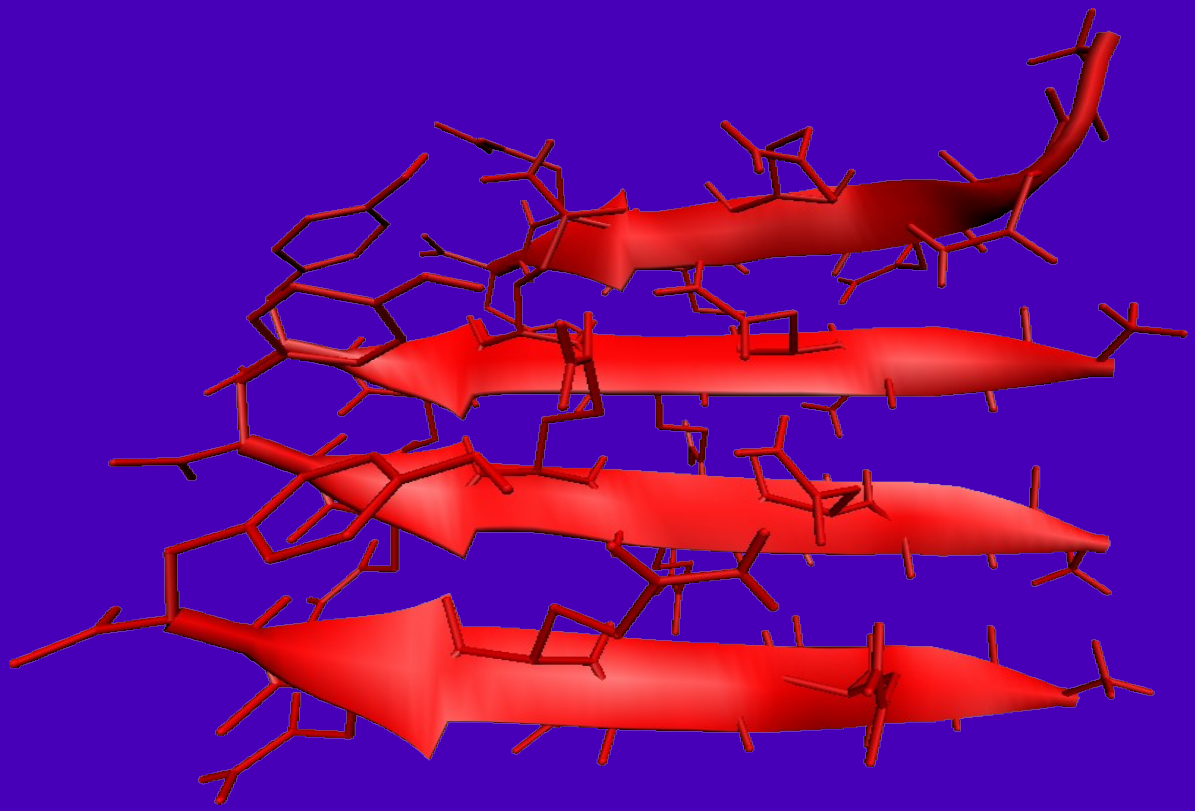
Exploring the Energy Landscape for Amyloid Formation



Telluride, 4th April 2007

Birgit Strodel
University of Cambridge
Department of Chemistry

What are Amyloid Fibrils ?

- **Amyloid** fibrils are encountered in several **diseases** such as **Alzheimer's**, **prion** diseases, or **Parkinson's**.

Sheep infected with **Scrapie**.

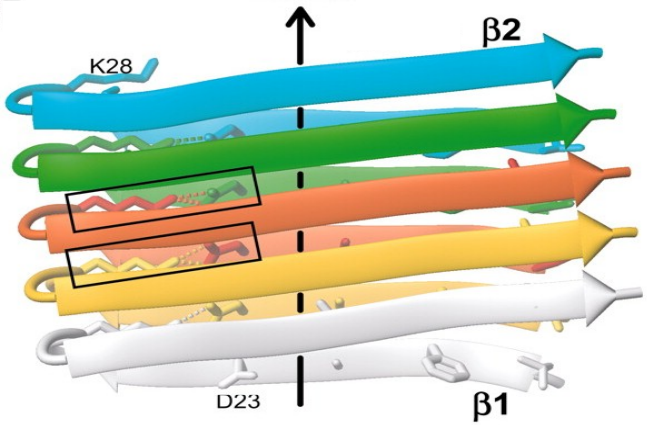
Fore people in Papua New Guinea infected with **Kuru** (“laughing death”) which is caused by their ritual cannibalism.

- Not only the 20 or so proteins associated with clinical disorders form amyloid structures; it now seems to be a **generic feature of polypeptide** chains.
- An increasing number of proteins have been found to form **amyloid fibrils** that have **functional** rather than disease-associated properties.
- For neurodegenerative diseases there is evidence that **soluble oligomers** - the **precursor** stage in fibril formation - are the **cytotoxic** agents.
- For a recent overview, see F. Chiti & C.M. Dobson, Annu. Rev. Biochem. **75**, 333-366 (2006)

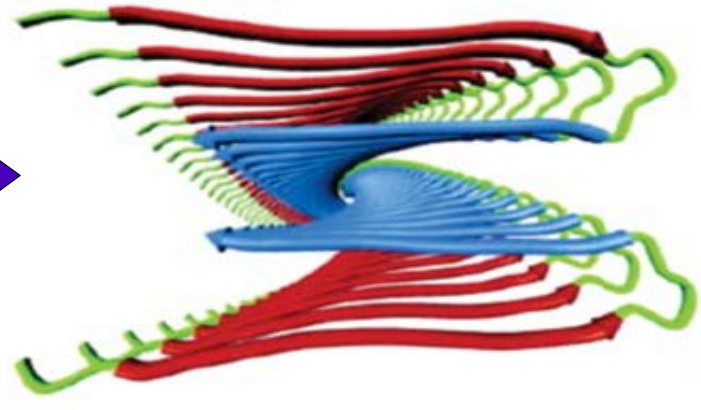
Structure of Amyloid Fibrils

Amyloid aggregates all share a common **cross β-sheet structure** with the β-strands perpendicular to the fibre axis and the β-sheets propagating along the direction of the fibre.

