Lund, October 2013

Modeling protein aggregation: from sticks to atomic representation

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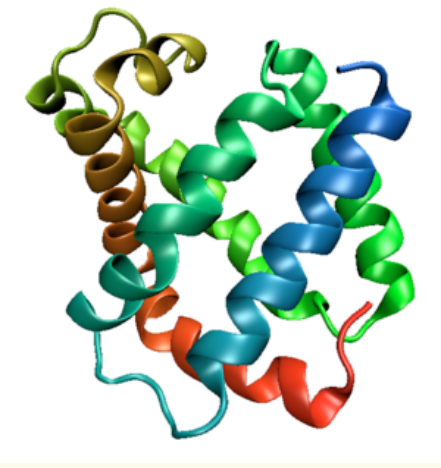
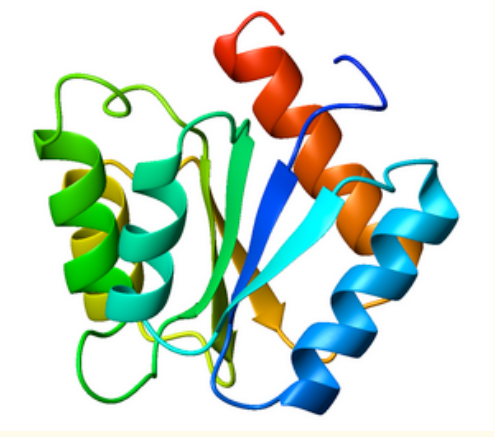
Computational Biology and Biological Physics

Protein structure

- Exponentially many possible states
- Unique “native” state
- Native structure by X-ray crystallography or NMR
- Local structure: α-helices and β-sheets





Protein folding

- Protein with 100 amino acids, 3 states per amino acid
  $\longrightarrow$ $3^{100} \sim 10^{47}$ possible structures
- Levinthal's paradox: a random search for the native state would take the protein longer than the age of the universe
- Forces driving folding: H bonding, hydrophobic attraction,...

Do we have

(i) sufficient knowledge of the driving forces, and

(ii) sufficiently fast computers

to be able to simulate the folding of a 100-amino acid protein?

Folding of the Top7 protein

- Computationally designed & experimentally verified protein with >90 amino acids and a novel fold
  Kuhlman et al. Science 2003;302:1364-1368
- Folding simulations started from random initial conditions. Atomic protein representation, implicit water. General force field, Monte Carlo methods
  Mohanty et al. Proteins 2013;81:1446-1456

Simulated structure and crystal structure

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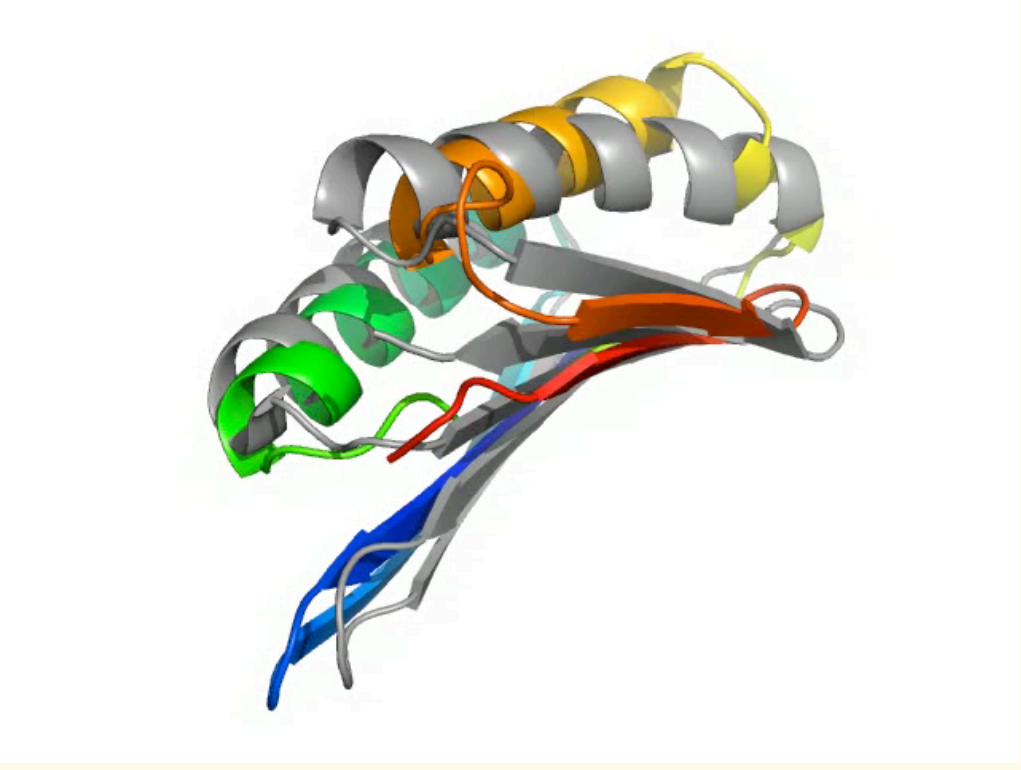

