Solvent and mutation effects on the nucleation of amyloid β-protein folding

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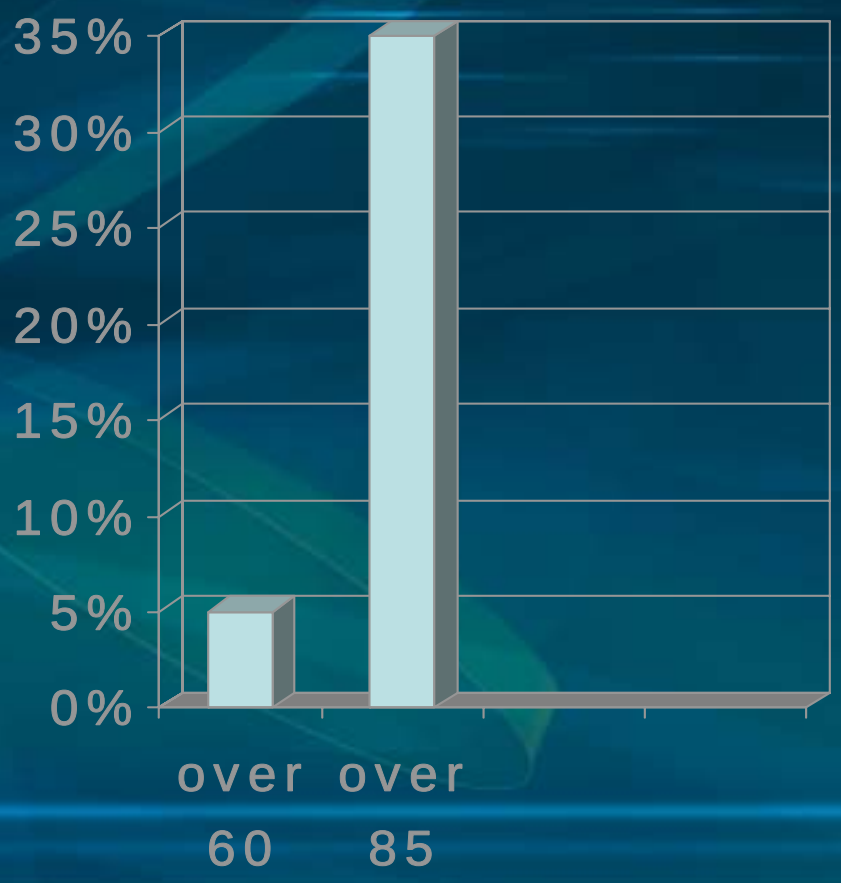
Background on Alzheimer's

- Progressive mental disorder
  - Confusion
  - Memory Failure
  - Disorientation
  - Restlessness
  - Agnosia
  - Speech disturbances
  - Inability to carry out purposeful movement

Understanding Alzheimer's

- Amyloid cascade hypothesis
  - the deposition of amyloid fibrils is the seminal event in the pathogenesis of Alzheimer's disease
  - Proposed in the 1990's



Amyloid β–protein Theory

- Developed around the millennium
- Studies suggest that amyloid β–protein could be responsible for Alzheimer's
  - Non-toxic in monomeric form
  - Inhibiting amyloid–protein polymerization into oligomers could prove to be an effective treatment
  - For this to occur there needs to be a better understanding of amyloid β–protein

Experimental difficulty

- Process is solvent dependent
  - Alpha-helical in ionic solution
  - Helixes and Beta sheets in aqueous solution

Stability and Folding

- Solution state NMR and diffusion ordered spectroscopy show variation in anionic strength in the buffer shifts equilibrium between monomer and oligomer
- Amino acid structure at specific sites influences ability to form oligomer
- Ile-41 – Ala-42 responsible for biophysical behaviors of $A\beta_{1–42}$ and $A\beta_{1–40}$
- Oxidation of Met-35 affects $A\beta_{1–40}$
- but not $A\beta_{1–42}$