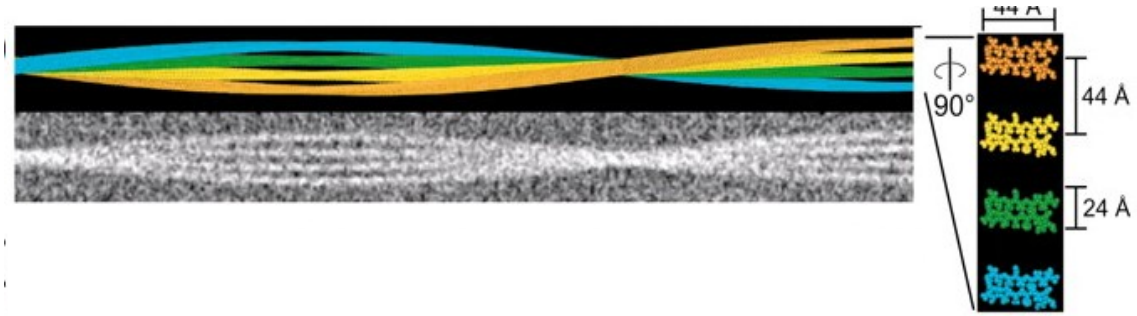
Example : Aβ(1-42)



β-sheet (β-tape)



protofibril



fibril

Lührs *et al.*, PNAS (2005) **102**, 17342 (PDB 2BEG)
Tycko, R. *et al.*, Biochemistry (2002) **42**, 3151

Methods

Methods

Motivation : We want to study the **early steps** of the **amyloid self-assembly**.
The questions which we would like to address are

i) What are the **driving forces** for amyloid formation?

ii) What are the **pathways** for amyloid formation? Are there **different** self-assembly **mechanisms** for different amyloidogenic peptides?

iii) Is a **reversal** of the early oligomerization process possible?

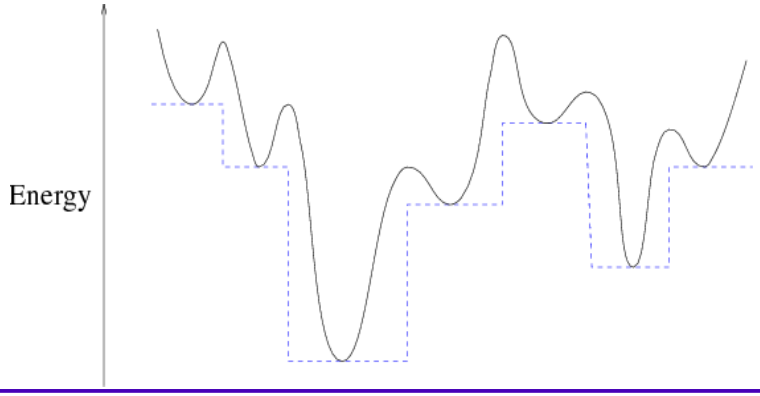
Our **approach** is as follows:

- All-atom description for the peptide: **CHARMM19** and the **EEF1** implicit solvent potential
- **Basin hopping (BH)** for global optimization of the oligomers.
- **Replica exchange molecular dynamics (REMD)** to calculate the **free energy surface** as a function of suitable order parameters.
- To identify the reaction coordiante we perform **Discrete Path Sampling (DPS)** between different minima of the free energy surface.
- From the DPS phenomenological **two-state rate constants** between ensembles of local minima can be obtained, which are **compared to experiment**.

Methods

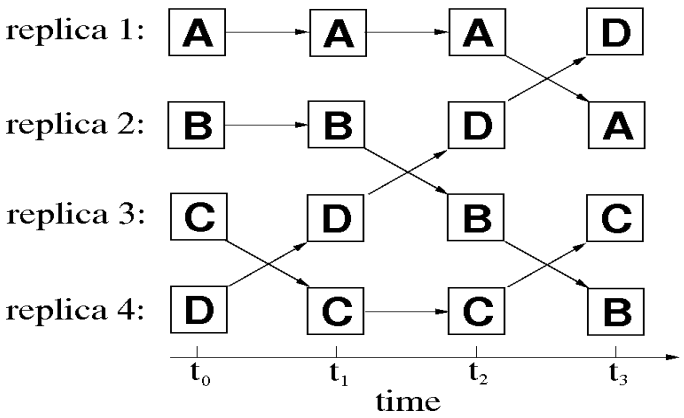
Basin hopping : Monte Carlo with minimization

$$\tilde{V}(\mathbf{Q}) = \min\{V(\mathbf{Q})\}$$



Wales, D. J.; Doye, J. P. K., JPCA (1997) 101, 5111-5116
Li, Z.; Scheraga, H. A., PNAS (1987) 84, 6611

Replica exchange molecular dynamics :



Replicas are exchanged according to the canonical **Metropolis criterion** for the exchange probability p :

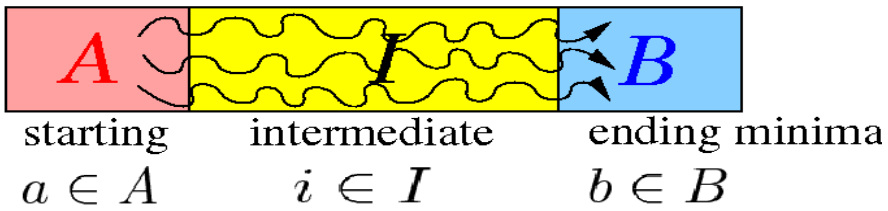
$$p = \begin{cases} 1 & : \quad \Delta \leq 0 \\ \exp(-\Delta) & : \quad \Delta > 0 \end{cases}$$

$$\Delta = \left(\frac{1}{k_B T_i} - \frac{1}{k_B T_j} \right) (V_i - V_j)$$

Free energy surfaces (FES) are constructed from the wheigted histogram analysis method (**WHAM**).
MMTSB : Brooks, C.L.; Feig, M.; Karanicolas, J., J. Mol. Graph. Model. **22** (2004), 377
WHAM: Kumar et al., J. Comp. Chem. 13 (1992), 1011

Discrete path sampling :

$$k_{BA}^{\mathrm{GT}} = \frac{1}{p_A^{\mathrm{eq}}} \sum_{a \in A} \frac{p_a^{\mathrm{eq}}}{\tau_a} \sum_{b \in B} P_{ba}$$

