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Monte Carlo studies of protein aggregation

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Comparing the folding free-energy landscapes of Aβ42 variants with different aggregation properties
S. Mitternacht, I. Staneva, T. Härd and A. Irbäck, *Proteins* **78**, 2600-2608 (2010)

Monte Carlo study of the formation and conformational properties of dimers of Aβ42 variants
S. Mitternacht, I. Staneva, T. Härd and A. Irbäck, *Journal of Molecular Biology* **410**, 357-367 (2011)

Introduction

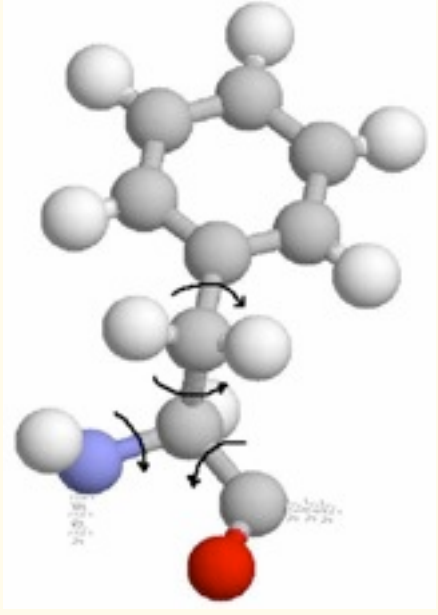
- Aβ fibrils main component of plaques in Alzheimer's disease (AD)
- Several single-amino acid mutations of Aβ linked to familial AD. Enhanced in vitro aggregation
- Small soluble Aβ oligomers more cytotoxic than fibrils
- Aβ oligomers a challenge to isolate and characterize

This talk:

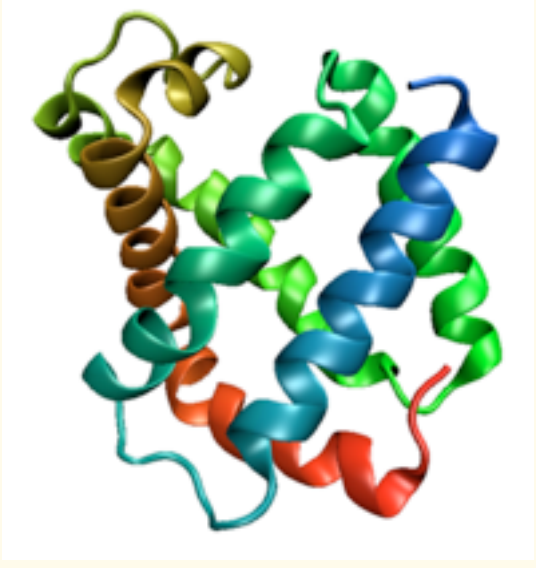
- Monomers and dimers of Aβ42 (42 amino acids)
- α-synuclein monomers (140 amino acids, Parkinson's disease)
- Oligomer growth for a fibril-forming 6-amino acid peptide

Building blocks

- Chains of ~50 to thousands of amino acids (20 types)
- Primary structure: amino acid sequence
- Secondary structure: α-helices and β-sheets



amino acid
(phenylalanine)



α

β

Computational model

- Implicit solvent all-atom representation, torsional degrees of freedom
- Effective force field
  - local potential
  - excluded volume
  - hydrogen bonding
  - effective hydrophobic attraction (pairwise additive)
  - charge-charge interactions
- Same force-field parameters for different proteins
- Monte Carlo dynamics
- Open source C++ package, PROFASI

Irbäck, Mitternacht and Mohanty, PMC Biophys., 2009

Folding

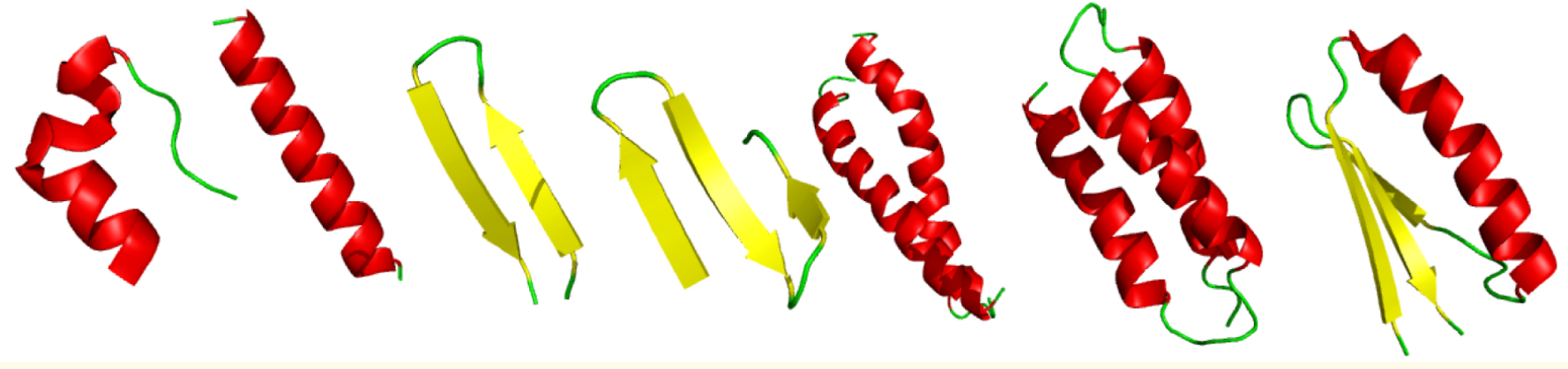
The force field was developed through folding studies of a diverse set of small proteins. Deliberately kept as simple as possible.

Peptides with 10-37 residues

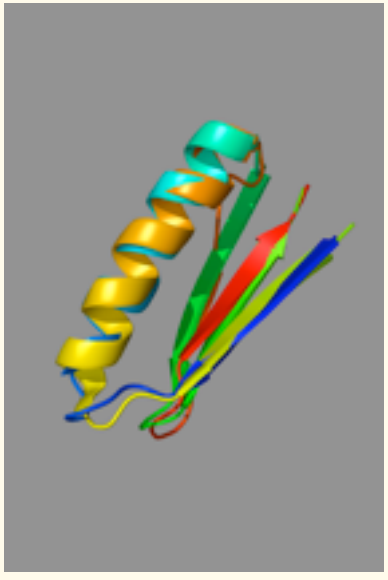
- Trp cage and 1RIJ, 5 α-helices, 7 β-hairpins, 3 3-stranded β-sheets

Small proteins

- Heterodimeric coiled-coil system, 2×30 residues (1U2U)
- Three-helix-bundle protein, 67 residues (1LQ7)
- Mixed α/β protein, Top7-CFr, 49 residues (2GJH)

