Aggregation & mechanical unfolding of proteins

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Outline

- Simple all-atom model for protein simulations
- Aggregation of a segment of Alzheimers Aβ peptide
- Mechanical unfolding of a fibronectin type III module

Protein model

- All atoms
- Only torsional degrees of freedom
- Implicit solvent
- Four main energy terms:

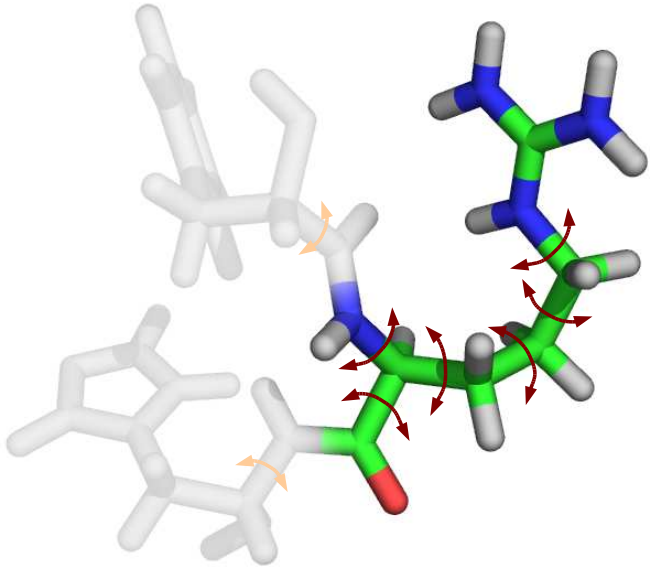
$$E = E_{\mathrm{loc}} + E_{\mathrm{exv}} + E_{\mathrm{hb}} + E_{\mathrm{sc}}$$

E_{loc}: Local electrostatic interaction between backbone dipoles

E_{exv}: Excluded volume, $1/r^{12}$ repulsion

E_{hb}: Hydrogen bonds

E_{sc}: Effective side-chain interaction, two parts: hydrophobicity and charged side chains



Protein model

Parameters calibrated by comparing to folding experiments

Latest version agrees reproduces experimentally obtained data on structure and thermodynamics of 15–20 different peptides, using one and the same parameter set

Projects:

- Mechanical unfolding
- Aggregation
- Properties of semiconductor-binding peptides
- Folding of peptides and proteins
- Available as free software, PROFASI: http://cbbp.thep.lu.se/activities/profasi/

Amyloid aggregation – Experimental background

- Aggregation of proteins linked to many diseases, e g Alzheimer's, Parkinson's & Huntington's
- Aggregation $\rightarrow$ amyloid fibrils $\rightarrow$ plaques in the brain
- Small oligomers important pathogenic agent? One proposed mechanism: ion-specific channels in neuronal cell membranes
- Oligomers transient species in vitro, difficult to characterize experimentally
- Certain fragments of these proteins share many characteristics with the full-size proteins

Aggregation – Simulations

We perform Monte Carlo simulations on a 7-residue fibril-forming peptide derived from Alzheimer's Aβ-peptide, Aβ(16–22)

Sequence: K LV F F A E

Important segment: experiments have shown that substitution of either of the phenylalanines in the full molecule reduces aggregation propensity dramatically

Simulations:

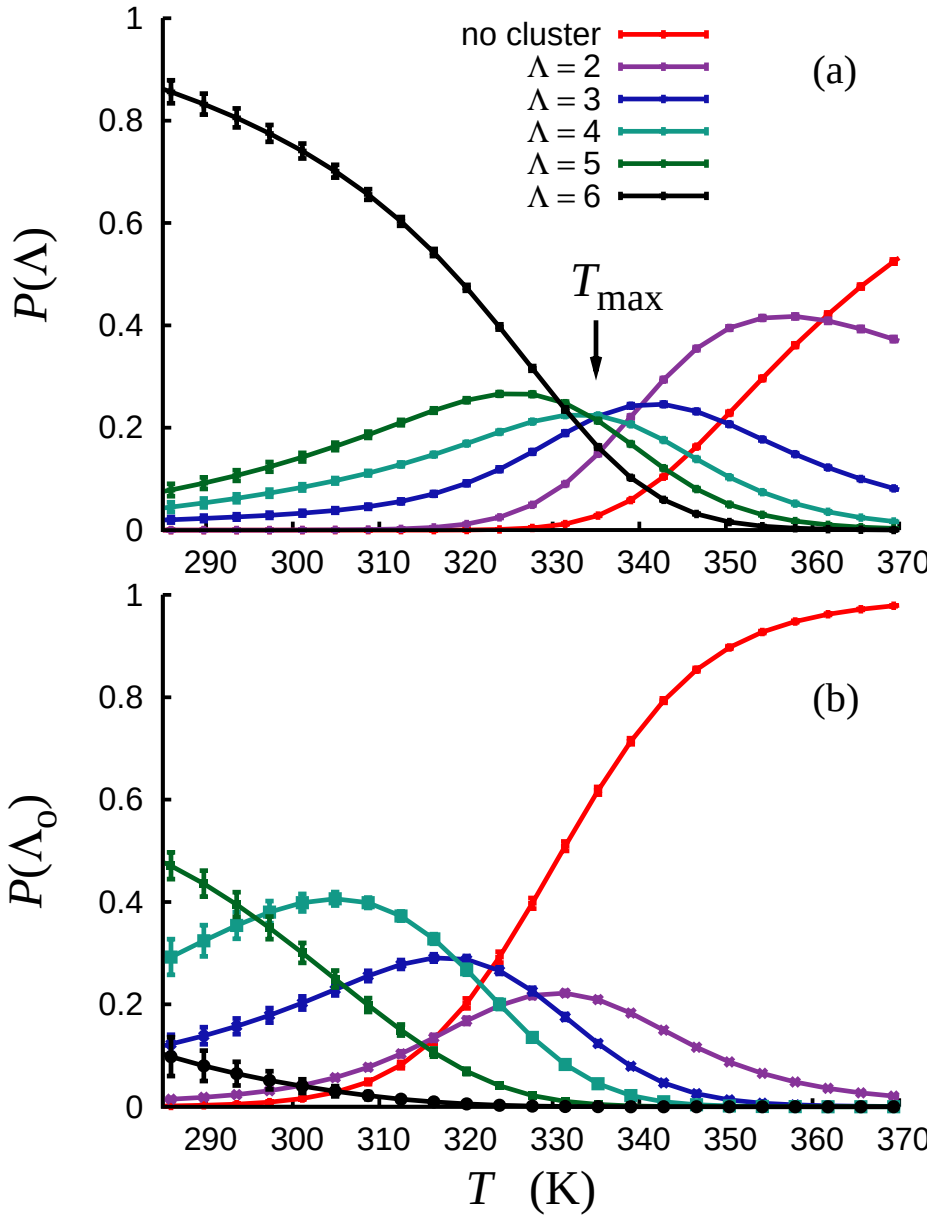
- Six chains in a periodic box, 50 Å
- Simulated tempering, temperature range 290 – 370 K
- Random starting configurations

Aggregate size analysis

Measure probabilities of different peptide-cluster sizes

- Λ: size of largest cluster
- Λ_o: size of largest *ordered* (β-sheet) cluster
- T_{max}: temperature at specific heat maximum

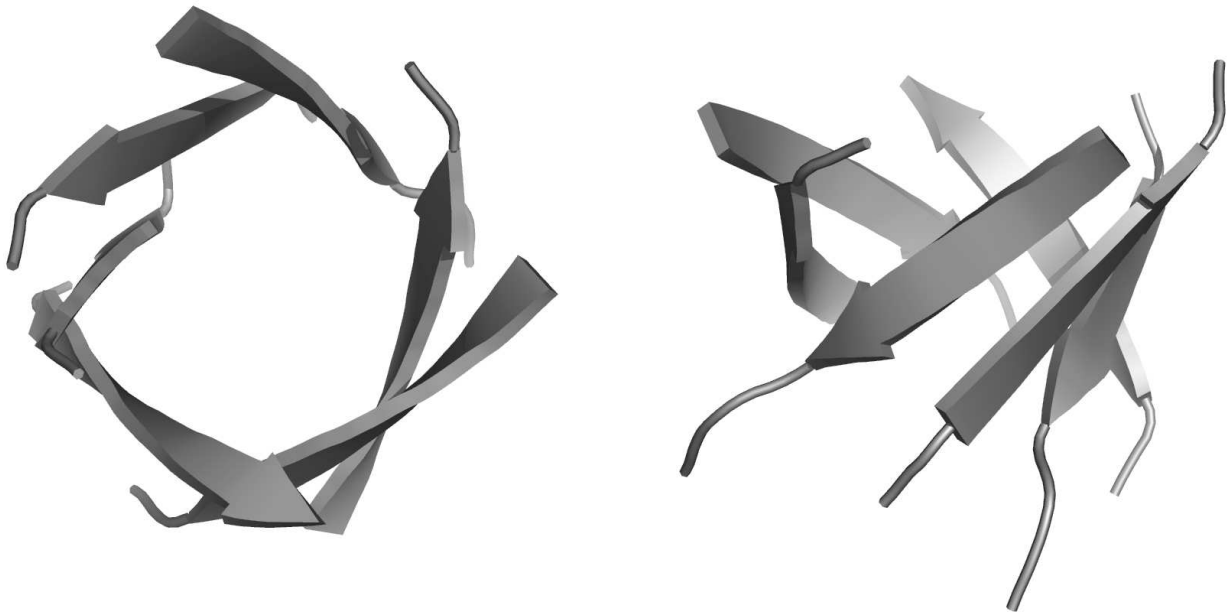
- $T > T_{max}$: Small clusters or no clusters
- $T \approx T_{max}$: Clusters of all sizes, only small ordered clusters
- $T < T_{max}$: Large β-rich clusters