Structural Basics

- A protease resistant segment has been found
  - Ala-21 –Ala30
  - Decapeptide shows same resistance
- A loop that is stabilized by hydrophobic interactions in the Val-24 –Lys28 region exists
- A high degree of flexibility in the termini
- Electrostatic interactions between the charged groups
  - Glu-22, Asp-23, and Lys-28 that modulate the stability of the folded structure

Purposes of the experiment

- Test whether the stability of the Val-24 –Lys-28 loop persists in all simulations
- To determine the effects of solvent alterations on the folding dynamics
- To investigate changes in dynamics caused by amino acid substitutions
- Study the dynamics of a monomer with a specific mutation

Molecular Dynamic Simulations

- Long-time MD simulations of monomer
  - In water at normal and reduced density
  - Normal density with dissociated salt ions
  - Mutated in normal density
- All atoms with potential energy given by CHARMM-27 force field were considered
- TIP3P model for water molecules
- Same temperature as *in vitro* tests

Molecular simulations cont'd

- Solvated each monomer
  - 43 angstrom cube of water
  - Around 2500 water molecules
- 25 dissociated molecules of NaCl put in resulting in a system of around 2400 water molecules

Structural Determinants

- 2 quantities used to characterize the structure of protein
  - Distance between the two alpha Carbon atoms of Ala-21 and Ala-30
  - The radius of gyration

$$R_g^2 = \sum_i m_i(|\vec{r}_i - \vec{r}_c|)^2 \Big/ \sum_i m_i,$$

Results

- Distances found to be fluctuating
- Events lasted for extended periods of time with small fluctuations are seen

Table 1. Total accumulated times of events in each of the five trajectories

	Trajectory				
Time/event	[RC]	[P1]	[P2]	[DU]	[RCS]
Total time, ns	102.6	65.0	83.6	80.0	145.0
Events, ns					
*S**	37.1	45.9	27.0	12.6	34.0
*R** (4,8)	35.2	46.6	21.4	9.6	30.8
*R** (2,8)	7.6	8.1	5.9	0.4	19.0
*R** (3,8)	5.9	14.7	1.7	1.7	18.0