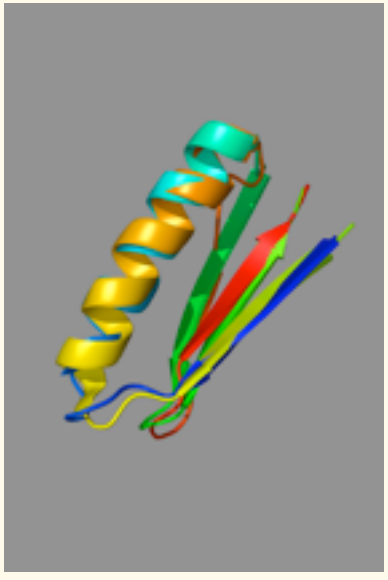


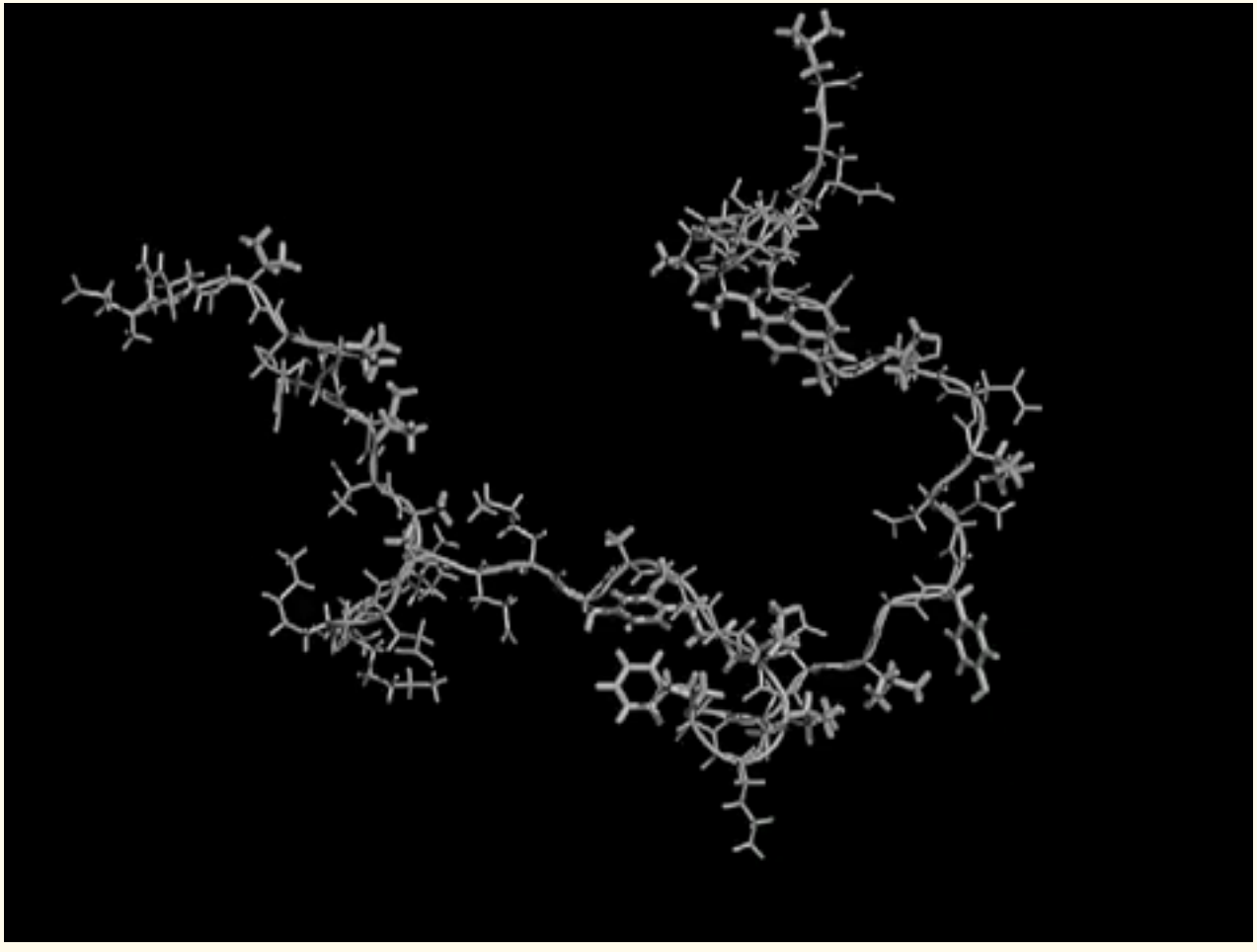
Top7-Cfr



[Mohanty et al., PNAS, 2008]

Top7-Cfr





[Mohanty et al., PNAS, 2008]

Monte Carlo methods

Conformational updates

- Rotations of single backbone and side-chain angles
- Semi-local backbone update, simultaneous rotation of 8 angles
- Rigid-body translations and rotations of whole chains

Generalized-ensemble techniques

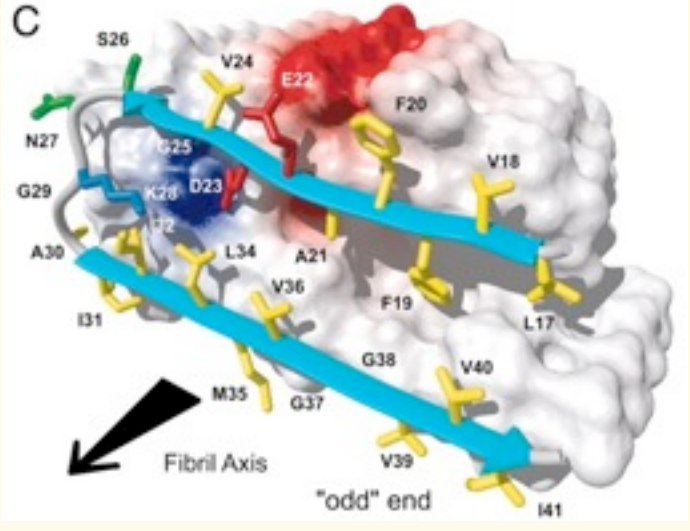
- Simulated tempering, parallel tempering/replica exchange
- Multicanonical, Wang-Landau ($P_\nu = \text{cst}/g(E_\nu)$, $P(E) = \text{cst}$)

Aβ conformations

- Monomer: natively unfolded
- Fibrils: β-loop-β motif, parallel in-register β-sheets
- Oligomers: less is known, but there have been recent studies of oligomeric species formed under various conditions