Aggregation – Barrel formation

The most stable structure seen in our simulations was a β-barrel that formed spontaneously in one of our runs

- Structure geometrically similar to barrels in proteins
- Barrels are interesting candidates for ion-channels



Mechanical unfolding – fibronectin

- Fibronectin: huge protein, converts chemical signals to mechanical signals (and vice versa) at interface between extra-cellular matrix and membrane
- Consists of many independently folding domains
- Single-molecule mechanical unfolding experiments have shown that one of the domains, $FnIII_{10}$, often unfolds via a semi-stable intermediate
- Previous simulations found intermediates, but of different kinds, only a few unfolding trajectories were analyzed
- Unfolding pathways and intermediates are relevant: $FnIII_{10}$ has interaction sites buried within the folded protein

Mechanical unfolding – simulations

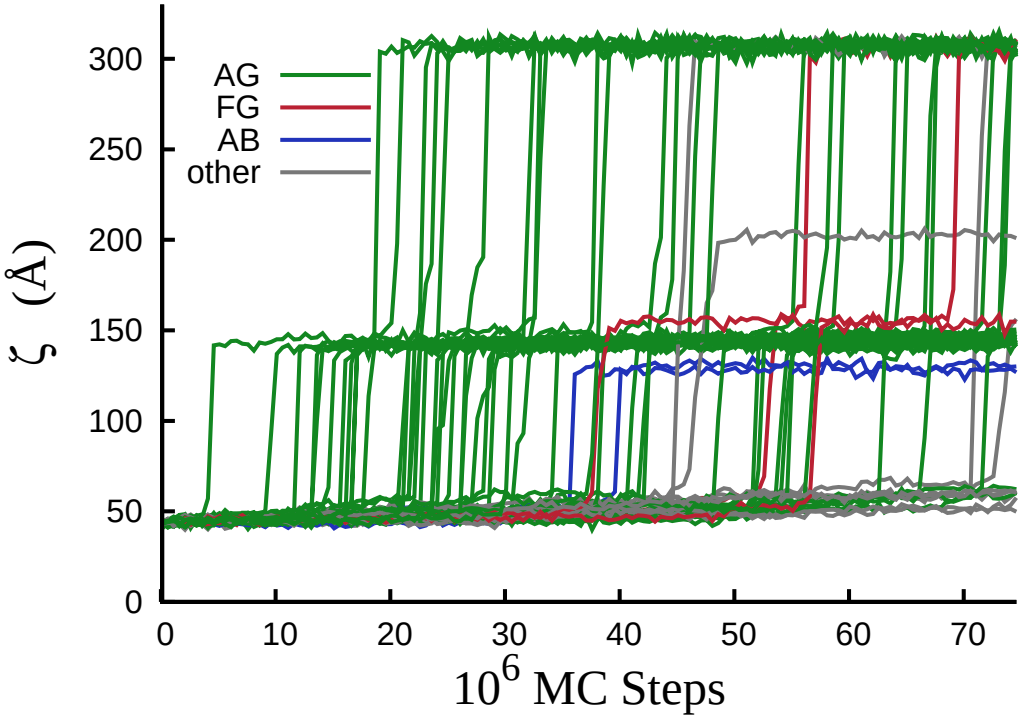
We perform two types of mechanical unfolding simulations

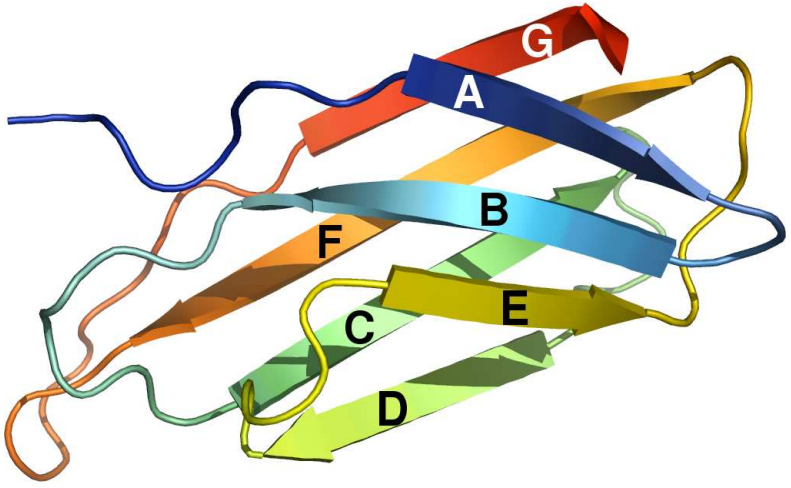
- pulling at both ends of the molecule with a constant force
- pulling at one end with constant velocity, in each MC step a pulling device (spring) is moved a microscopic step