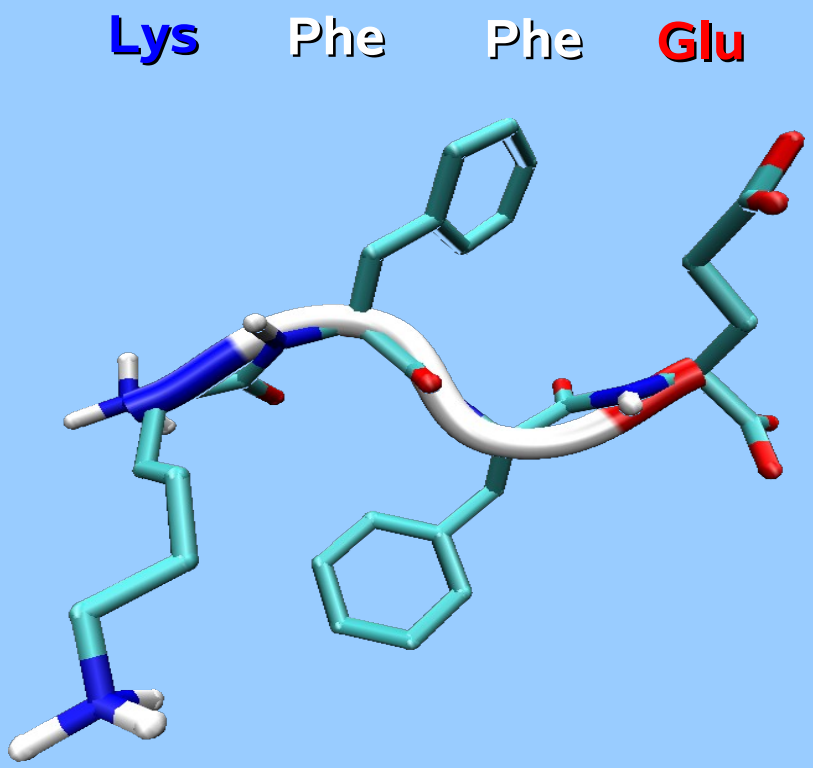


Wales, D. J., Int. Rev. Phys. Chem. (2006), 25 237-282

Example I :

KFFE

KFFE in experiment



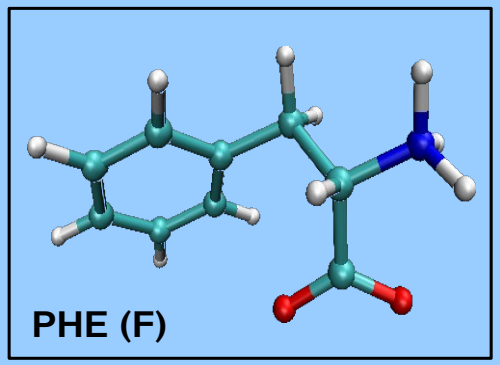
Experimental study by Tjernberg et al. showed:

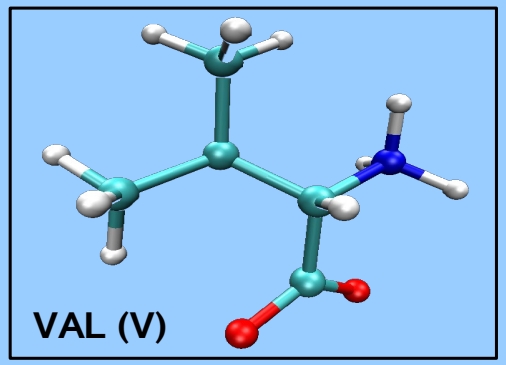
- KFFE and KVVE form amyloid fibrils.
- KLLE and KAAE do not form fibrils.
- KFFK and EFFE do not form fibrils.
- KFFK + EFFE (1:1) form amyloid fibrils.

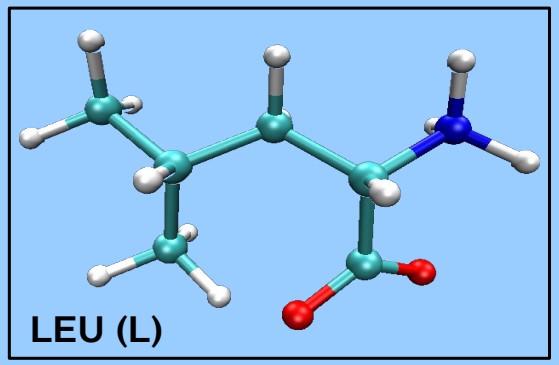
- KFFE and KVVE show partial β-strand conformation in solution.
- KLLE and KAAE show random structure only.

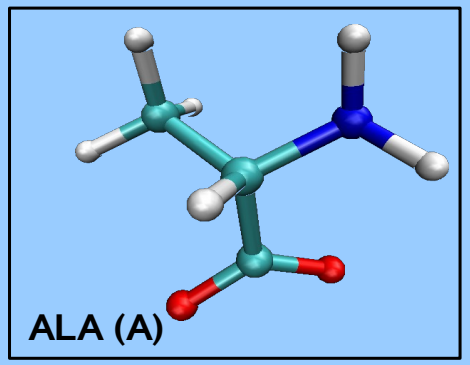
➔Formation of amyloid fibrils is promoted by complementary interpeptide **electrostatic interactions** and a high **β-propensity** of the monomer.

- No comment on the hydrophobic effect !