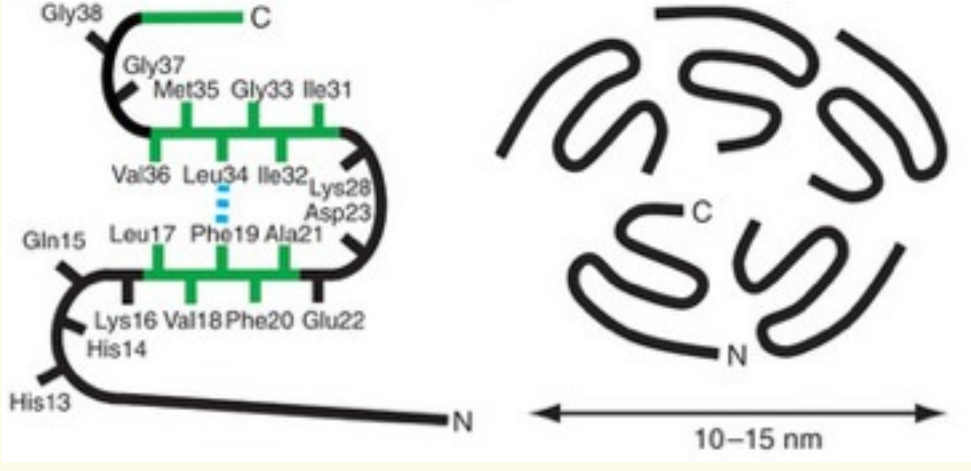
  Yu et al., Biochemistry, 2009 Cerf et al., Biochem. J., 2009

  Ahmed et al., NSMB, 2010 Sandberg et al., PNAS, 2010



Aβ42 fibril

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



Low-salt, low-*T* Aβ42 pentamer

Ahmed et al. NSMB 2010;17:561-567

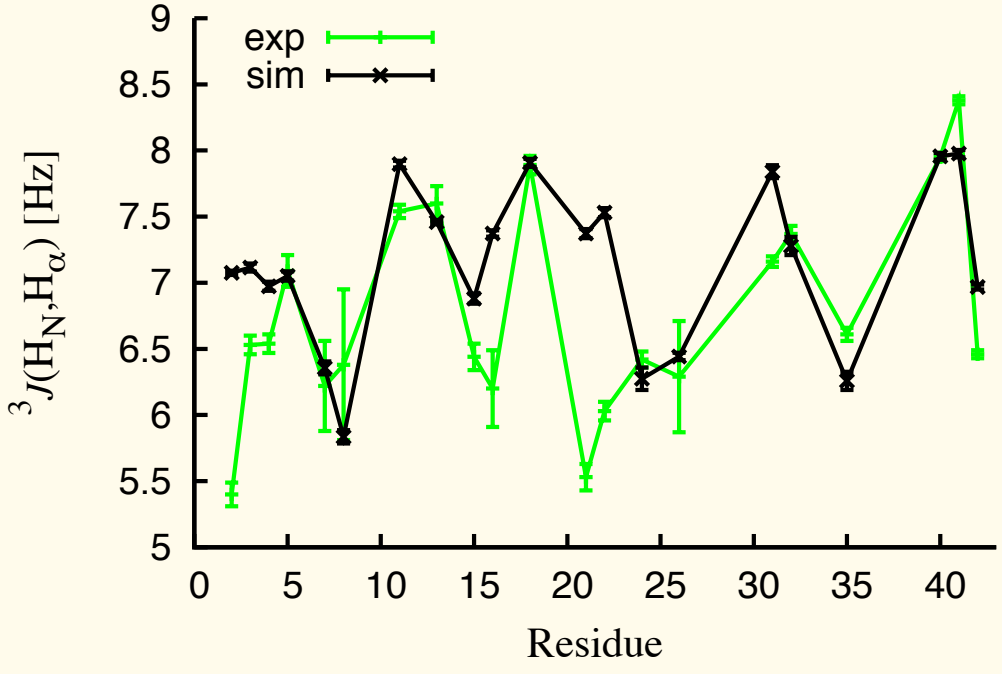
Our Aβ study

- Aβ42 monomers and dimers. Equilibrium simulations
- Four variants with very different aggregation properties:
  - WT
  - F20E, reduced aggregation
  - E22G (`Arctic´ mutant), enhanced aggregation
  - E22G/I31E, fibrils ↗, prefibrillar species ↘

  [Luheshi et al., PLoS Biol., 2007; Brorsson et al., Biophys. J., 2010]
- Dimer: 40 independent simulated-tempering runs for each variant, 60 days per run
- Previous work: mainly stability of preformed dimer structures

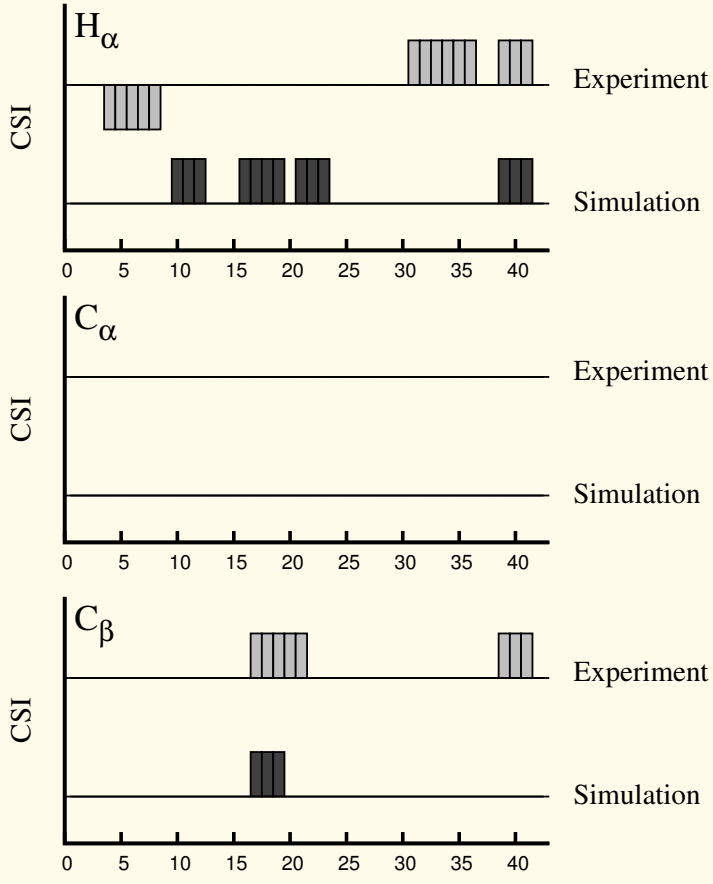
Monomer simulations against NMR data — 1. *J*-couplings

- Aβ42 WT
- 21 experimental $^{3}J(H^{N},H^{\alpha})$-couplings [Sgourakis et al., JMB, 2007]
- Simulated values obtained by using the Karplus equation
  (coefficients from Schmidt et al., J. Biomol. NMR, 1999)



