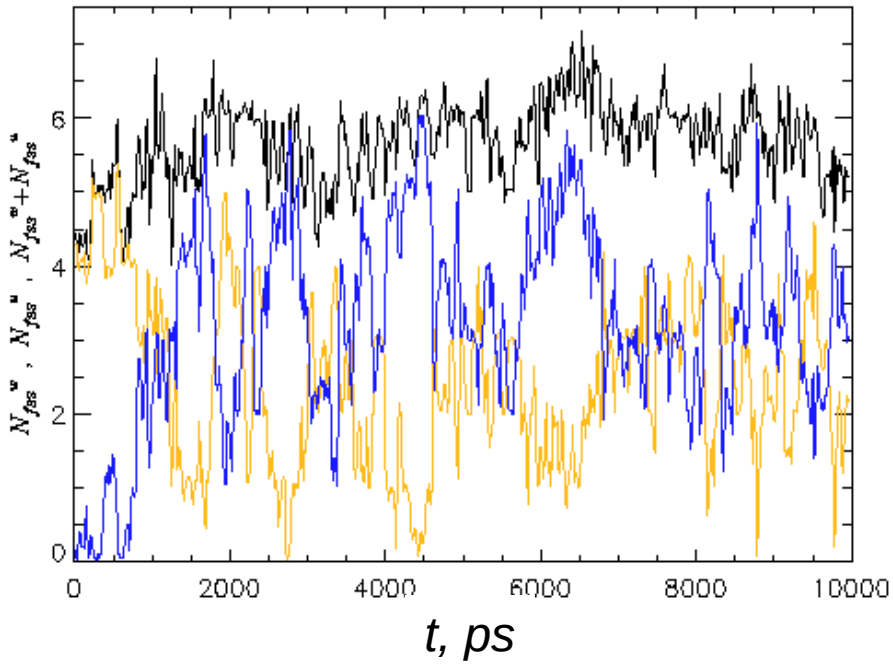
Backbone FSS: [W]/[U]≈1.5

$A\beta_{16\text{-}22}$ monomers in 8M urea

Enhanced β-propensity is due to urea-backbone hydrogen bonding

Solvation of backbone amide hydrogens:

- $\langle N_U \rangle + \langle N_W \rangle = 2.2 + 3.1 = 5.3$
- Urea cross-bridges backbone better than water



$A\beta_{16\text{-}22}$ monomers in 8M urea

*β-propensity is **not** caused by the solvation of side-chains*

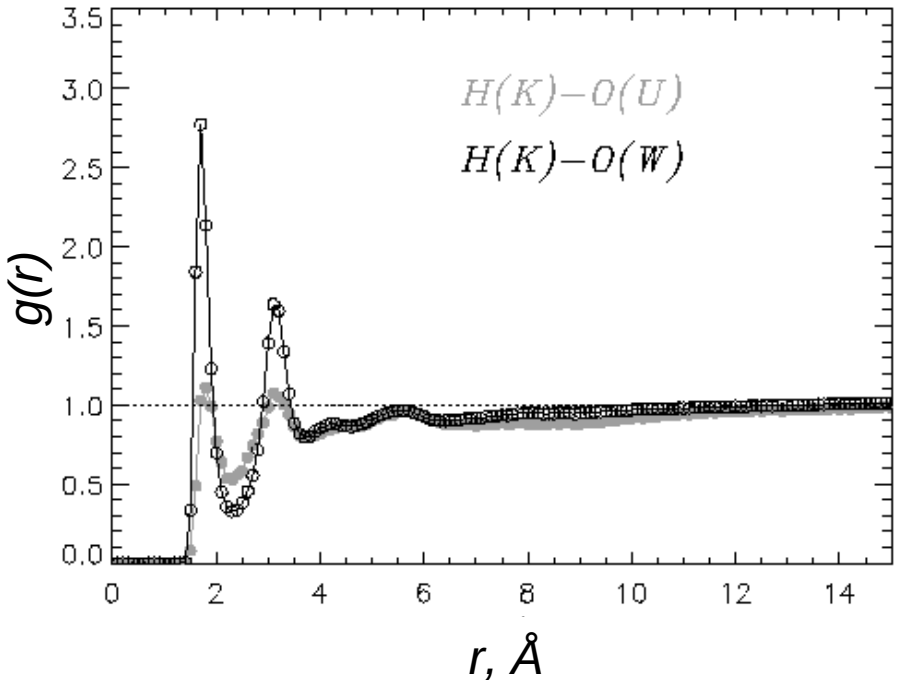
Water solvates *charged* side chains better than urea