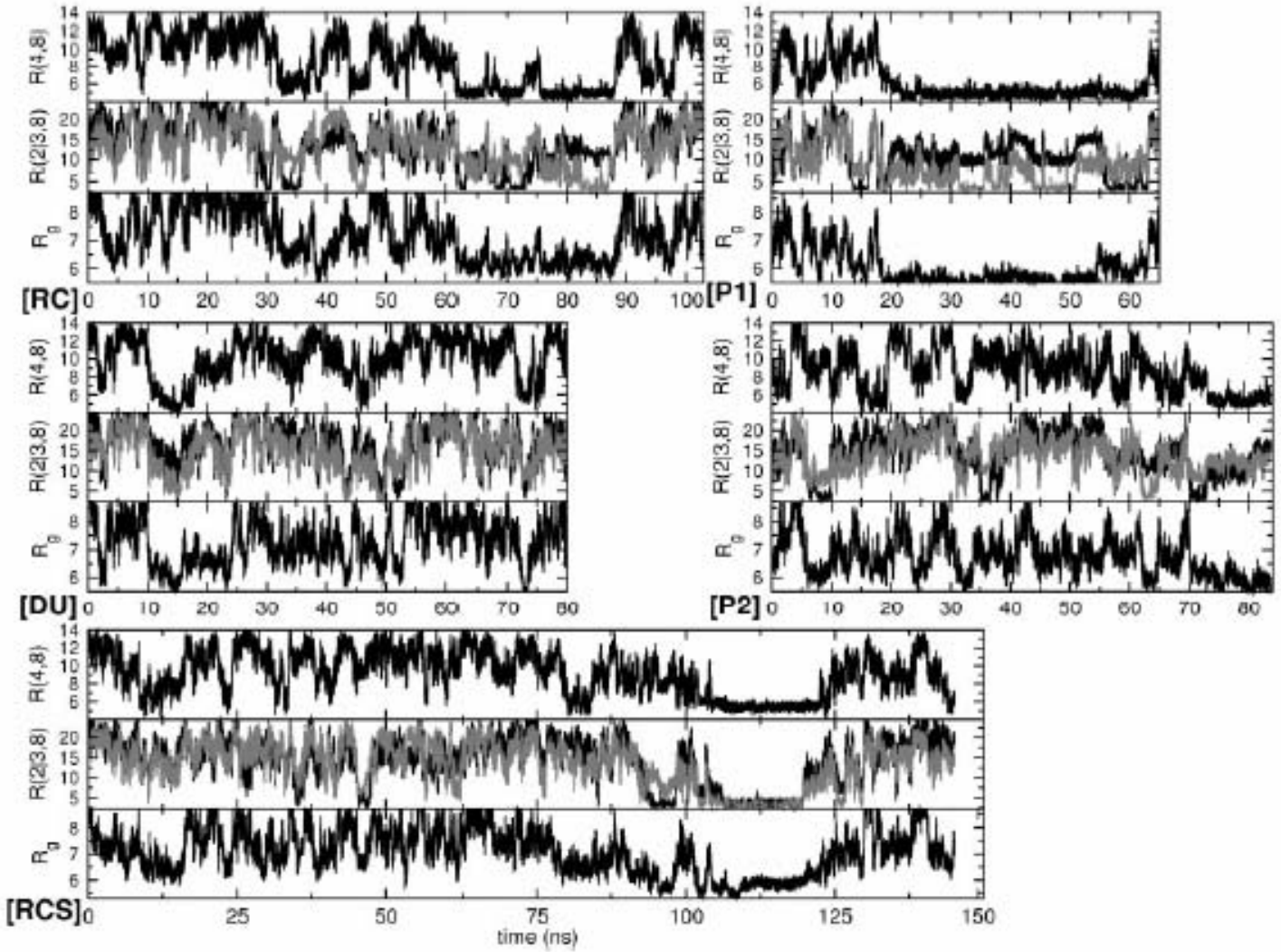


Fig. 1. Distances between C_α atoms of Val-24 and Lys-28 [R(4,8)], charged atoms of Glu-22–Lys-28 [R(2,8), black], Asp-23–Lys-28 [R(3,8), gray], and radius of gyration R_g as a function of time for each trajectory. All of the distances are measured in Å.

Temporal overlap

- Each overlap divided into 2 columns
  - One for each of the events
- The first overlap column shows that S* and R* (4,8) are correlated
  - Proximity of Val-24 and Lys-28 linked to hydrophobic interaction

Table 2. Percentage of overlap between pairs of events per trajectory

	Overlaps							
	$S^* \cap R^*$ (4,8)		R^* (4,8) ∩ SB		R^* (4,8) ∩ R_g^*		R^* (2,8) ∩ R^* (3,8)	
Trajectory	S^*	R^* (4,8)	R^* (4,8)	SB	R^* (4,8)	R_g^*	R^* (2,8)	R^* (3,8)
[RC]	91.9	96.7	34.9	90.8	72.3	79.7	0.0	0.0
[P1]	97.1	95.6	40.6	84.9	95.9	85.8	5.3	2.9
[P2]	76.0	95.8	1.2	3.4	73.9	45.7	0.0	0.0
[DU]	72.6	95.7	4.3	19.7	68.1	41.3	5.7	1.4
[RCS]	85.7	94.6	54.1	70.4	81.1	60.2	70.1	73.9

Secondary Structure

- The pi-helix correlates with with lowered values of all the R(2,8) R(3,8) and R(4,8) distances
  - Similar correlation found during helix formation
  - Helices formed in both [P1] and [P2] formed under a pre-existing R*(4,8) event

Discussion and conclusions

- For five trajectories hydrophobic events predominate over electrostatic events
  - Hydrophobic caused by packing of isopropyl groups
  - Highly correlated with smaller value for radius of gyration
- Loop is formed in reduced density water
- In normal density water SBs play a prominent role in stabilization of loop