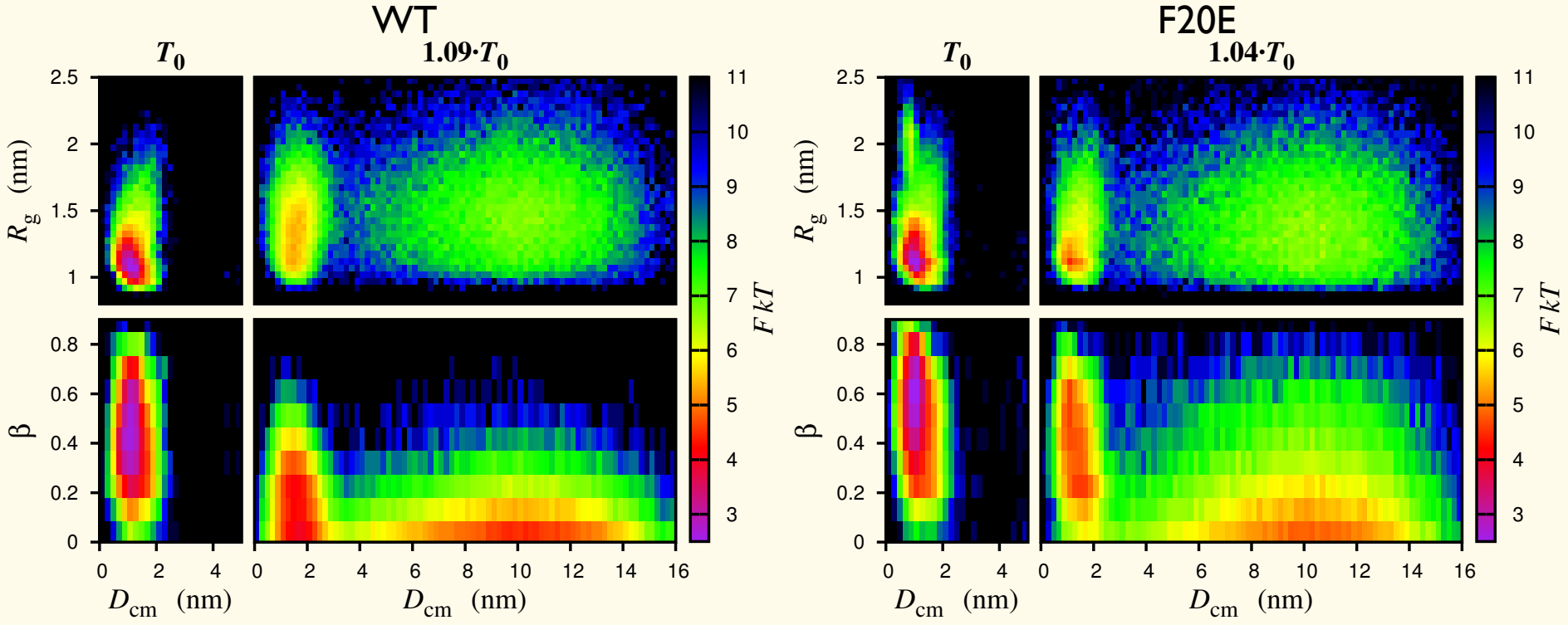
Monomer simulations against NMR data — 2. Chemical shifts

- Aβ42 WT
- H^{α}, C^{α}, C^{β} chemical shift indices (-1, 0 or 1) [Hou et al., JACS, 2004]
- Simulated primary shifts obtained by using the SHIFTS program, CSI conversion with high-*T* data as random coil reference values



(H^{α} and C^{β}: -1 helix, 1 strand)

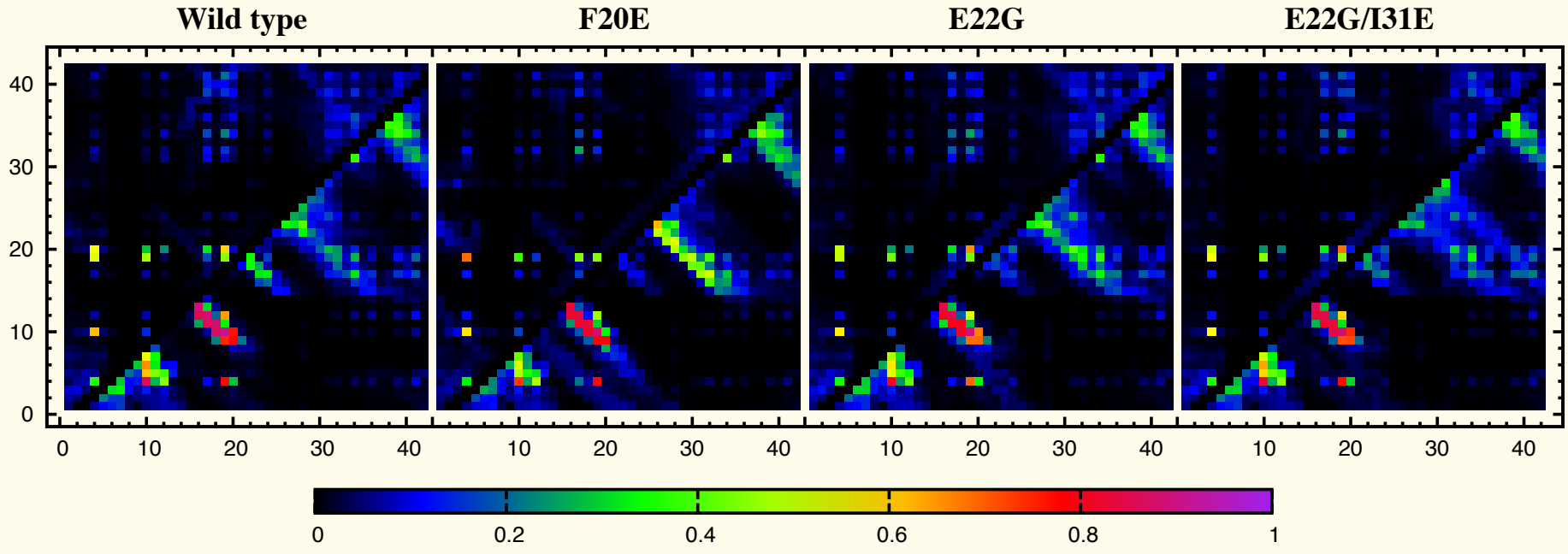
Aβ42 dimers — free energies of binding



D_{cm} = cm distance, β = strand content, R_g = radius of gyration of individual chains