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Folding of Top7

Mohanty et al. Proteins 2013;81:1446-1456

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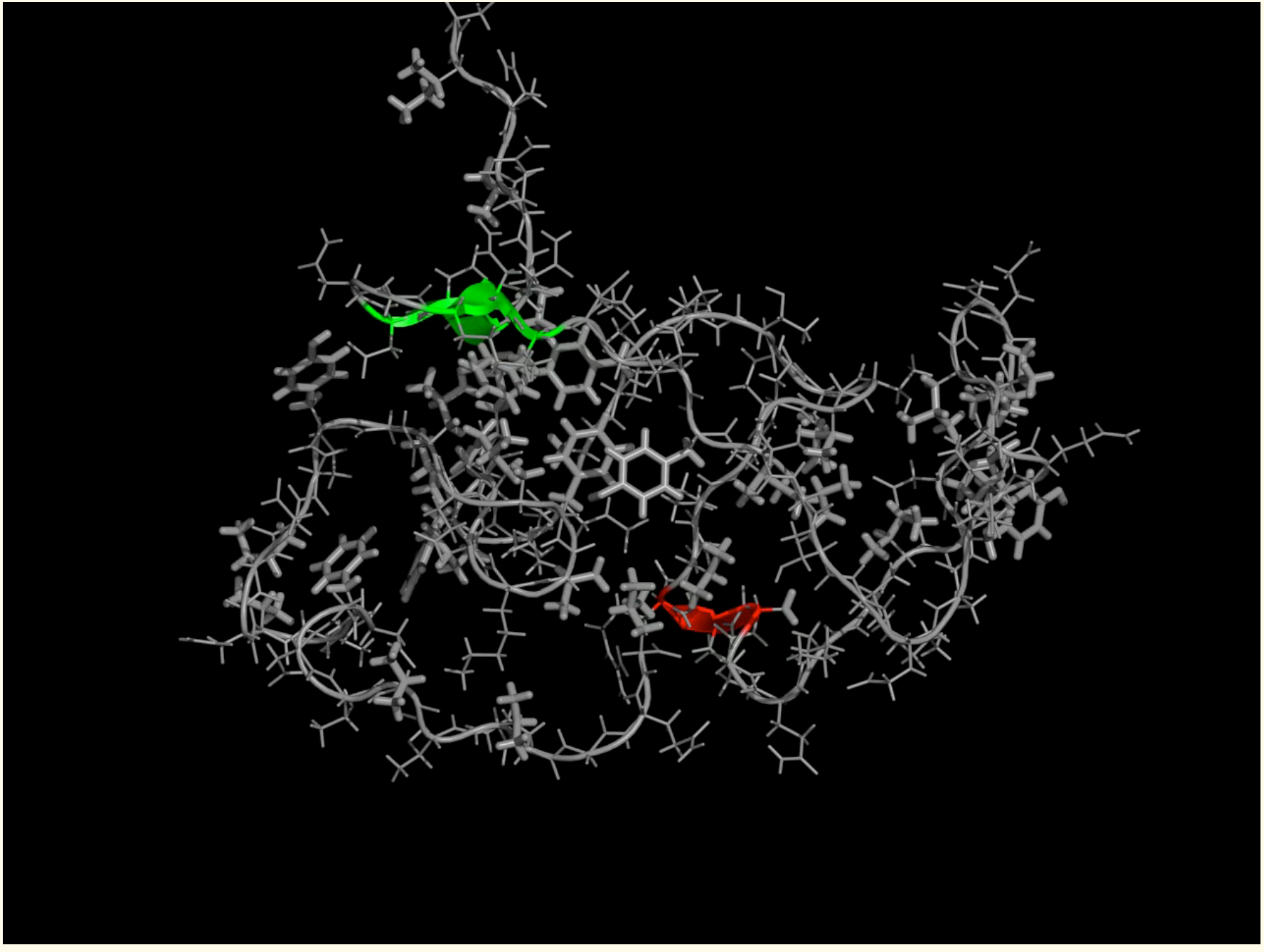
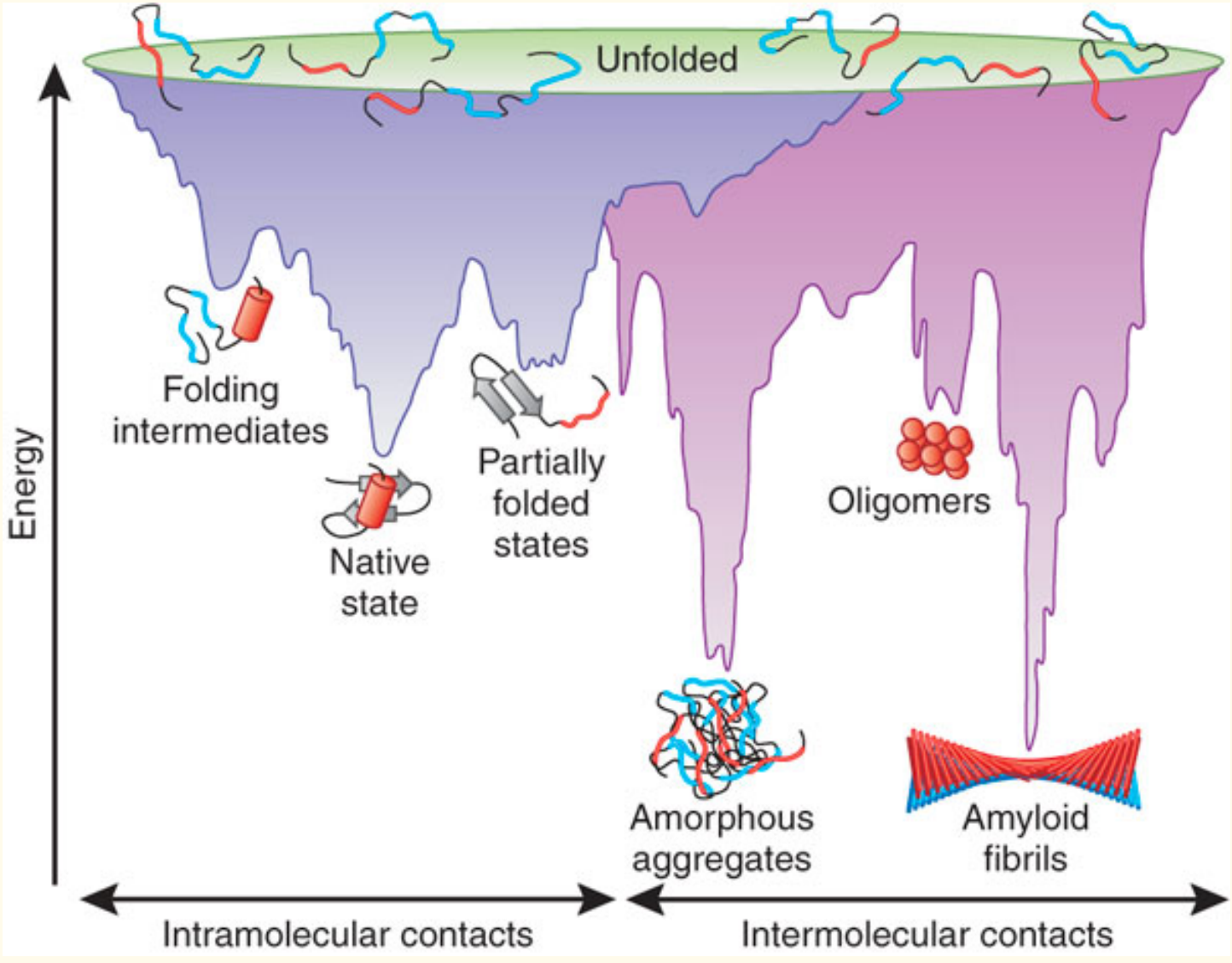


Mohanty et al. Proteins 2013;81:1446-1456

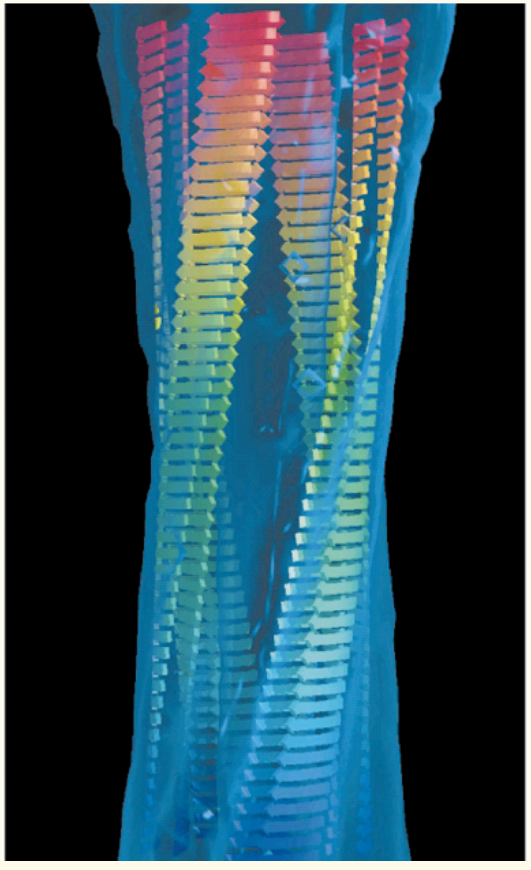
Protein aggregation



Hartl and Hayer-Hartl NSMB 2009;16:574-581

Amyloid fibrils

- Characteristic core of β-sheets
- “Universal”: formed by many proteins
- Interesting material properties
- Linked to many diseases, can be functional