Increase in [W]/[U]

H(K) FSS: 50%

O(E) FSS: 30%

Lysine

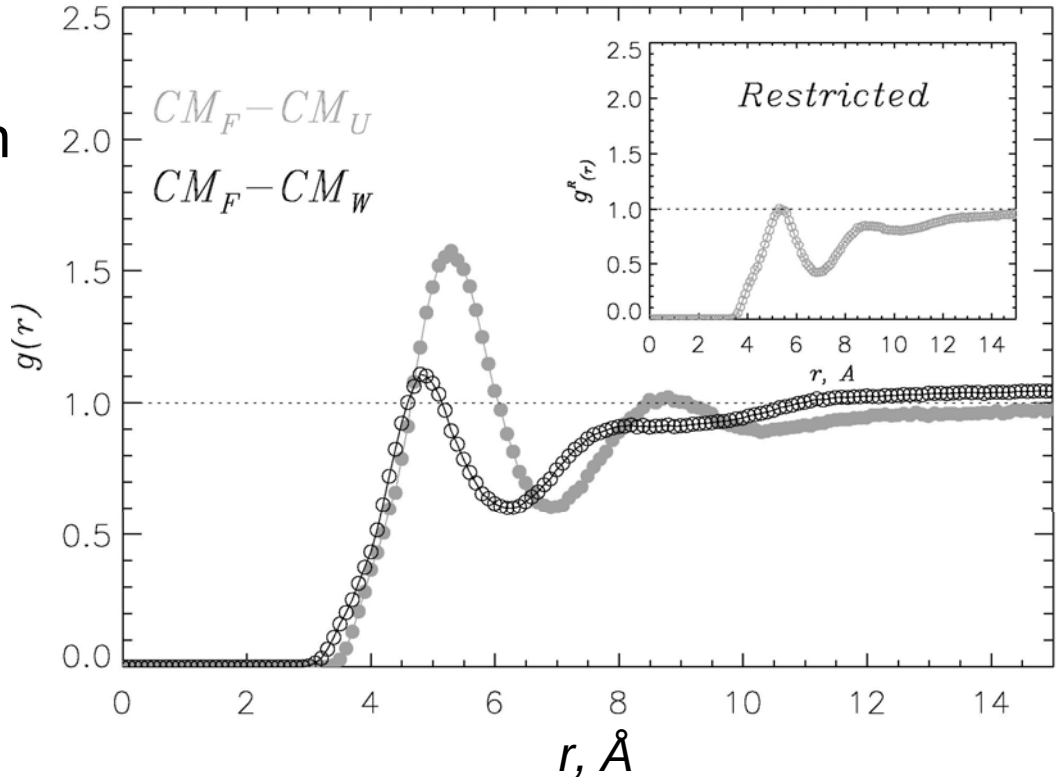


$A\beta_{16\text{-}22}$ monomers in 8M urea

β-propensity is **not** caused by the solvation of side chains

Urea - hydrophobic side chain interactions *are driven* by urea-backbone hydrogen bonding