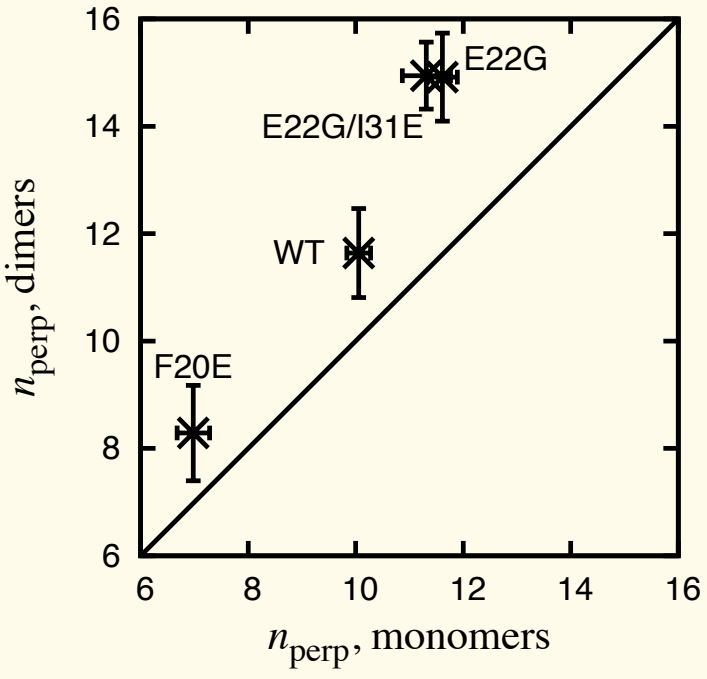
- Two phases: monomers and dimers
- F20E: aggregation sets in at a lower *T*, no disordered dimers
- E22G and E22G/I31E results similar to those for WT

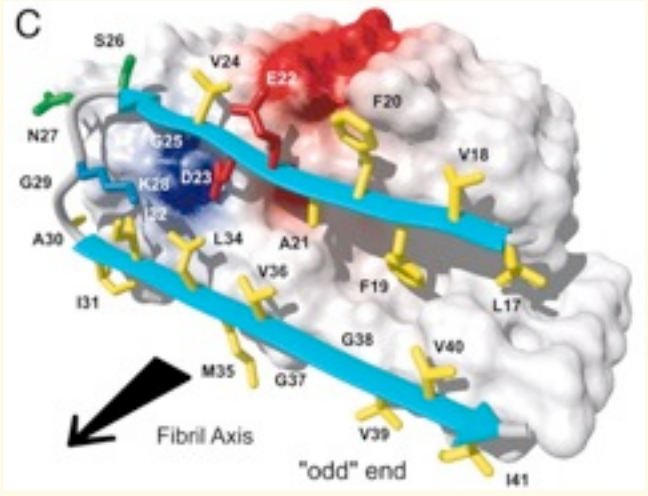
Dimer contact maps



- Intra-chain contacts (below the main diagonal)
  - several bands corresponding to turns
  - turns located in the fibril loop region altered by the mutations
- Inter-chain contacts (above the main diagonal)
  - no clear bands indicating parallel or antiparallel β-structure
  - a few hydrophobic residues responsible for all strong contacts

The fibril loop region 25-30





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

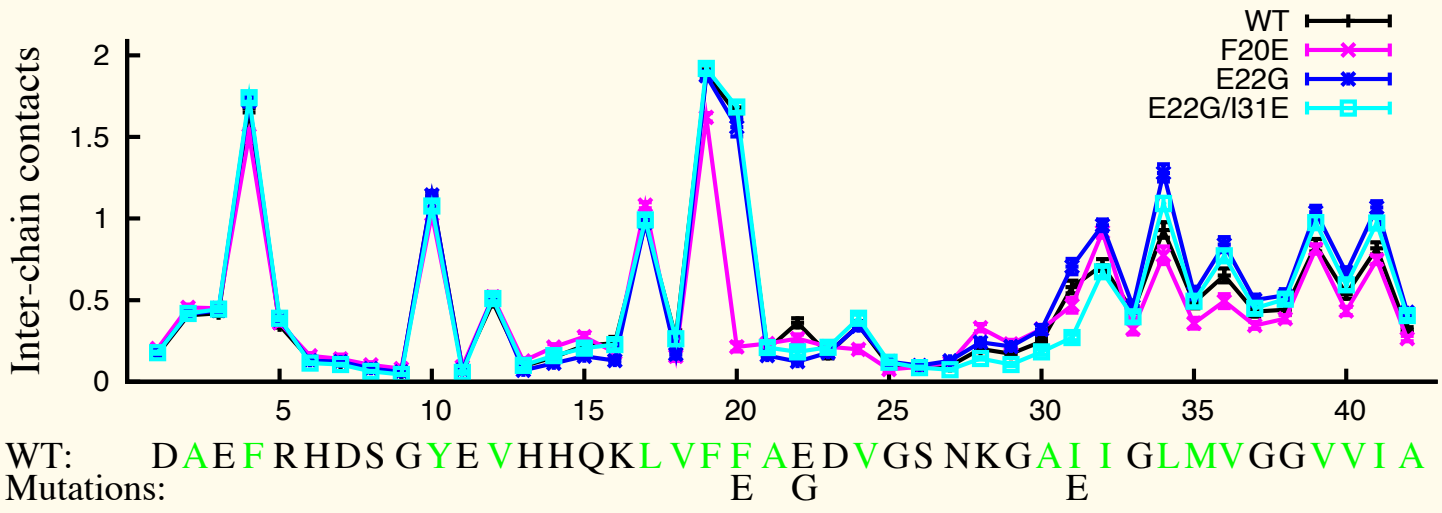
Frequency of turns centered in the 25-30 region

- Simple contact-based measure, n_{perp}
- (i) n_{perp}↗ upon dimerization, (ii) F20E<WT<E22G/I31E~E22G

Correlates with experimental fibril formation rates

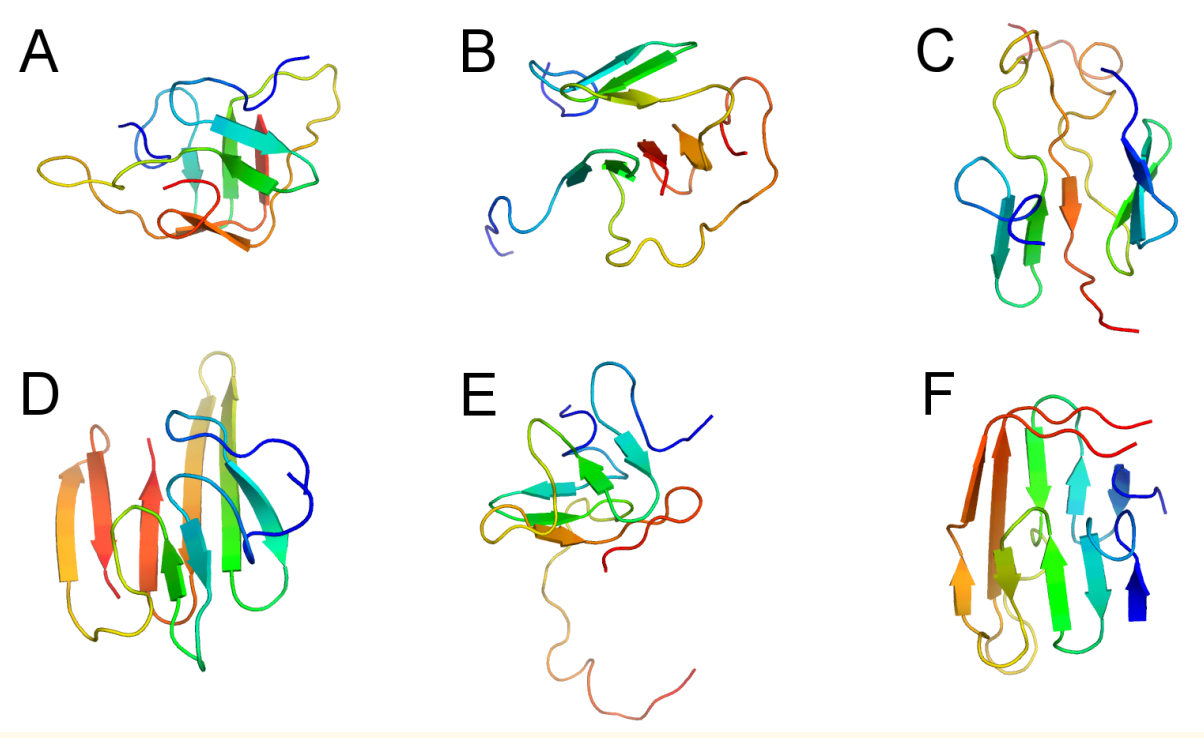
Exp. with a D23-K28 bridge: fibrillation enhanced 1000-fold
[Sciarretta et al., Biochemistry, 2005]