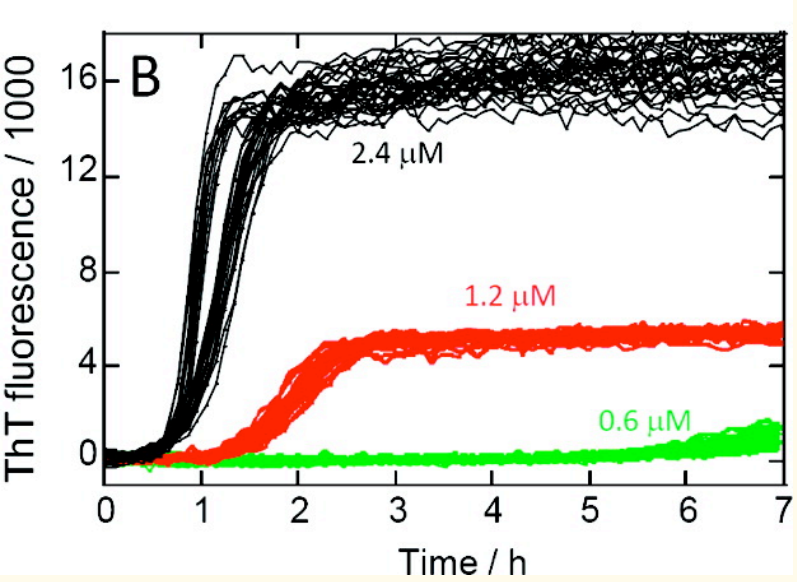


Dobson TBS 1999;24:329-332

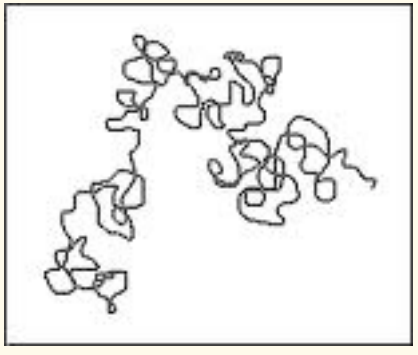
Amyloid formation

- Fibril formation occurs with sigmoidal kinetics. Critical nucleus?

Hellstrand et al. ACS Chem Neurosci 2009; 1:13-18



- Fibril growth occurs by monomer addition. Aggregation-competent form of the monomer?



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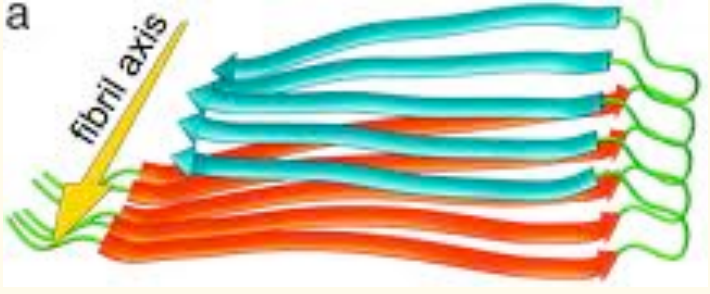


Petkova et al. PNAS 2002;99:16742-16747

Fibril nucleation

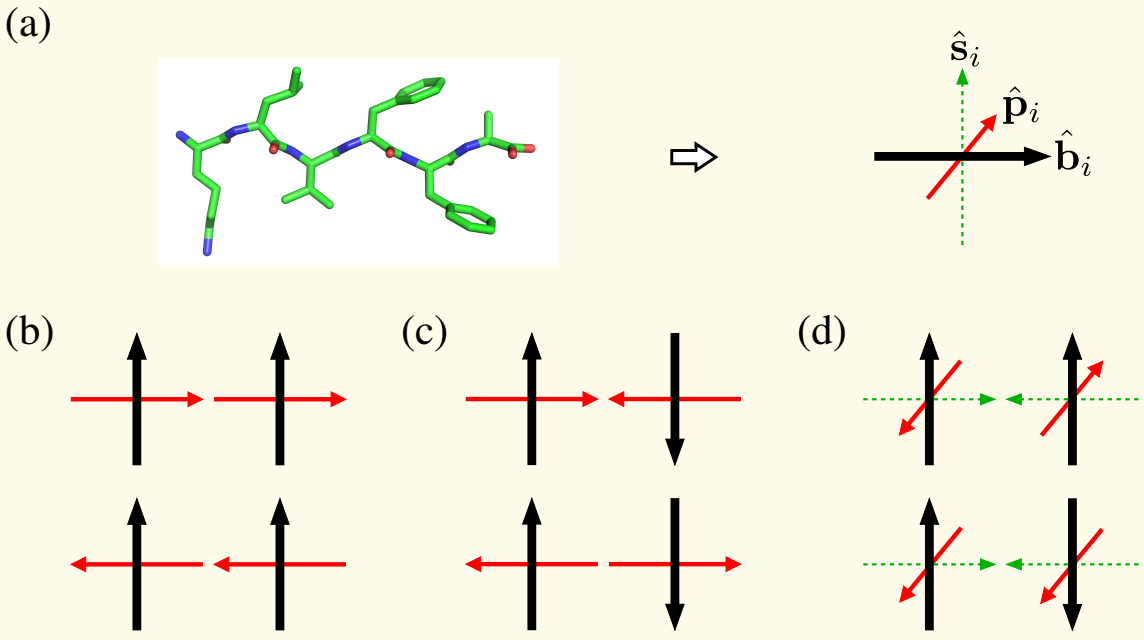
- Experimentally difficult, transient species
- Simulations: system size limitations
- Simple nucleation (3D): balance volume, surface terms.
  No critical size in 1D

- Need to study the interplay between fibril length and width
- Sigmoidal kinetics independent of sequence details

$\longrightarrow$ coarse-grained modeling

Minimalistic model for amyloid formation

- Assume internal dynamics fast and can be averaged out
- Each peptide unit-length stick, **b,** on a cubic lattice
- H bonds in a direction **p** perpendicular to **b**
- Hydrophobic side **s** = **b** x **p**