Phenylalanine



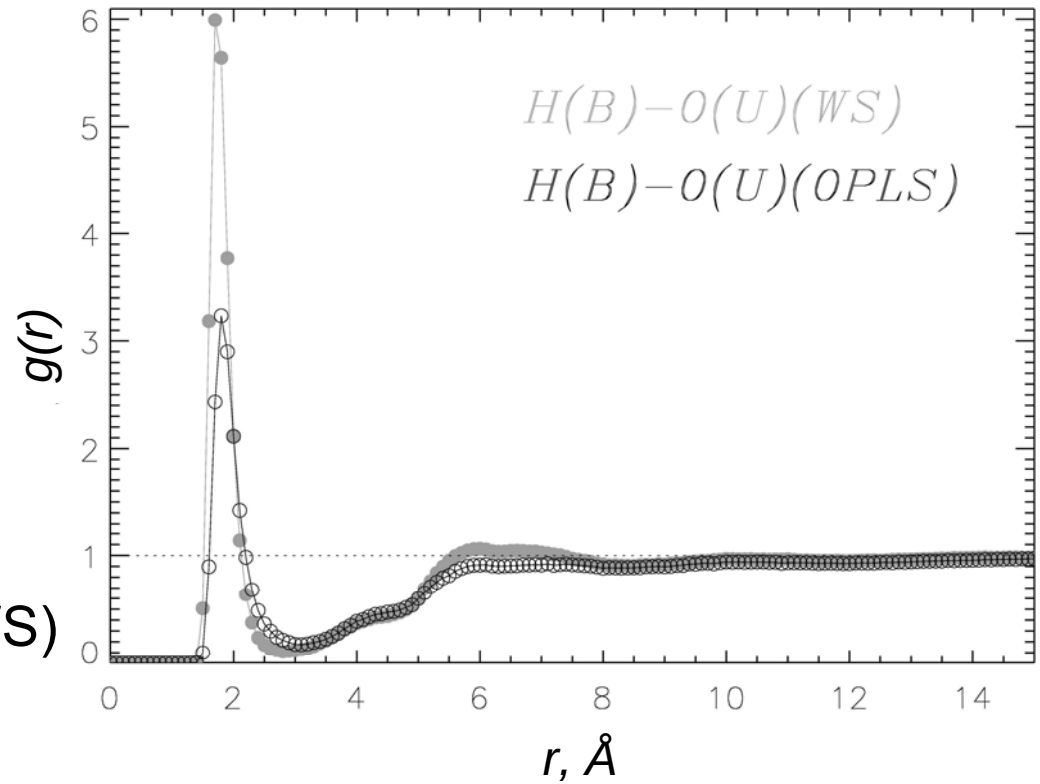
$A\beta_{16\text{-}22}$ monomers in 8M WS urea

Modifying urea partial charges (Weerasinghe and Smith (WS))

- O(U): - 0.390 → -0.675
- H(U): +0.333 → +0.285

Effect of WS potentials:

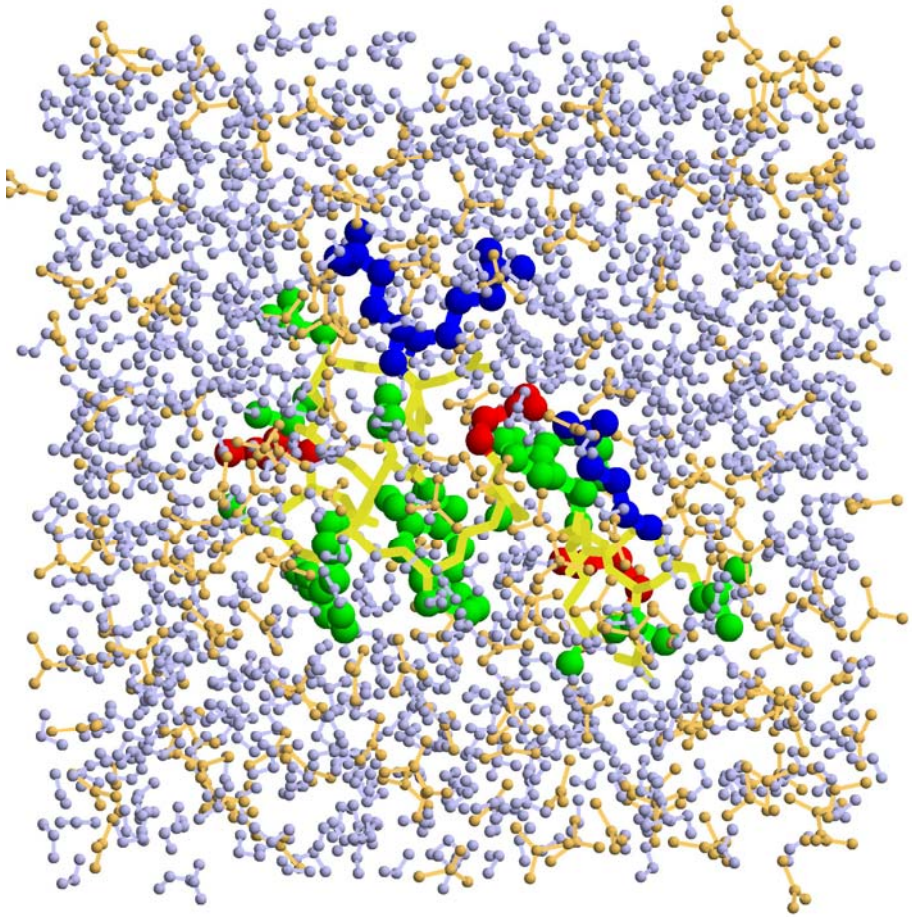
1. further enhance β-propensity
2. improve backbone solvation with urea
   - relative gain [U]/[W] = 5.5
   - $<\tau_U>$: 14 (OPLS) → 25 ps (WS)