Hydrophobicity drives dimer formation



- Clear correlation no. of inter-chain contacts ↔ hydrophobicity
- The no. of inter-chain H bonds is small

Examples of dimer structures

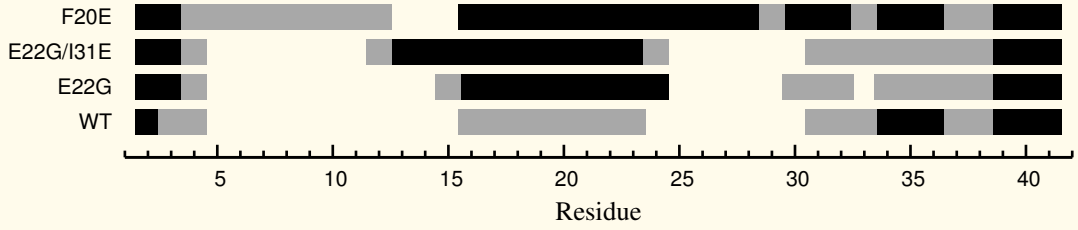


Aβ42 WT. A-E: cluster centroids (populations 1-6%). F: lowest energy

- The simulated dimer ensemble conformationally diverse
- Secondary structure: mainly intramolecular antiparallel β-sheets
- Parallel in-register β-sheets (as in fibrils) are rare

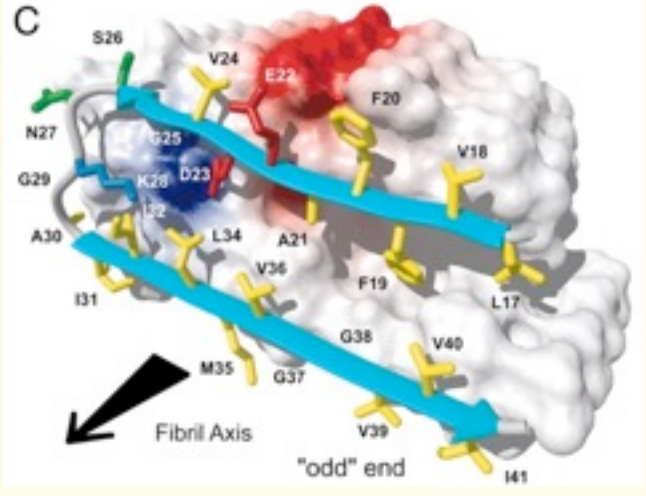
Parallel in-register β-sheets

- Parallel in-register β-sheets are rare
- Frequency of in-register parallel β-sheets (<6% everywhere):



white <0.1%, grey 0.1-1%, black >1%

- Common pattern for WT, E22G and E22G/I31E that matches well with the fibril structure



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

Summary Aβ dimers

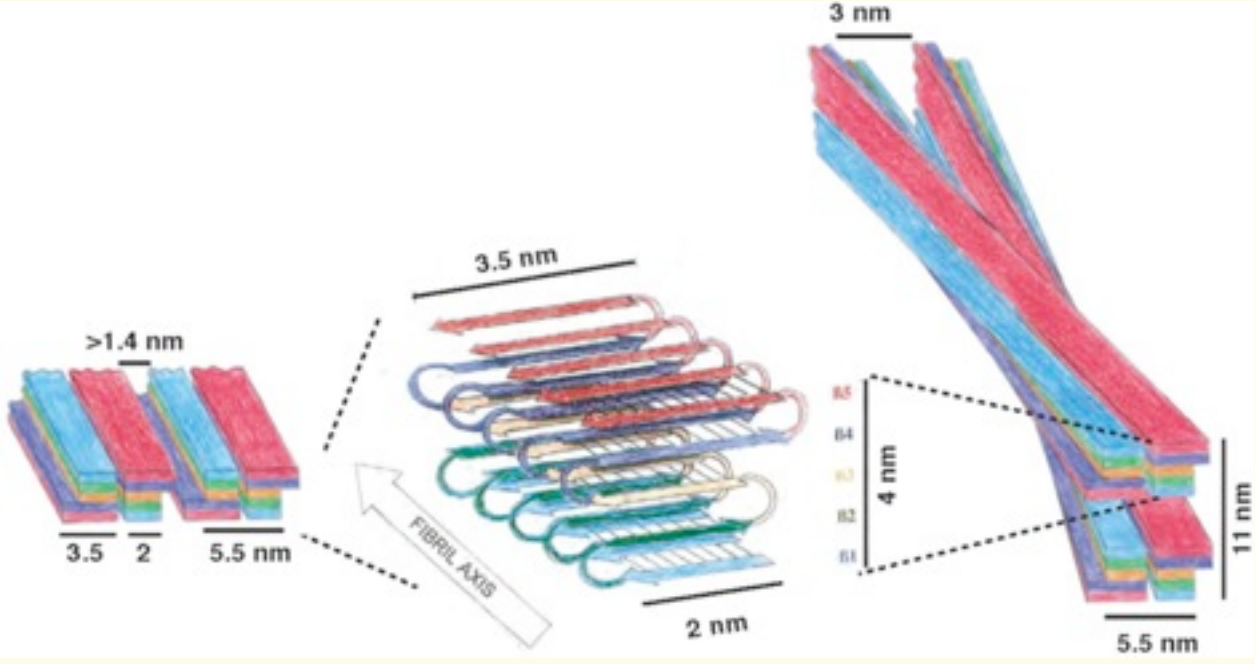
- Aβ42 dimerization computationally feasible (with implicit water)
- Our simulated dimers are diverse and not fibril-like. Intramolecular antiparallel β-sheets are the main type of secondary structure
- Hydrophobicity is the main driving force
- The probability of finding turns centered in the fibril loop region
  - increases upon dimerization
  - correlates with fibrillation rate (F20E<WT<E22G/I31E~E22G)
- Parallel in-register β-sheets are rare, but their location match well with the fibril β-loop-β motif (WT, E22G/I31E, E22G)
- Outlook:
  - trimers,...
  - interaction of Aβ with small-molecule aggregation inhibitors