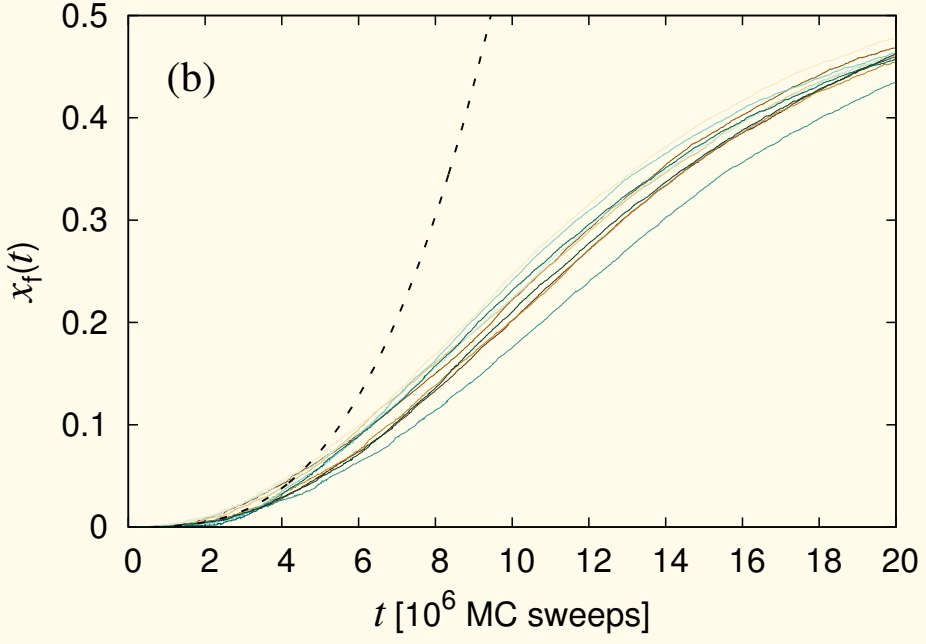


$E_b < E_c < E_d < 0$

A Irbäck, S Æ Jónsson , N Linnemann, B Linse, S Wallin Phys Rev Lett 2013

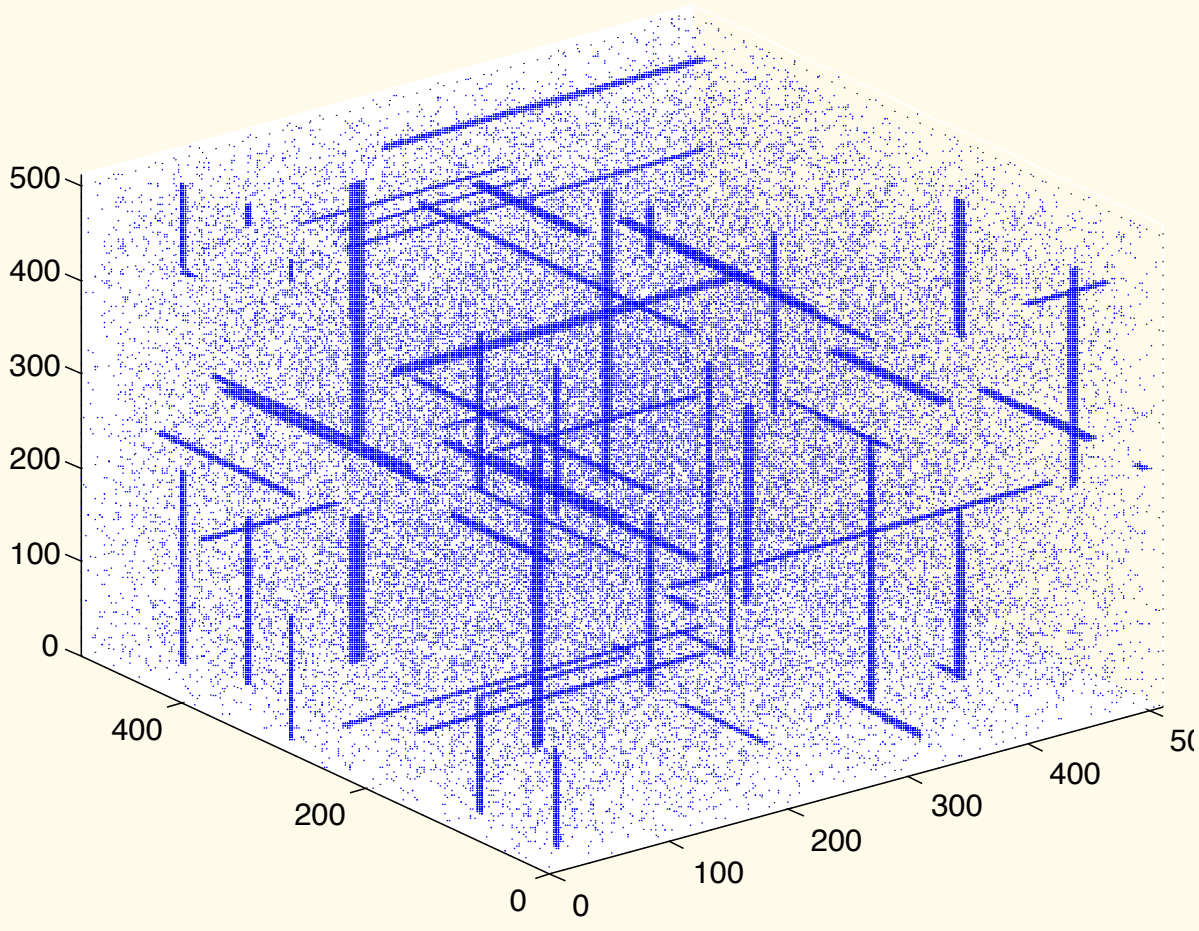
Kinetics

- Monte Carlo single-peptide moves
- >100,000 peptides, 10 runs from random initial conditions
- Total fibril mass against Monte Carlo time
- Sigmoidal kinetics