Constant velocity simulations allow measurement of free energy using the Jarzynski relation

Constant force unfolding – preliminary results

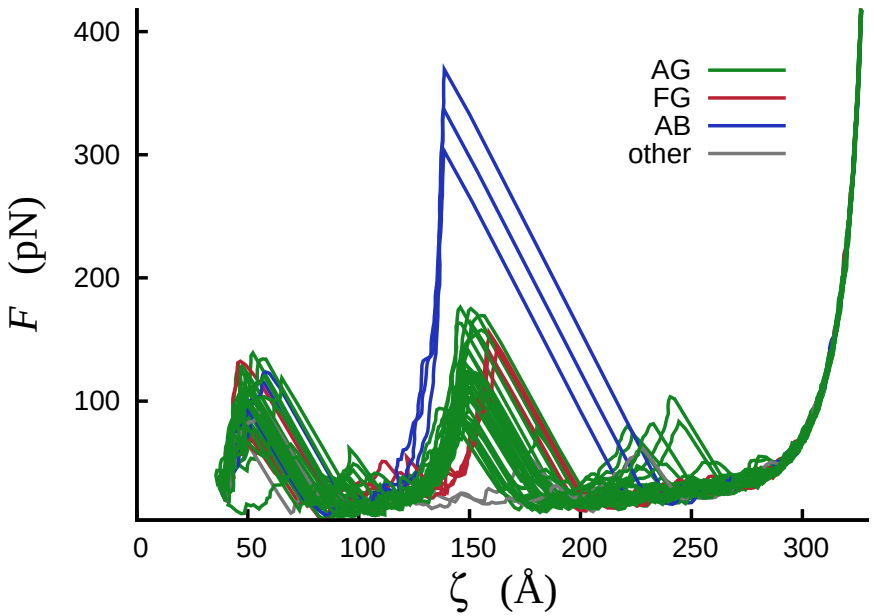
- Three distinct unfolding pathways, different intermediates
- The intermediates differ slightly in extension
- One intermediate is more stable than the others
- Relative frequencies of intermediates are force-dependent

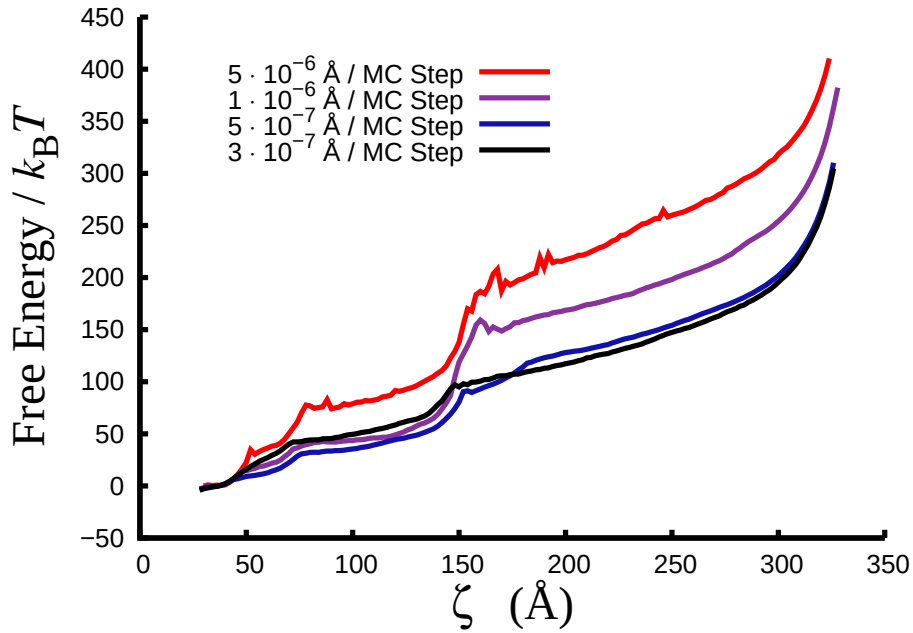




Constant velocity pulling – preliminary results

- Same three unfolding pathways (and intermediates)
- Frequency of pathways speed-dependent
- Free energy from Jarzynski-relation seems to converge at slow velocities





Thank you for your attention

Model: A. Irbäck and S. Mohanty, *Biophysical Journal* 88, 1560-1569 (2005)

Aggregation: A. Irbäck and S. Mitternacht, *Proteins* DOI: 10.1002/prot.21682 (2007)

Mechanical unfolding: work in progress

Software: http://cbbp.thep.lu.se/activities/profasi/