α-synuclein (αS)

- Aggregated αS present in Parkinson's disease brains (Lewy bodies).
  Three known αS mutations linked to familial PD

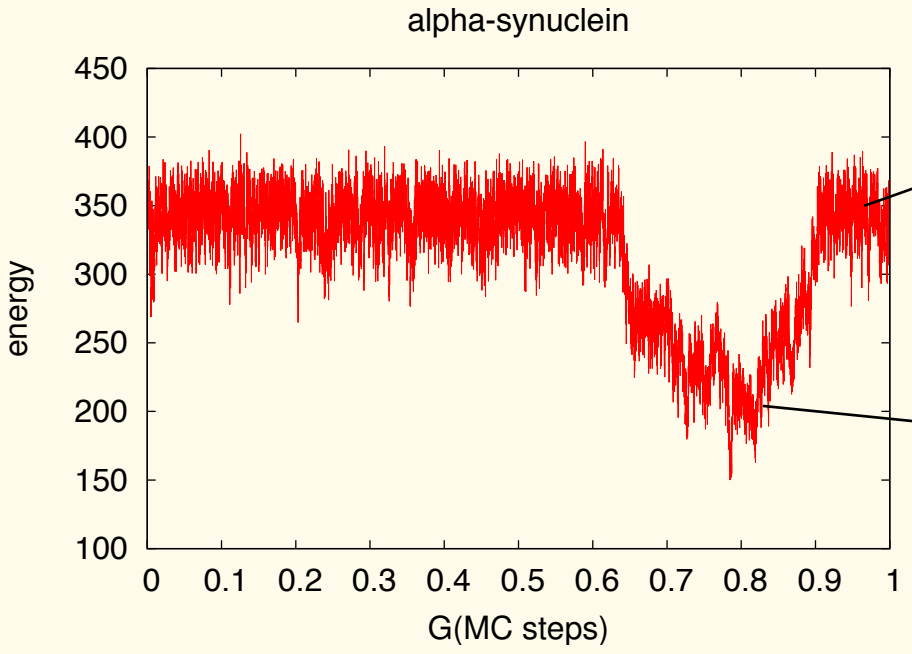
- αS is a natively unfolded 140-amino acid protein.
  Experiments suggest a coil-like behavior.
  But indications of populated compact states have been found
  [Sandal et al, PLoS Biol., 2008; Frimpong et al., Proteins, 2010]

- Proposed fibril fold:

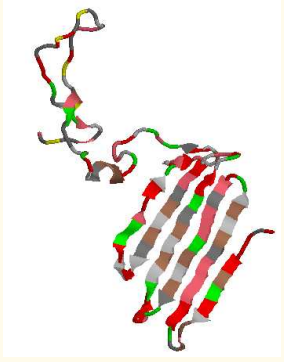


Vilar M et al. PNAS 2008;105:8637-8642

Incomplete simulations of the αS monomer



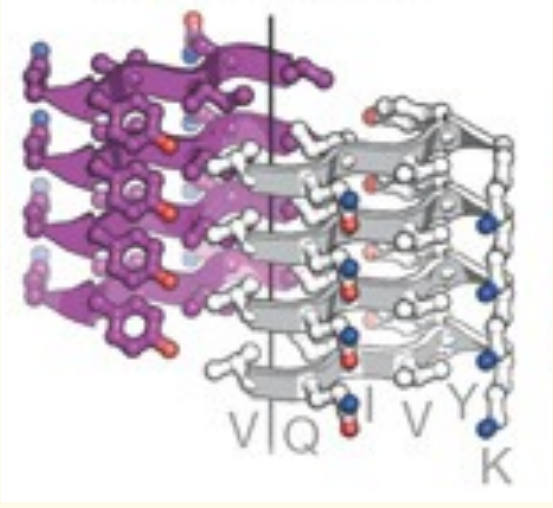
random coil



- Parallel-tempering simulations:
  Compact state observed, but coil ↔ compact transitions rare
  Relative populations impossible to estimate
- Flat-histogram techniques help, but more simulations still needed

Oligomer growth

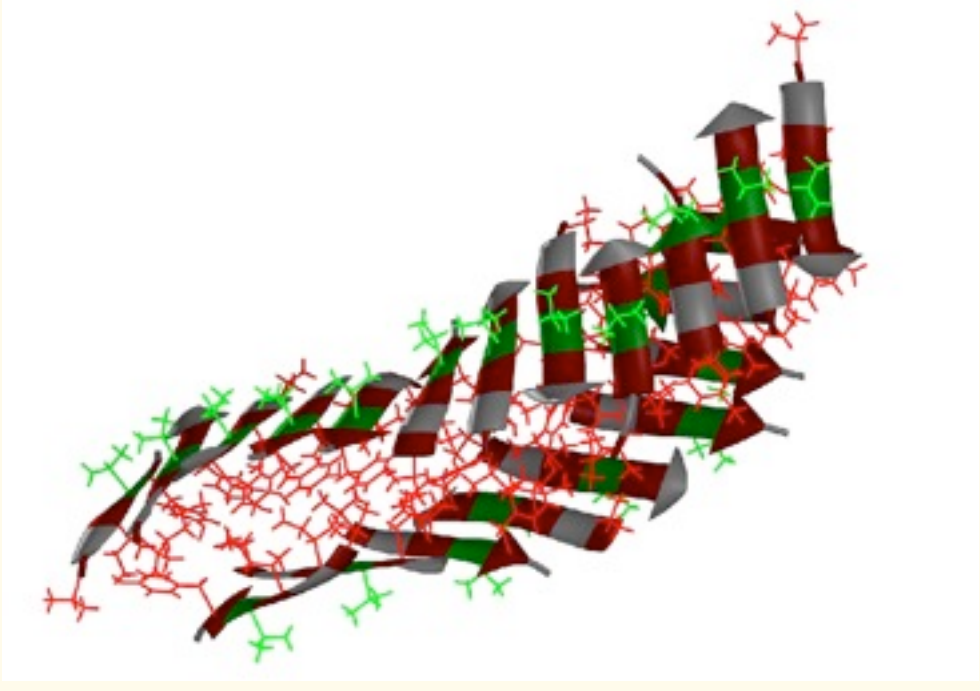
- Small fibril-forming peptide, PHF6
- 6-amino acid fragment (VQIVYK) of protein tau
- PHF6 fibrils consist of pairs of tightly packed parallel β-sheets. `Dry steric zipper´