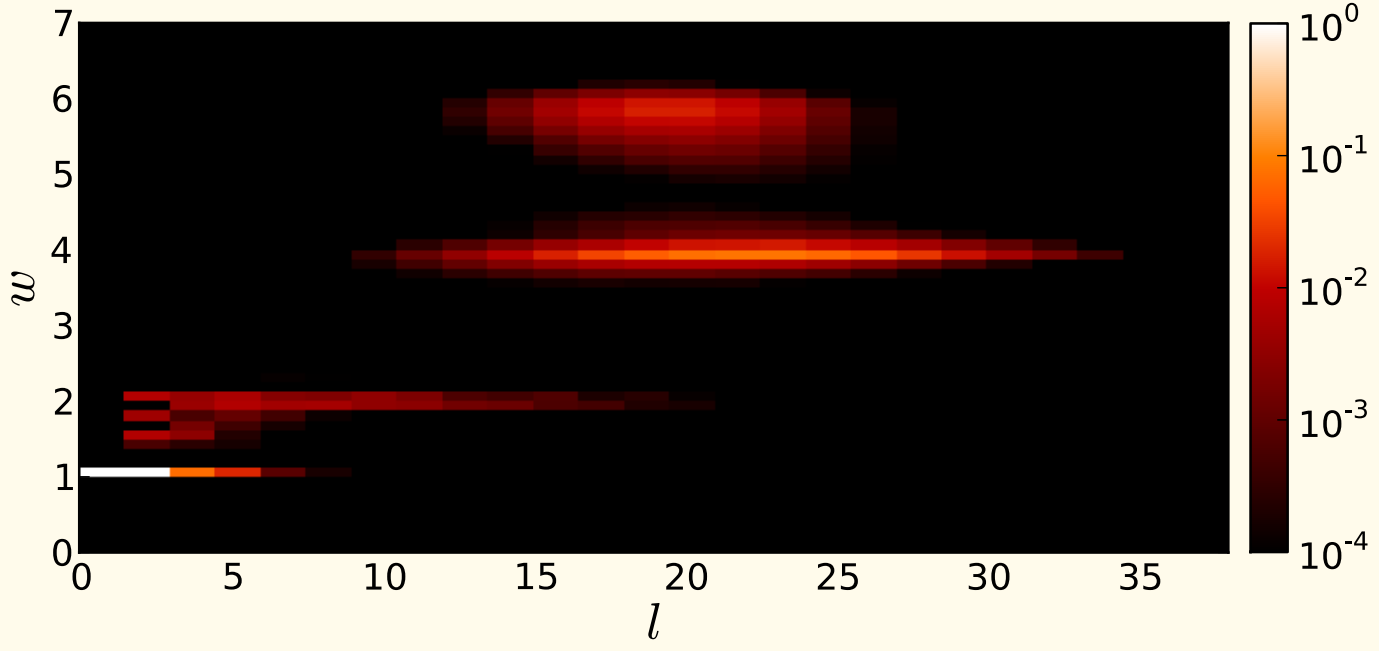
Final configuration in a kinetic run

- ~40-50 fibrils, average mass ~1400



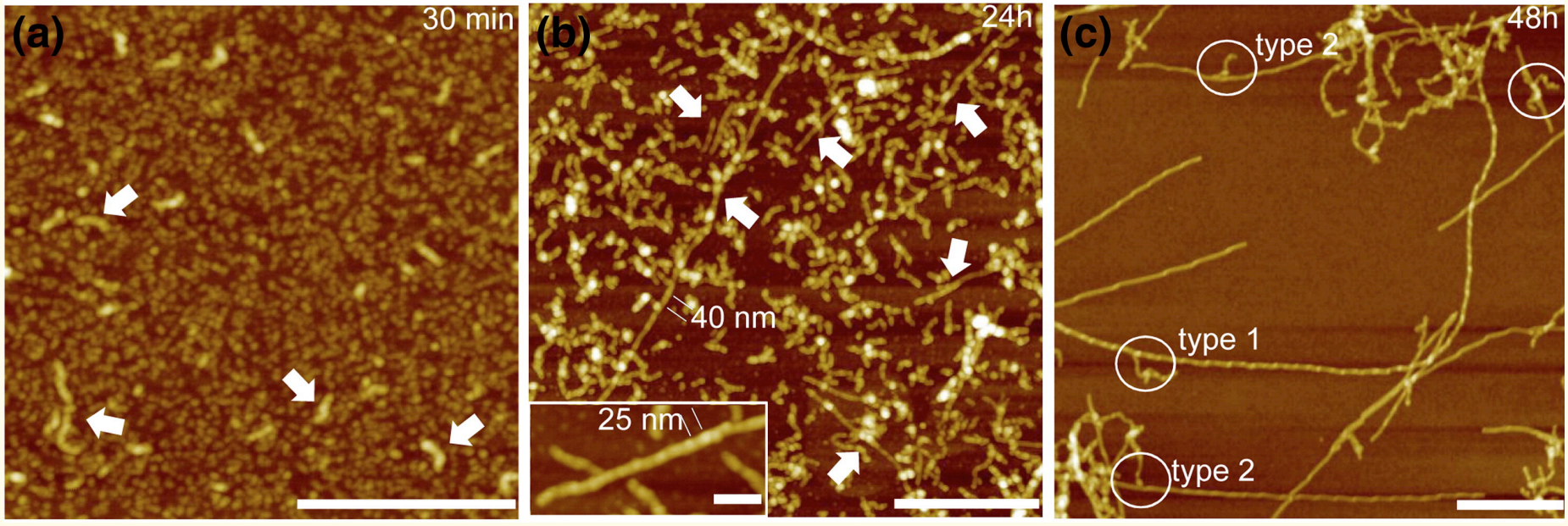
Aggregate geometry

- Equilibrium Monte Carlo simulation, 256 peptides
  Cluster moves and generalized-ensemble techniques
- Aggregate length *l* and width *w* from inertia tensor
- Probability for a peptide to be part of an *l* x *w* aggregate



- To reach a given *l*, a minimum *w* is required
- Multistep process

AFM images of fibril formation



Jeong et al. JMB 2013; 425:1765-1781

Specific proteins

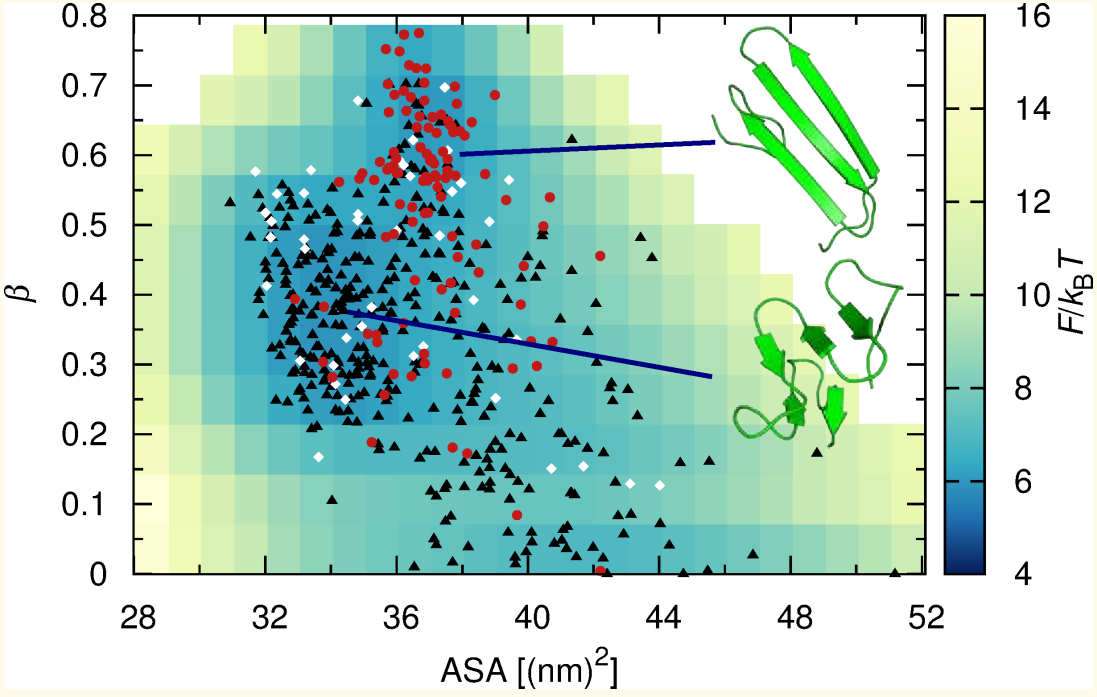
- amyloid β, Aβ (Alzheimer's disease)
- α-synuclein, αS (Parkinson's disease)
- superoxide dismutase 1 (amyotrophic lateral sclerosis)
- apolipoprotein A-I

Modeling:

- all-atom protein representation, implicit solvent
- one and the same force field (same as in Top7 study)
- Monte Carlo methods

Aβ

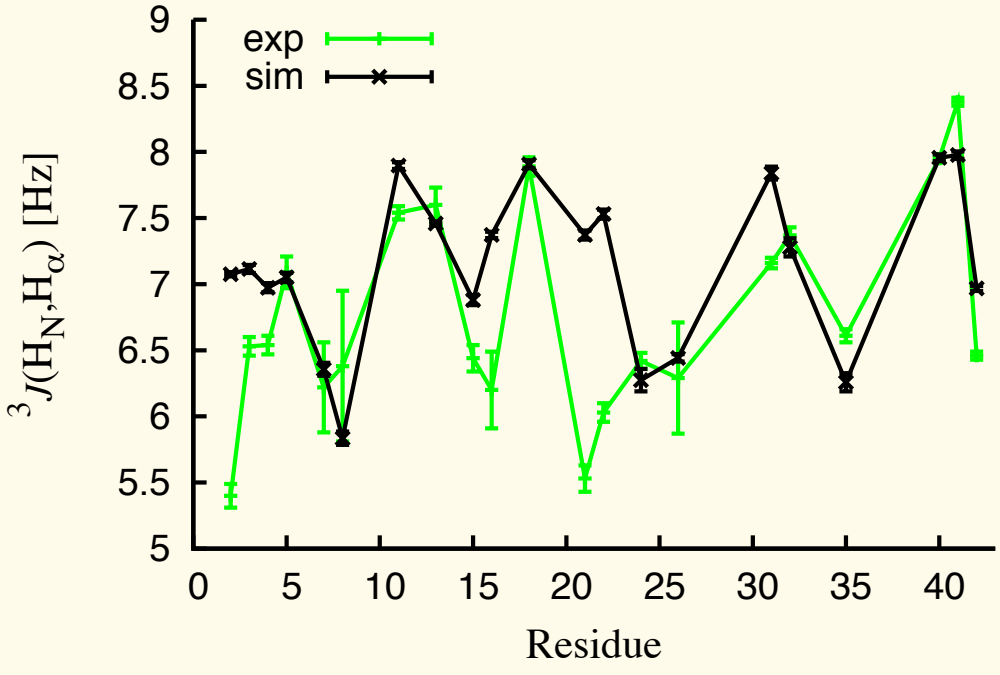
- Major constituent of Alzheimer plaques
- “Intrinsically disordered”, 42 amino acids
- Simulated free energy *F*(surface area,β-content):



- Typical simulations: more disorder, smaller β-content

Comparison with NMR experiments

- $^3J(H^N,H^\alpha)$-couplings: functions of a single backbone torsion angle (Karplus equation)