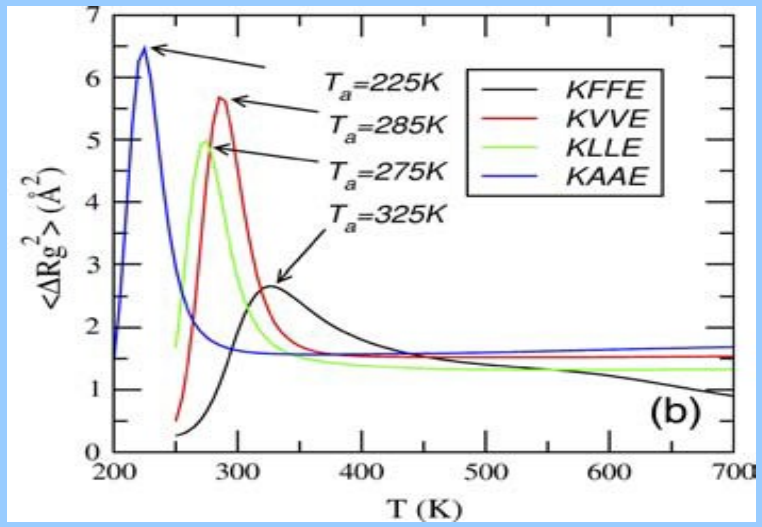


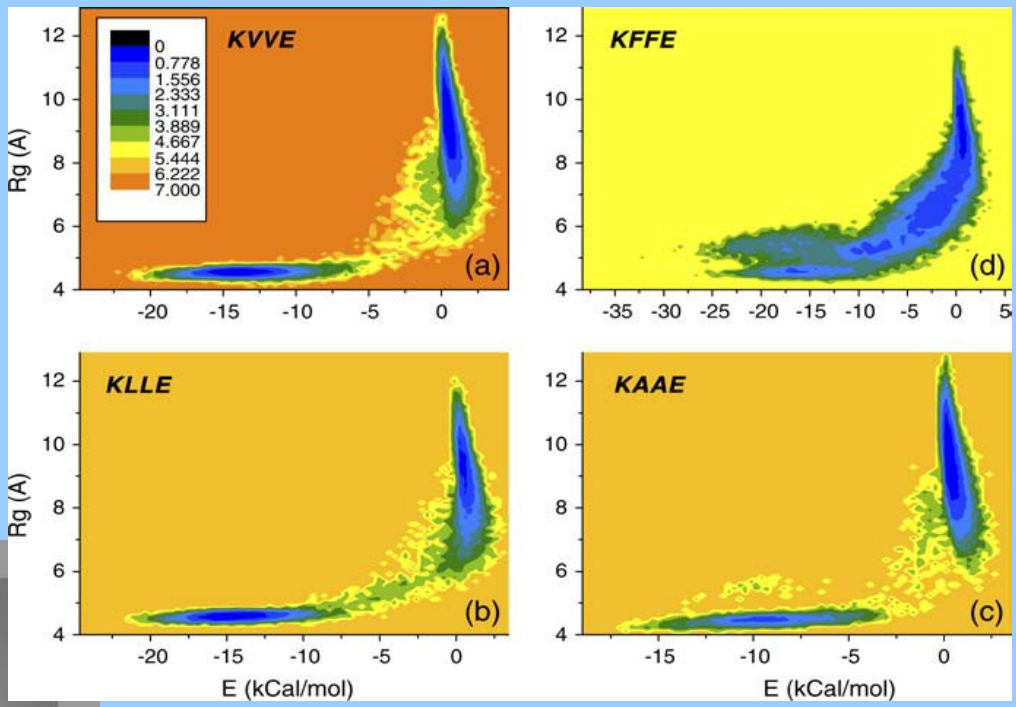
Tjernberg, L. *et al*., J. Biol. Chem. 45, 43243 (2002)

The KFFE dimer

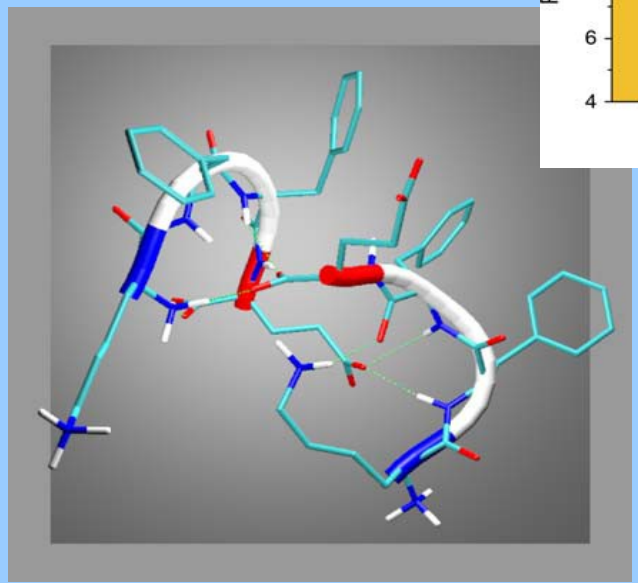
A **theoretical study** by A. Baumketner & J.-E. Shea, in which the **dimerization** of KFFE, KVVE, KLLE and KAAE is investigated, mainly supports the experimental results.

The method is **REMD** and as potential was the **CHARMM19** forcefield chosen together with the **Generalized Born** solvation model.





But the most stable KFFE dimer structure is **not sheet-like**:



Baumketner, A. & Shea J.-E.,
Biophys. J. 89, 1493-1503 (2005)

The KFFE dimer

Our approach : **Global optimization** with different potentials

1) CHARMM19 + EEF1

2) CHARMM19 + GB

3) CHARMM22 + GBSW

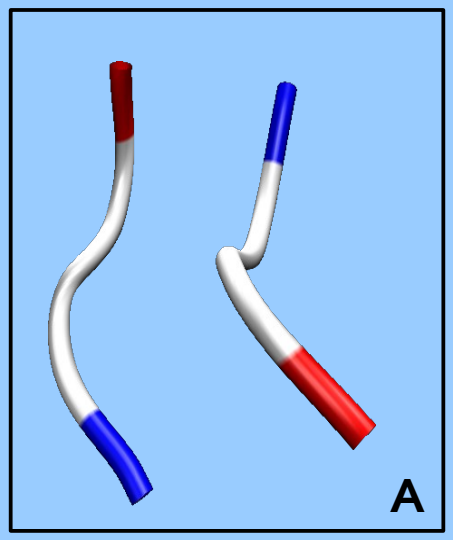
The resulting **lowest-energy structures** from each global optimization run 1) to 3) were then minimized using the other potentials to get an **energy ranking** as a function of the potential.

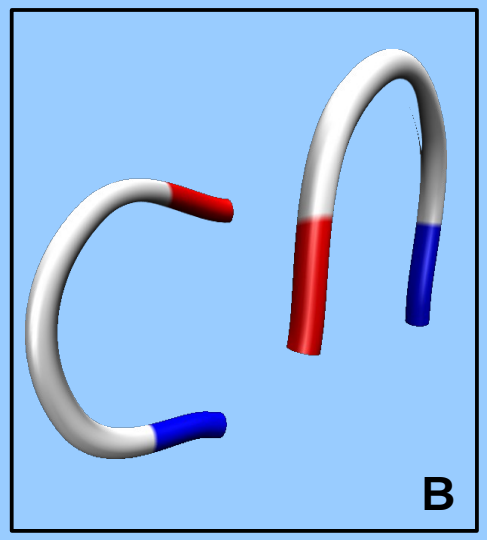
In addition to the classical force field potentials also **DFT geometry optimizations** were performed for the lowest-energy structures from 1) to 3).