Conclusion:

urea-induced structural changes are electrostatic in origin

$A\beta_{16\text{-}22}$ oligomer in aqueous 8M urea solution

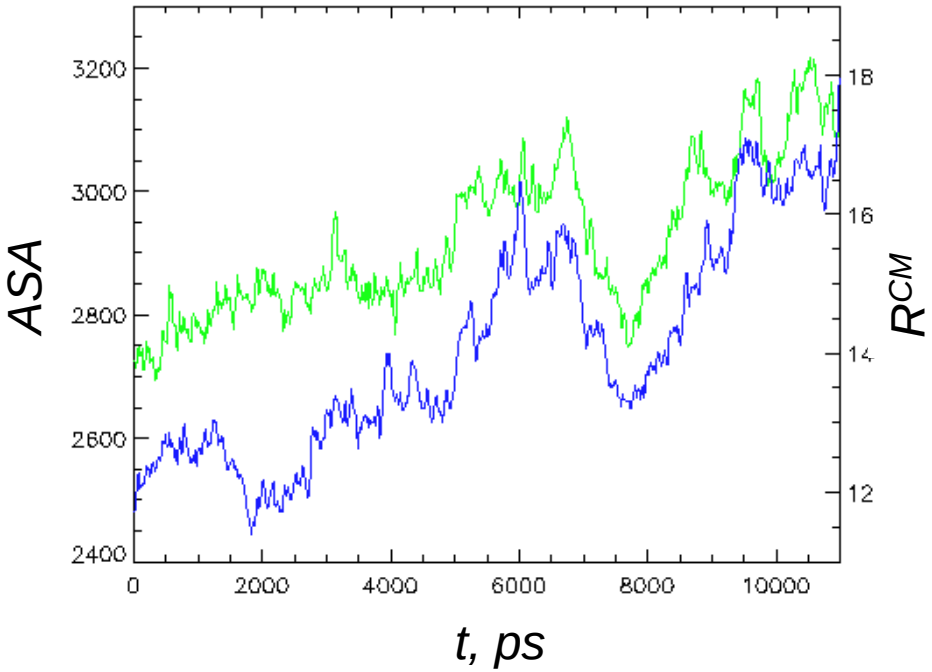


Molecular dynamics simulations of Alzheimer's Aβ peptides

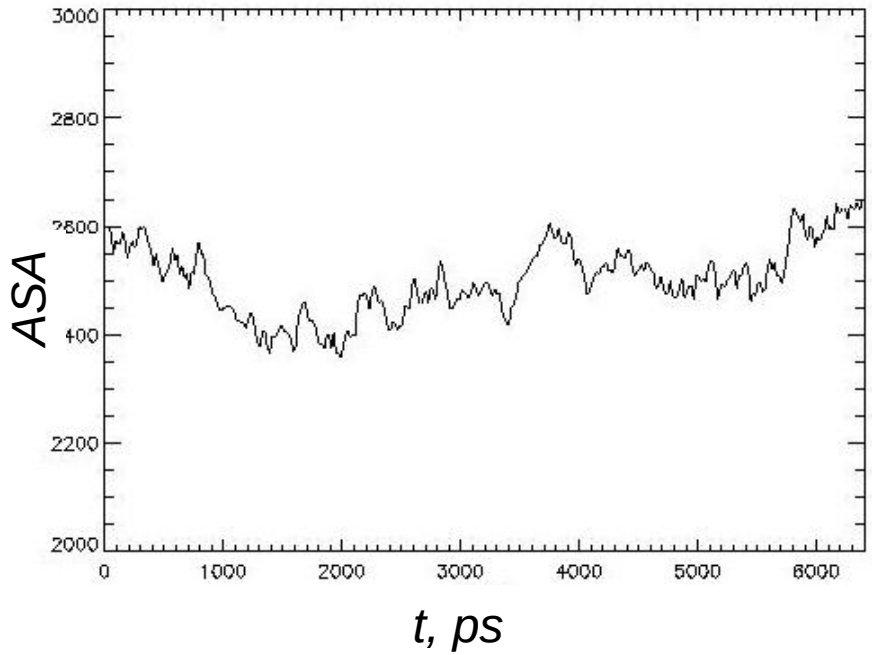
$A\beta_{16\text{-}22}$ oligomers in 8M urea