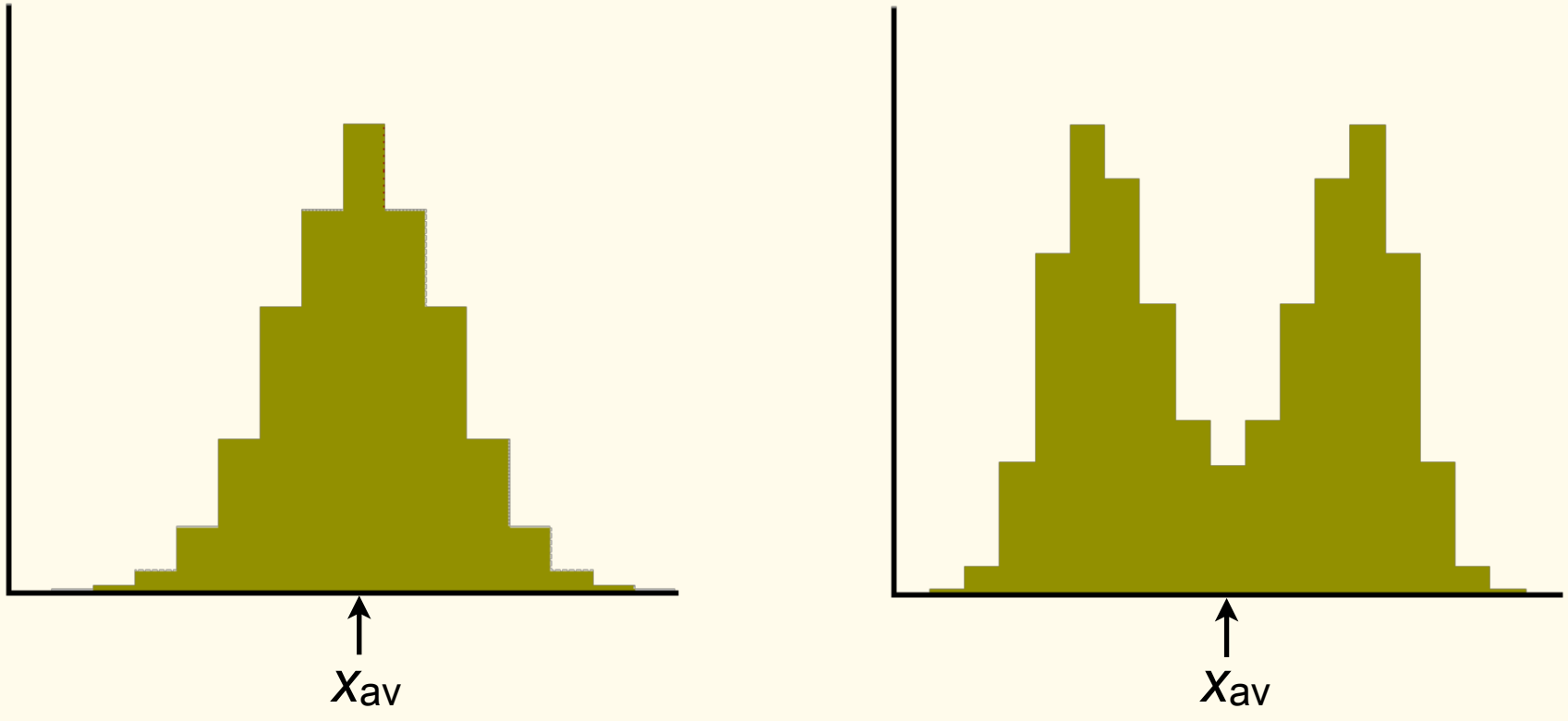
Exp.: Sgourakis et al. JMB 2007; 368:1448-1457

- Comparatively good agreement (alanine outlier)

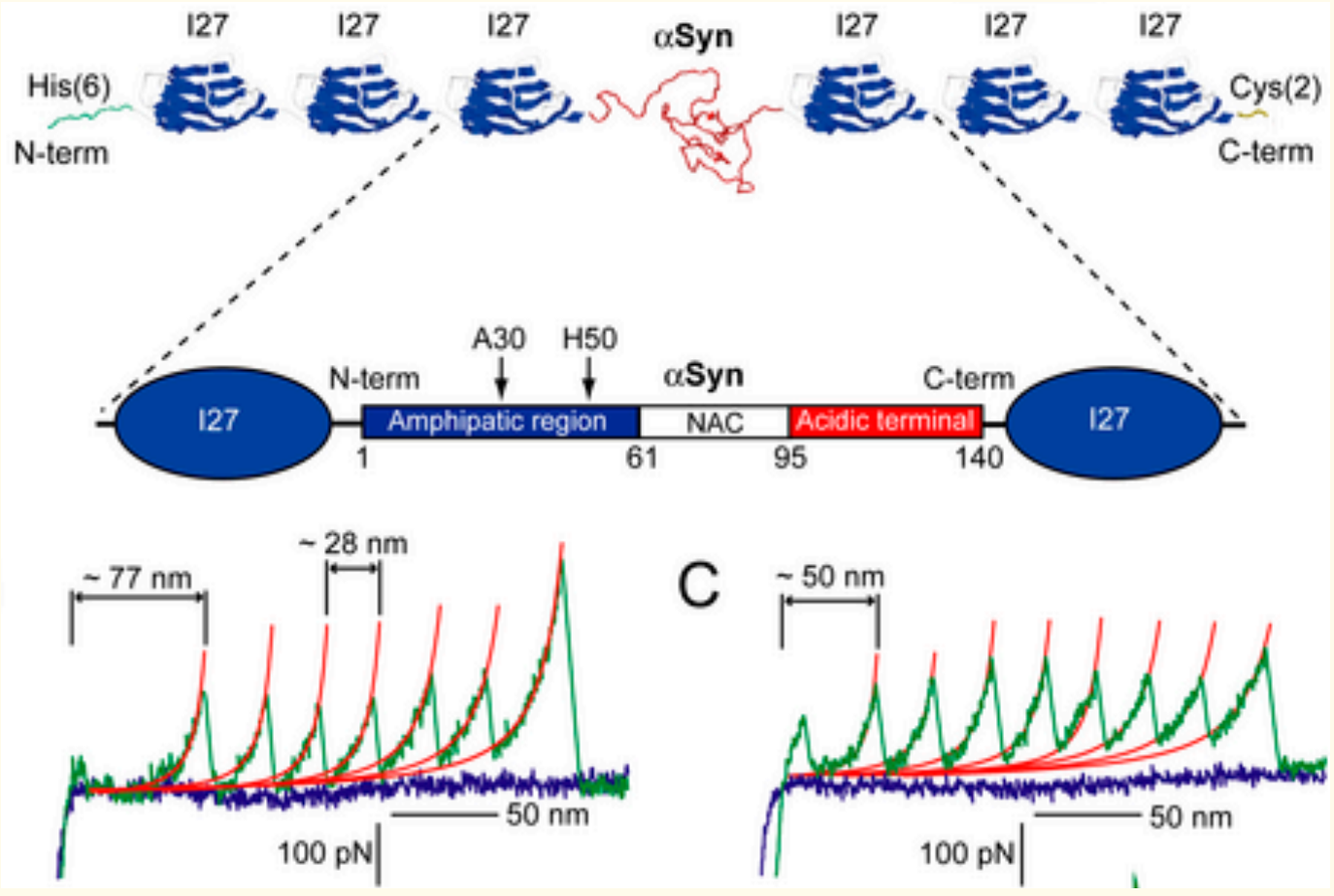
S Mitternacht, I Staneva, T Härd, A Irbäck Proteins 2010, J Mol Biol 2011

Experimental characterization of unstructured proteins

- Mainly bulk experiments, which must be interpreted with care
- Single-molecule experiments are becoming available