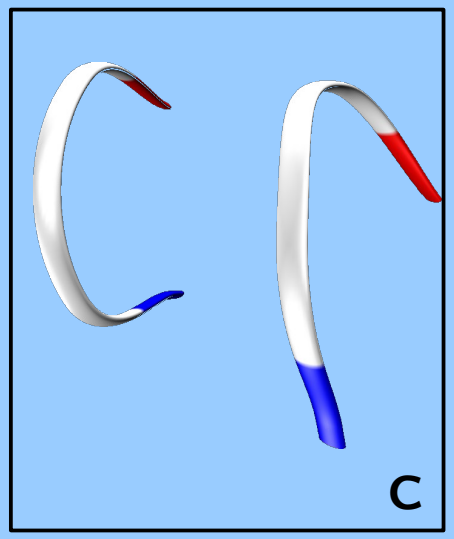
Details of the DFT calculations:

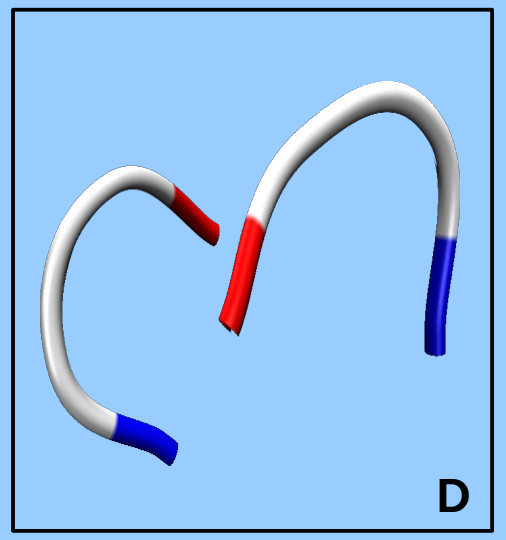
- pbe functional + dnp basis set (= double-zeta basis) as implemented in DMOL3
- COSMO as implicit solvent model

The KFFE dimer









	A	B	C	D
EEF1 energies	0	37.17	14.30	24.12
GB energies	26.36	0	26.25	20.35
GBSW energies	8.18	0.22	0	3.02
DMOL3 energies	1.51	22.34	9.71	0

(all energies in kcal/mol)

The KFFE dimer

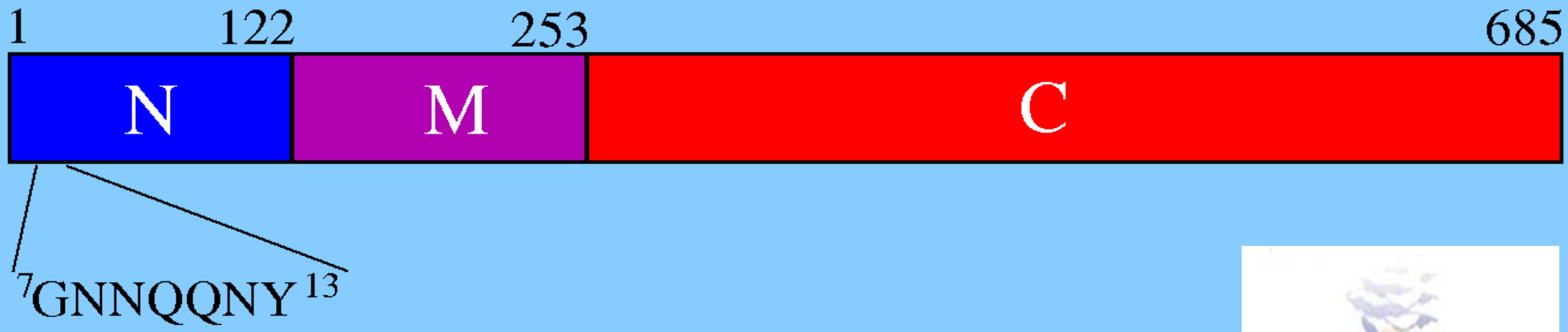
What next ?

- Global minima for **in-vacuo** potentials, i.e. CHARMM19 (with EEF1 parameter file), CHARMM19, CHARMM22 and the DFT energies without COSMO)
- **Free energy surfaces** for different solvent models? (CHARMM19+EEF1, CHARMM22+GBSW, CHARMM22+TIP3P)
- Origin of different global minima for different potentials / solvent models? (**Structure analysis**)
- Intermolecular interaction calculated by SAPT/DFT or the distributed-multipole-analysis potential of S. Price & A. Stone
- **Methodological developments** for the MC step taking scheme to improve the sampling of peptide oligomers.

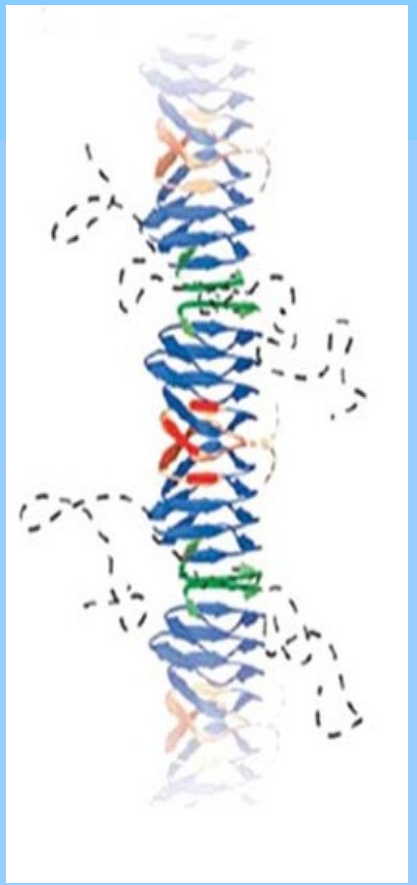
Example II :