8M urea ***dissolves*** $A\beta_{16\text{-}22}$ oligomers

- <ASA>: 2700 →3200 A^2 in 11 ns
- $<R^{cm}(t)>$: a 50% increase

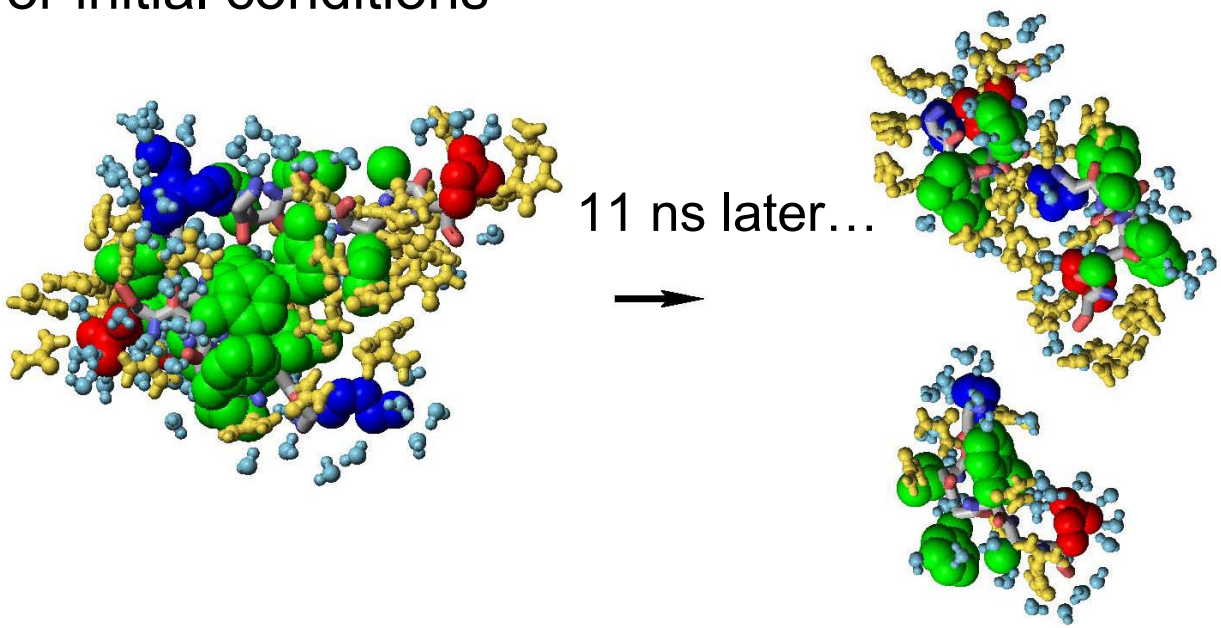


Comparison:
in pure water <ASA(t)>≈const



$A\beta_{16\text{-}22}$ oligomers in 8M urea

8M urea dissolves $A\beta_{16\text{-}22}$ oligomers ***irrespective*** of urea model or initial conditions