AFM single-molecule pulling experiments



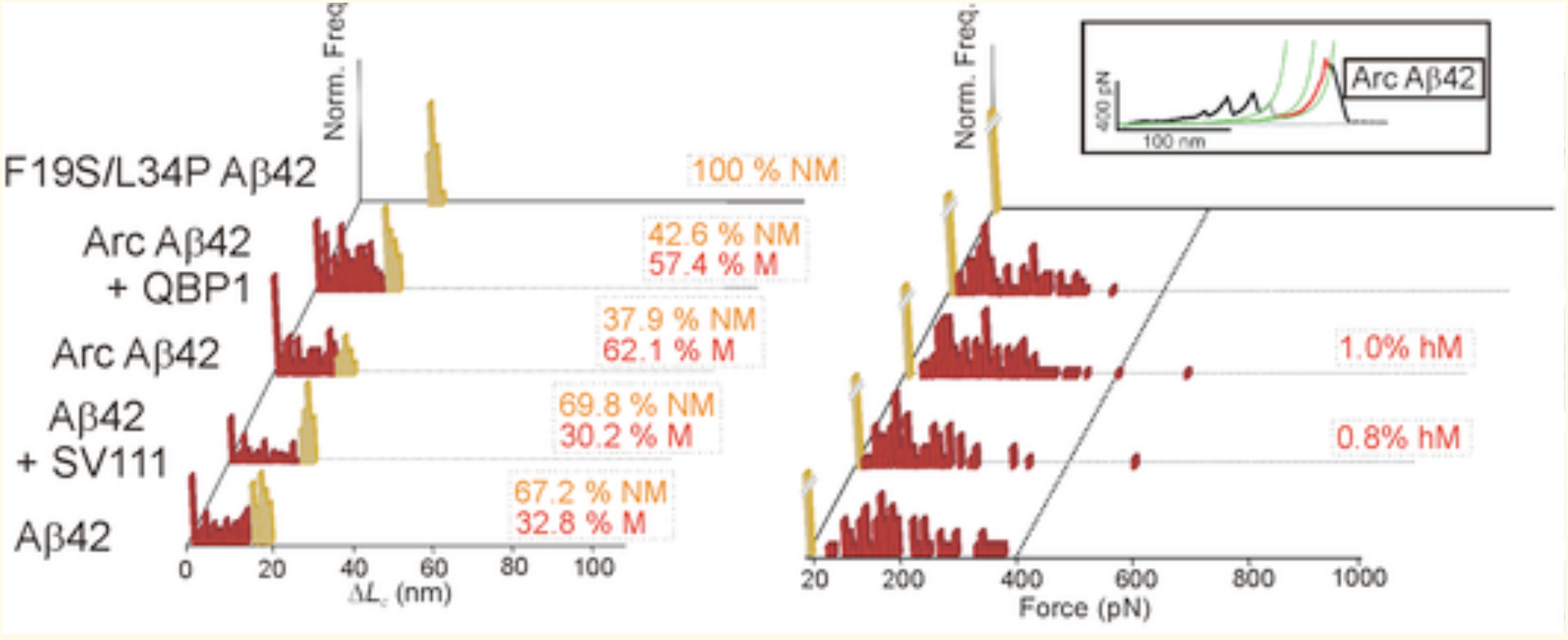
Sandal et al. PLoS Biology 2009; 6:e6

- Force versus distance
- Force peaks signal rupture events
- Aβ and αS: unexpected force resistance

  Hervás et al. PLoS Biology 2012; 10:e1001335

AFM results for Aβ

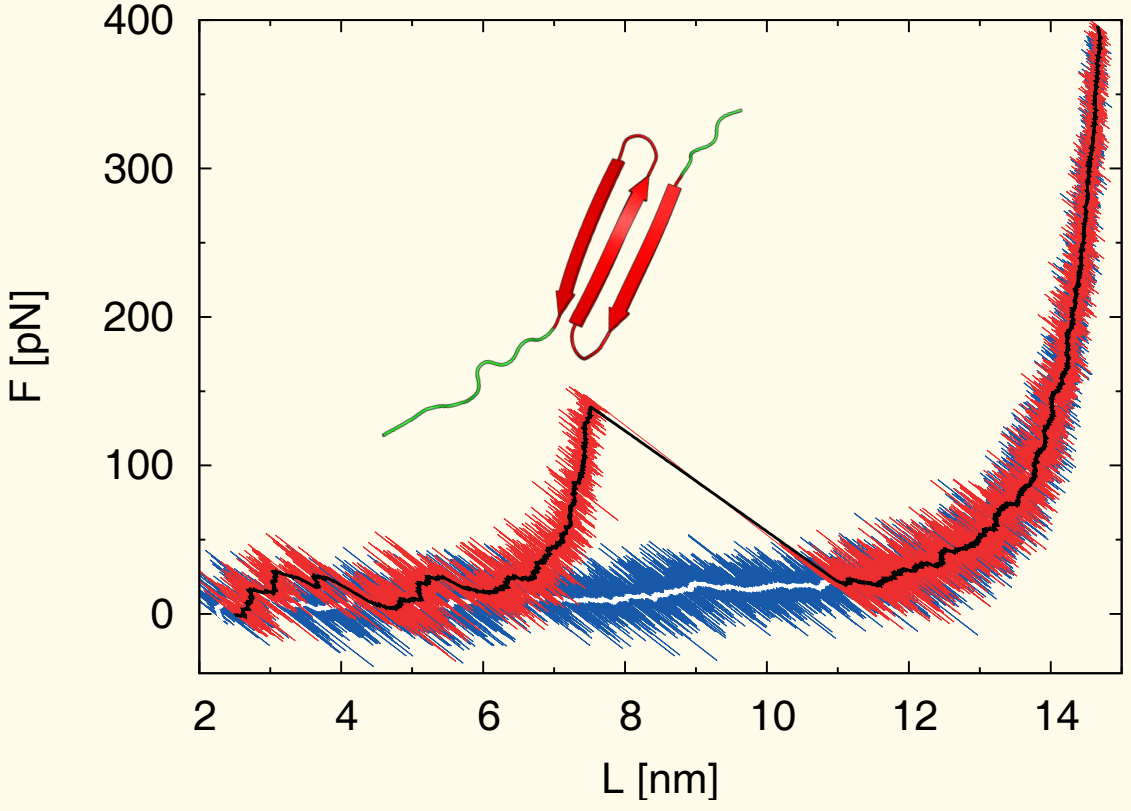
- Sometimes no resistance, sometimes rupture forces as high as those for unusually stable, folded proteins
- Can a small 42-amino acid protein like Aβ be that force-resistant? If so, what do the structures look like?