GNNQQNY

GNNQQNY – a 7-residue peptide from Sup35



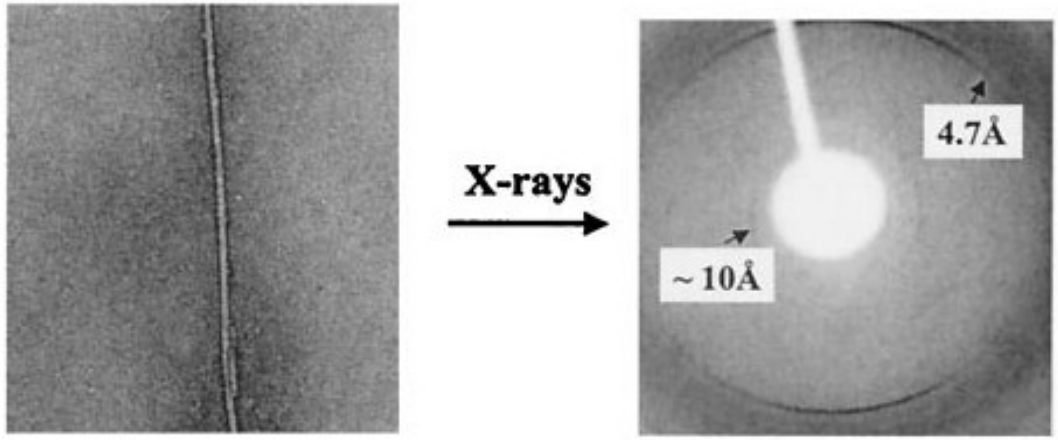
- **Sup35** is a 685-residue **prion-like** protein in yeast
- Residues **1-122** are the **prion-determining** domain (**N region**), which contains a series of 9-residue repeats with sequence PQGGYQQYN.
- Residues 123-253 are the highly charged M region, and residues 254-685 function in **translation termination** (Sup35's **normal** cellular role).
- **GNNQQNY** is a 7-residue polar peptide **from the N region** that exhibits the **amyloid** properties of full-length Sup35:
  - cooperative kinetics of aggregation with a concentration-dependent lag time
  - binding of the dye Congo red
  - the characteristic cross-β- x-ray diffraction pattern



Amyloid fibrils from the NM region of Sup35.
Nature 435 (2005), 765

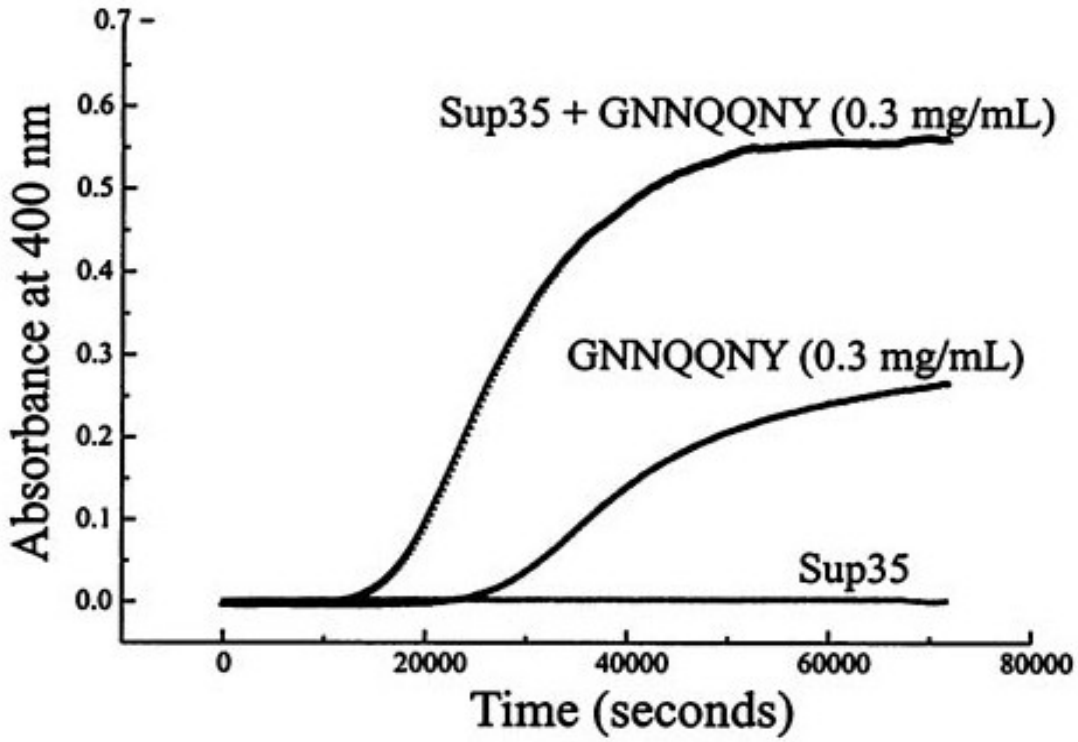
Amyloid fibrils of GNNQQNY

An electron micrograph of the GNNQQNY amyloid fibrils and their x-ray diffraction pattern.



Fibril diameter ~ 75 Å

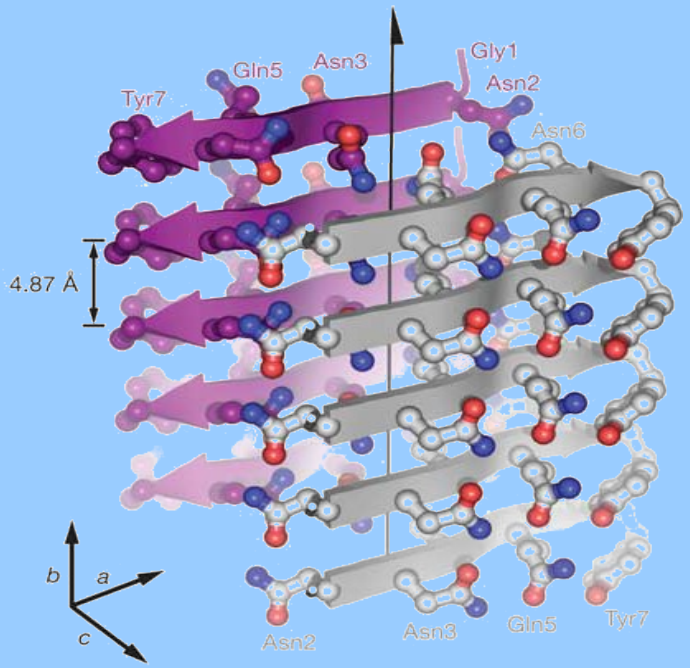
Kinetics of amyloid formation for GNNQQNY, Sup35 and a mixture of both.