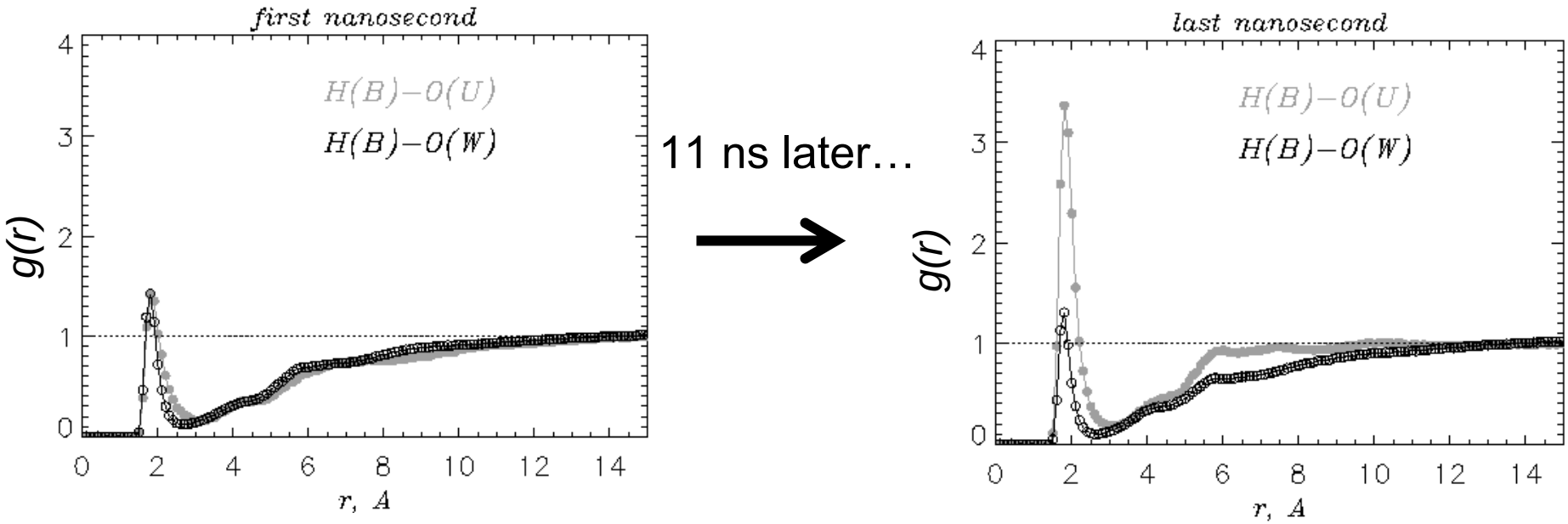


Oligomer is disrupted within 11ns

$A\beta_{16\text{-}22}$ oligomers in 8M urea

Dynamics of solvation of peptides' backbones:

Urea "invades" into $A\beta_{16\text{-}22}$ oligomers

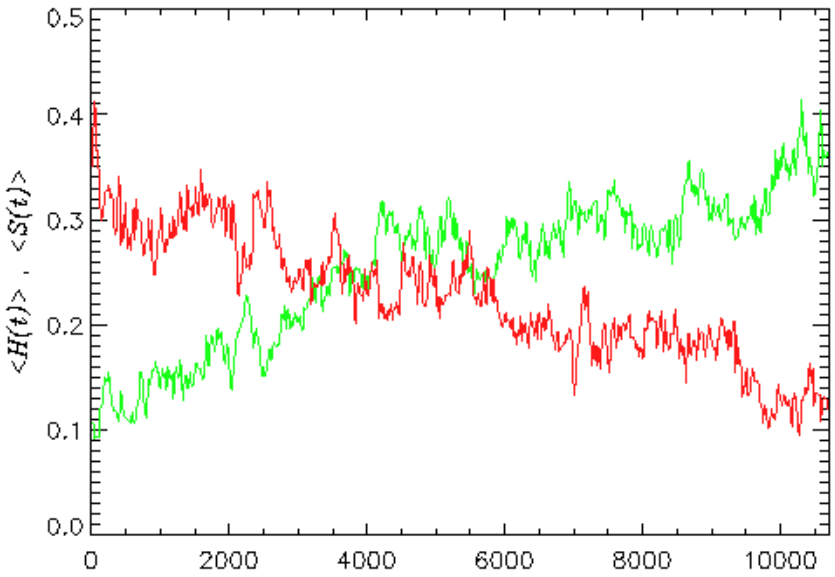


Result: urea covers hydrophobic residues ([U] increases by 50%)