Sawaya et al., Nature 447, 453-457 (2007)

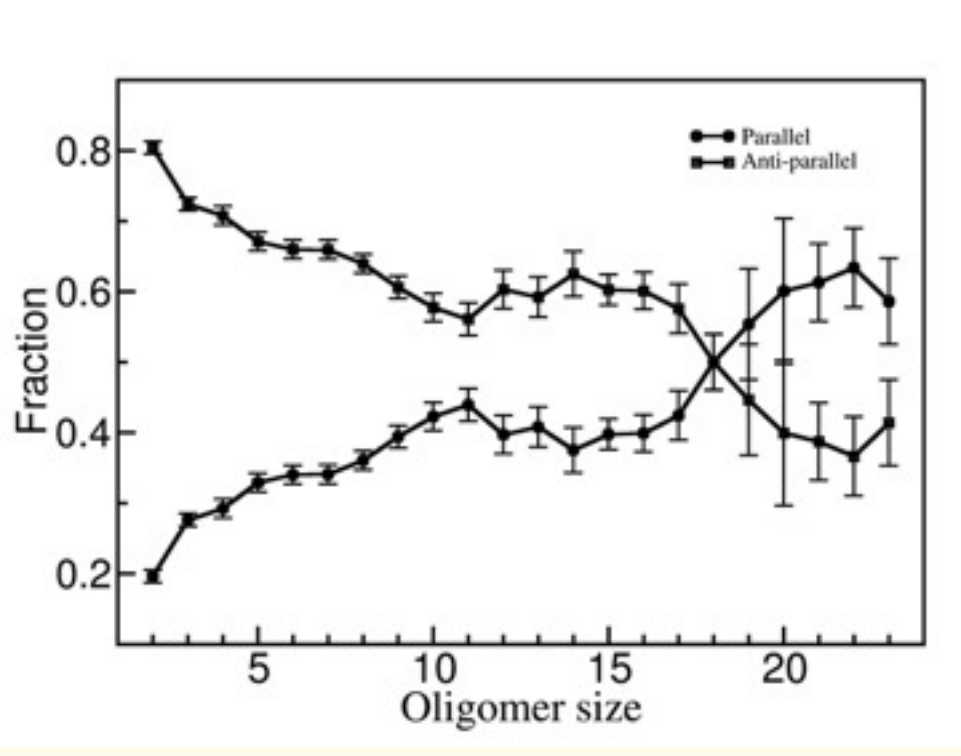
Simulations with up to 36 PHF6 peptides

- A multitude of small oligomeric states
- All larger aggregates (>20 chains) have β-sandwich structure



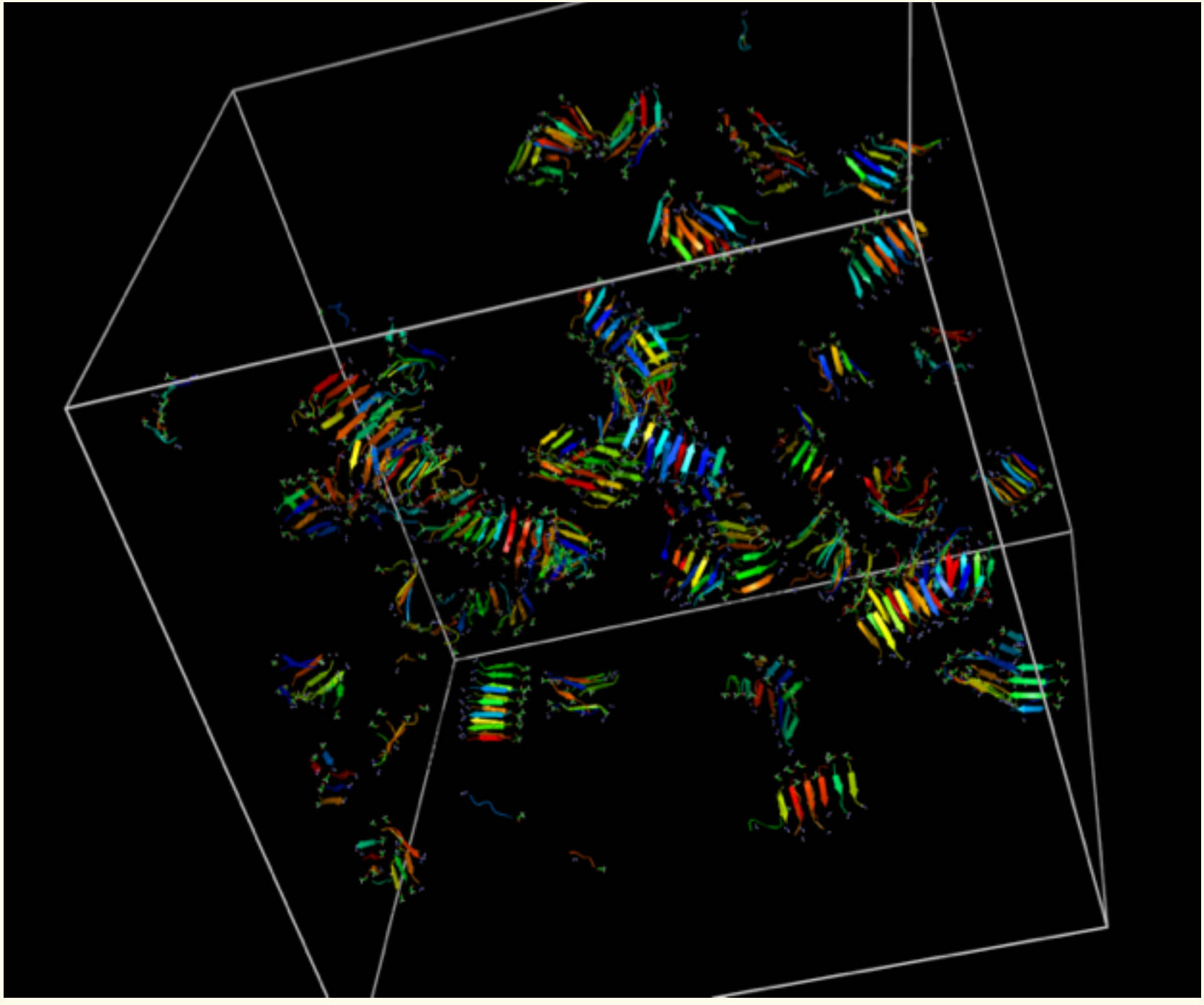
β-sheet organization against oligomer size

- PHF6 fibrils: parallel β-sheets
- Our simulated oligomers: mixed parallel/antiparallel β-sheets
- The fraction of parallel structure increases with oligomer size:



[Li, Mohanty, Irbäck and Huo, PLoS Comp. Biol. (2008)]

Snapshot from 500-chain run



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Shuanghong Huo, Clark U (PHF6)

Jan Meinke, Jülich (PHF6)