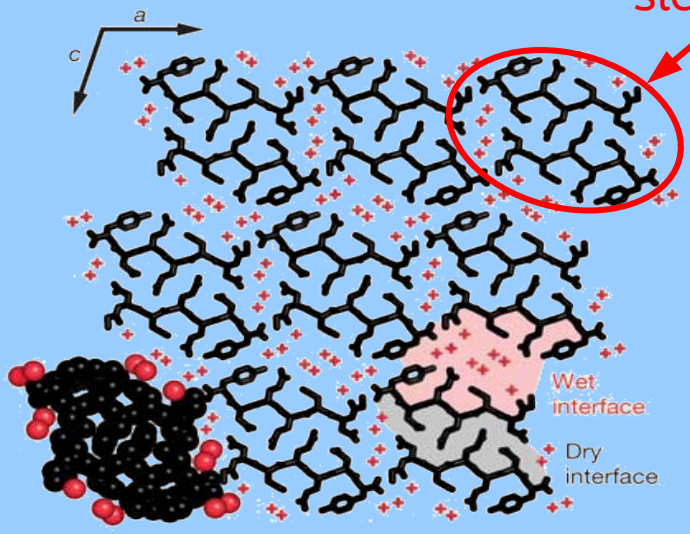


Balbirnie, M.; Grothe, R.; Eisenberg, D.S., PNAS 98 (2001), 2375

Amyloid fibrils of GNNQQNY

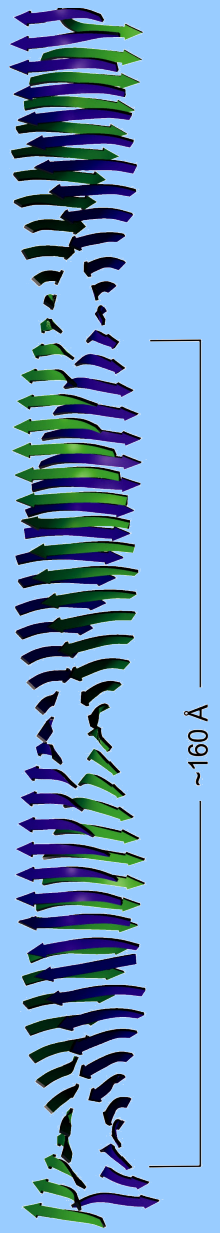
Experiment : (PDB 1YJP)

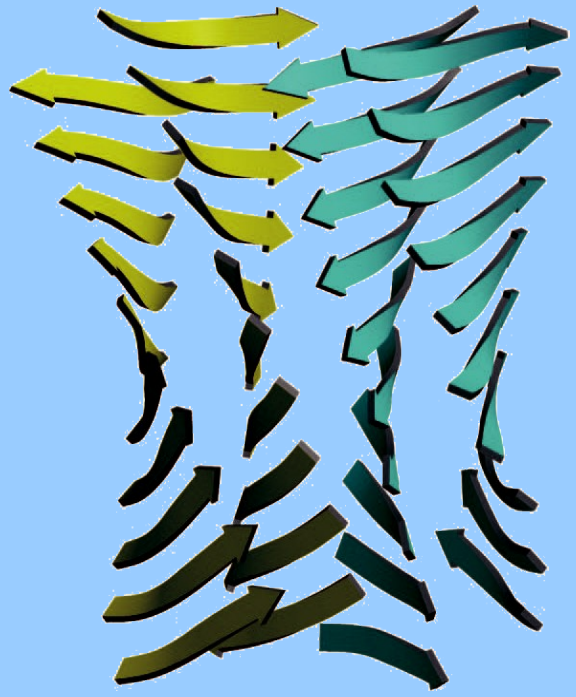


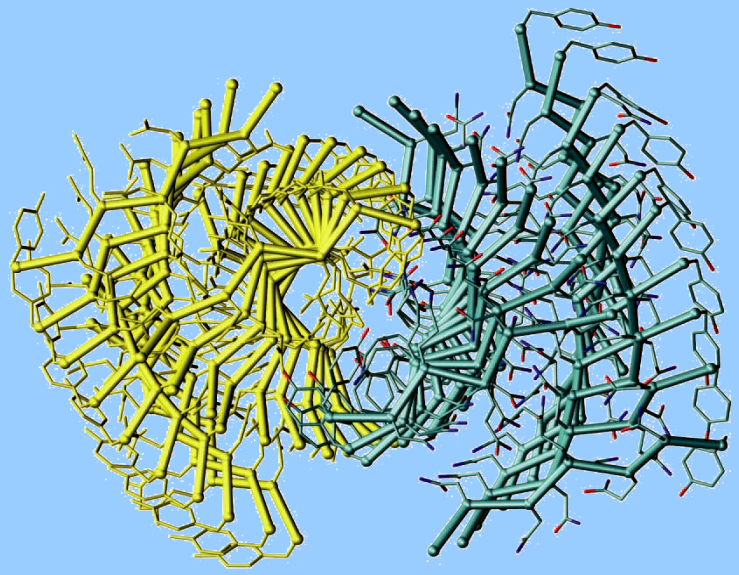


Nelson, R. *et al*., Nature 435, 773 (2005)

Theoretical study :