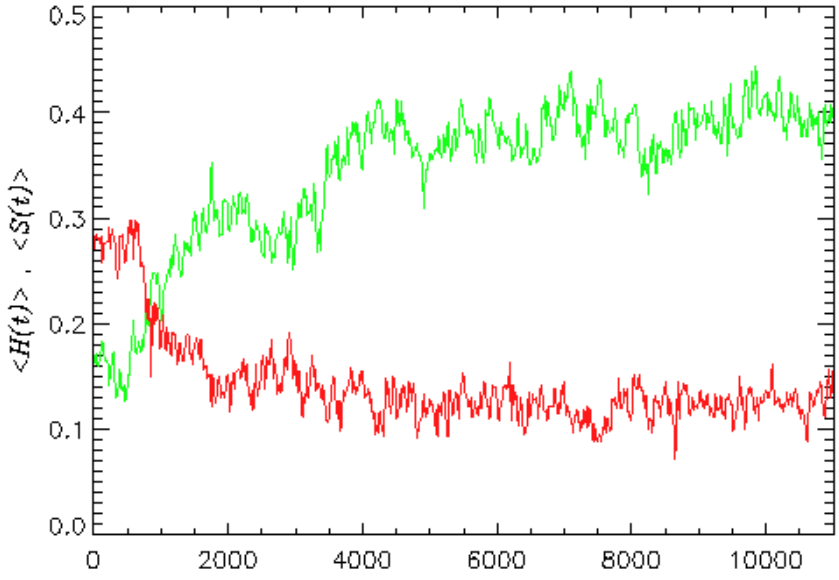
$A\beta_{16\text{-}22}$ oligomers in 8M urea

Urea ***accelerates*** α-helix →β-strand conformational transition

water



8M urea



Summary of urea effect on $A\beta_{16\text{-}22}$ oligomers

The impact of urea on $A\beta_{16\text{-}22}$ oligomers is two-fold:

- Destabilizes and disrupts $A\beta_{16\text{-}22}$ oligomers
- Accelerates and enhances β-structure formation

Through

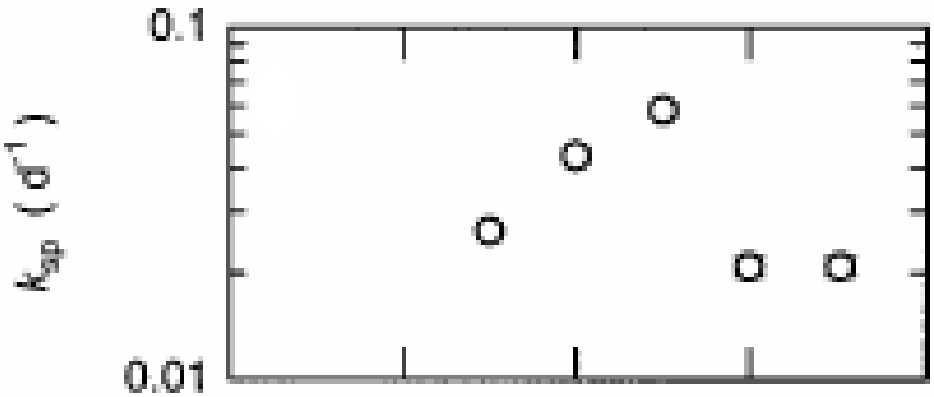
- Hydrogen bonding to backbone amides
- Disruption of hydrophobic interactions

Prediction:

1. High [U] is likely to block amyloid formation
2. Moderate [U] *may accelerate* amyloid assembly

Outlook

Experiments on β-lactoglobulin



Outlook

MD simulations of $A\beta_{16\text{-}22}$ peptides:

1. Consistent with experiments (*Prot. Sci*. **11**, 2417(2002))
2. Probes the mechanism of amyloid assembly
3. Applicable to other amyloidogenic polypeptides
4. Probes the mechanism of protein unfolding

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Talk to be presented at 2005 ACS meeting