Hervás et al. PLoS Biology 2012; 10:e1001335

Pulling simulations of Aβ

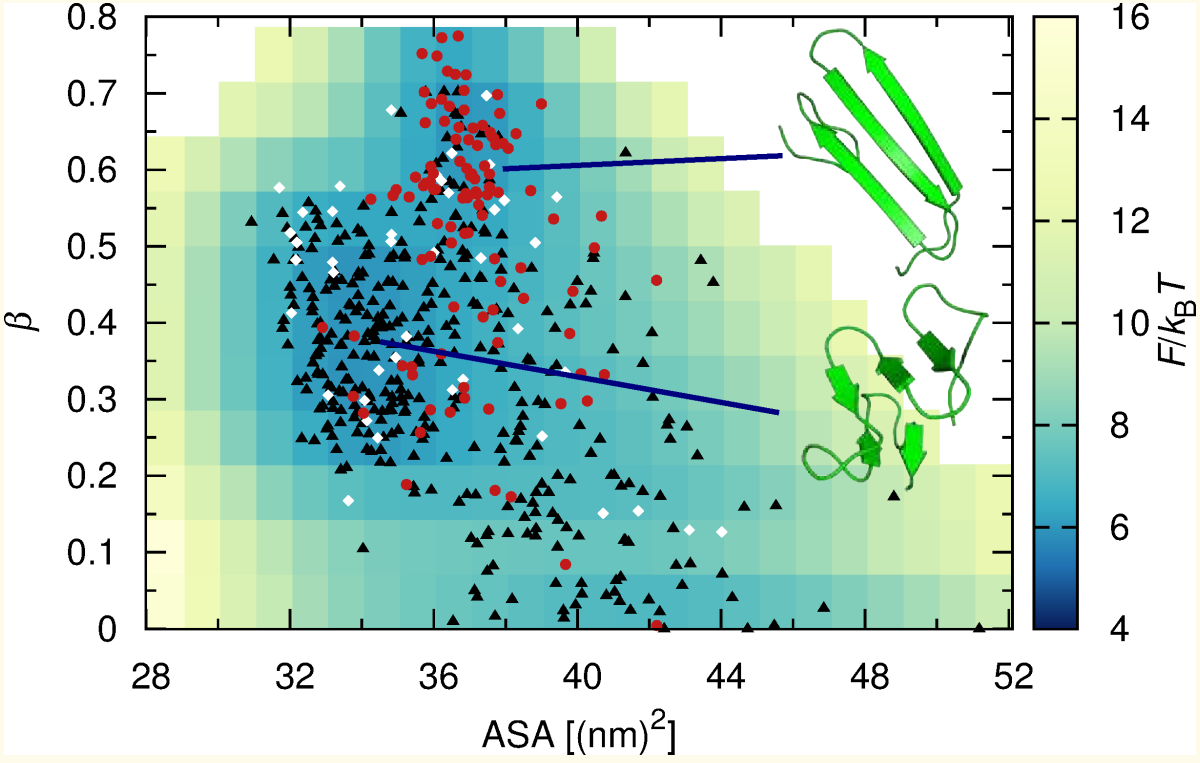
- Initial structures randomly drawn from simulated ensemble
- Two examples of force vs distance trajectories (in total >500)



S Æ Jónsson, S Mitternacht & A Irbäck Biophys J 2013

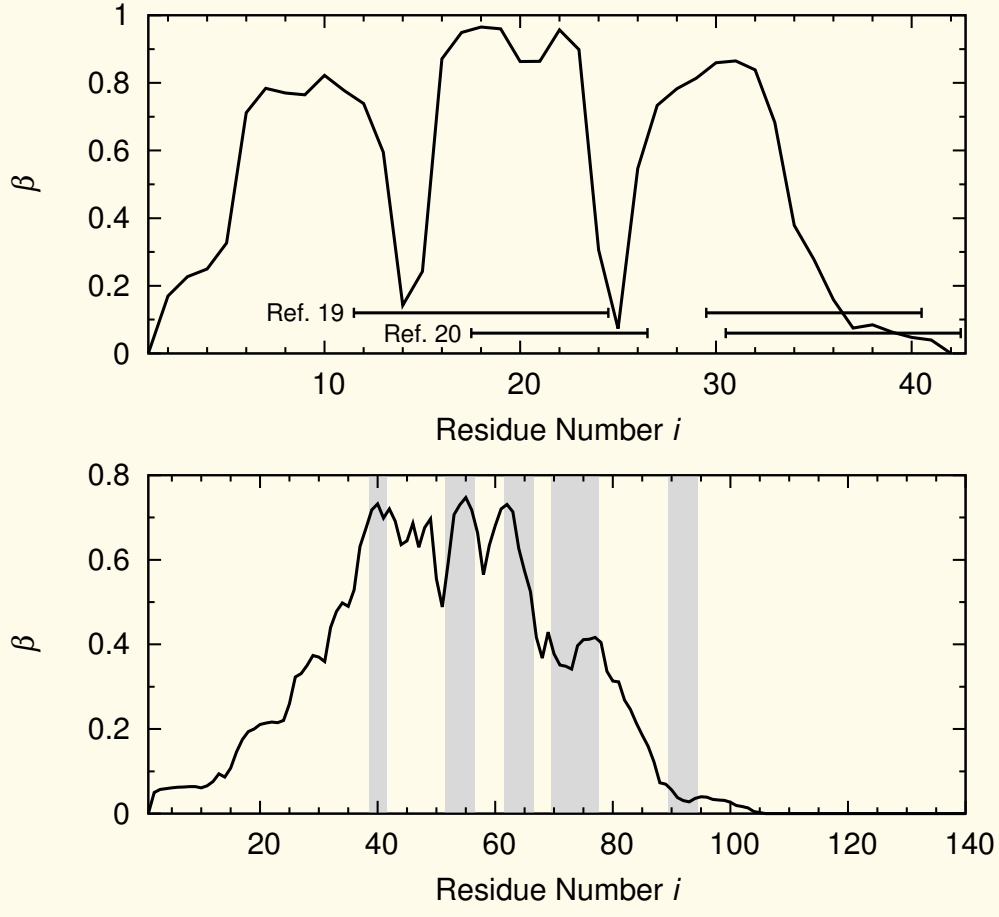
Pulling simulations of Aβ

- Plot symbols indicate initial structures
- The color indicates maximum rupture force. Black <20 pN, white 20-150 pN, red >150 pN



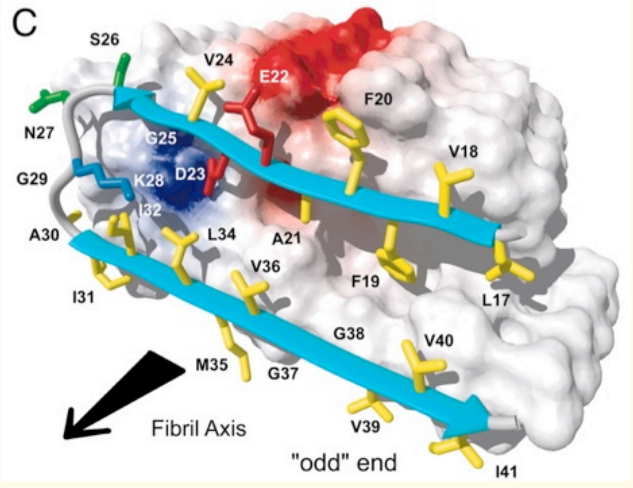
Comparison with fibril conformations

- β-structure profiles for force-resistant Aβ and αS structures
- Similarities with β-strand locations in fibrils

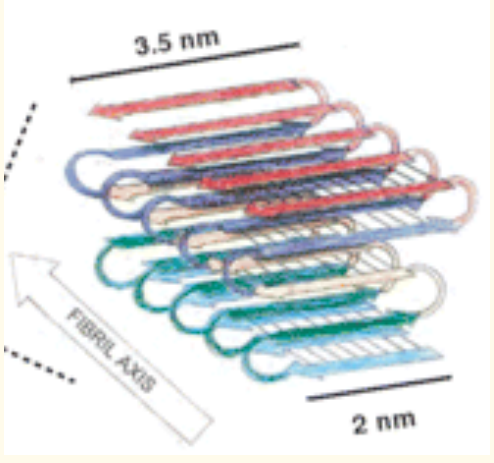


Aβ

αS



Lührs et al. PNAS 2005;102:17342-17347



Vilar et al. PNAS 2008; 105; 8637-8642

Summary — Aβ and αS studies

- Aβ and αS pulling simulations reproduce the surprisingly high rupture forces observed in AFM experiments
- The mechanically most resistant structures
  - share a common architecture:
    a β-sheet with three strands in a meander pattern
  - show similarities with the fibril folds
- Fibril-like structures might play a key role in aggregation

To do:

- Study fibril growth (monomer + fibril template)
- Study aggregation-inhibiting small molecules