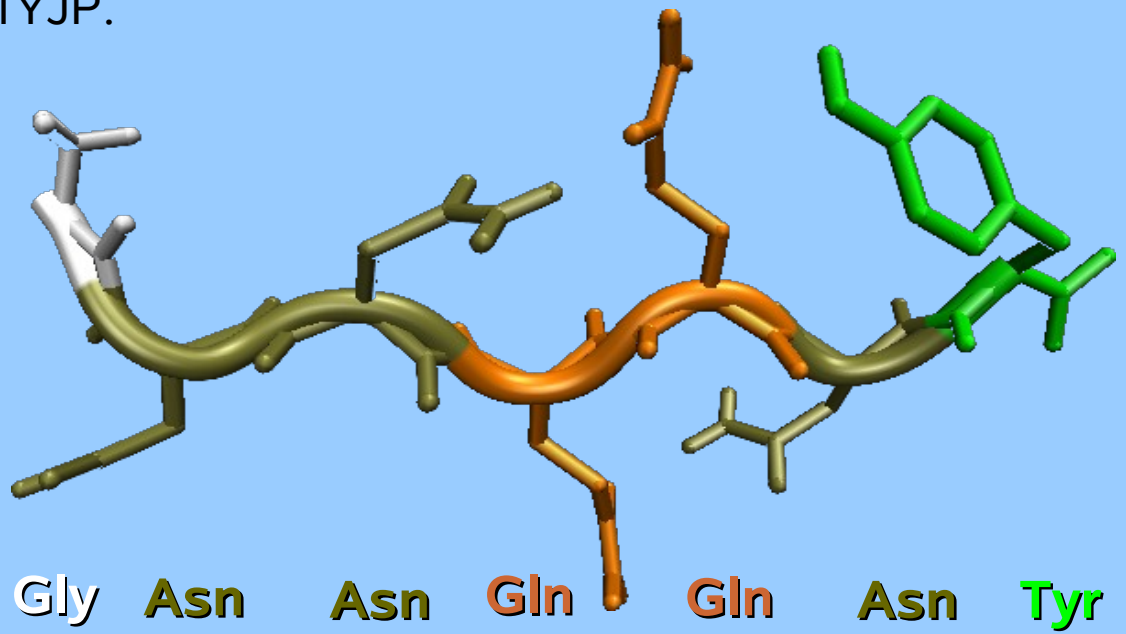
Esposito, L. *et al*., PNAS 103, 11533-11538 (2006)

The GNNQQNY monomer

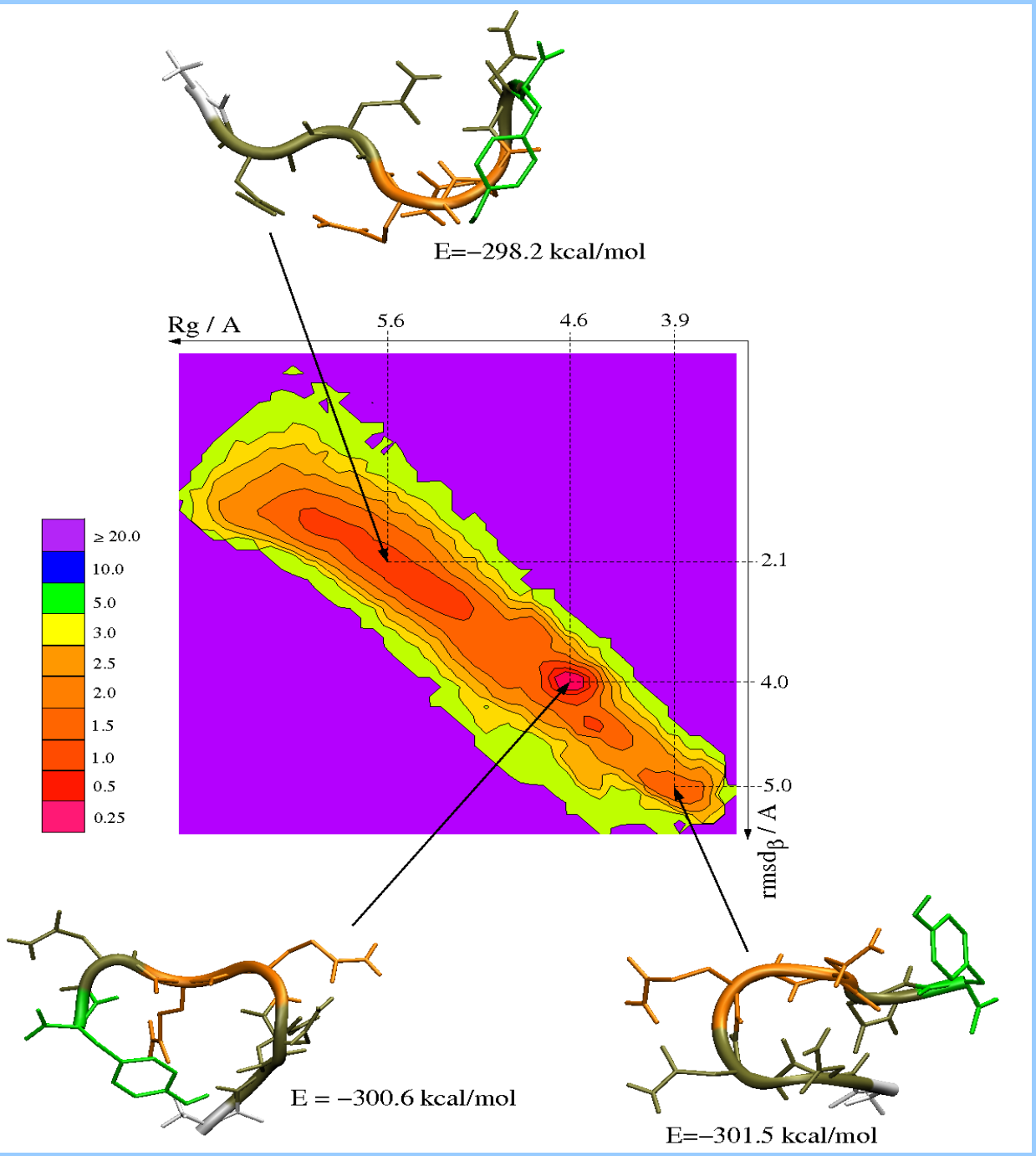
Motivation :

- To study the monomer was motivated by a simulation done by R. Pellarin & A. Caflisch in which they had used a coarse-grained model of an amphipathic polypeptide to investigate **kinetics of aggregation and the pathways of fibril foramtion**.
- They found that with **increasing** relative **stability of the β-prone state** of the polypeptide, **disordered aggreagation** changes into **fibrillogenesis** with oligomeric **on-pathway** intermediates, and finally **without intermediates** for very stable β-prone peptides.
- R. Pellarin & A. Caflisch suspect **GNNQQNY** belonging to the class of very stable **β-prone** peptides and thus should exhibit very **fast fibrillogenesis**.

Starting strucure : A β-strand which is the building block of the amyloid structure, taken from PDB 1YJP.

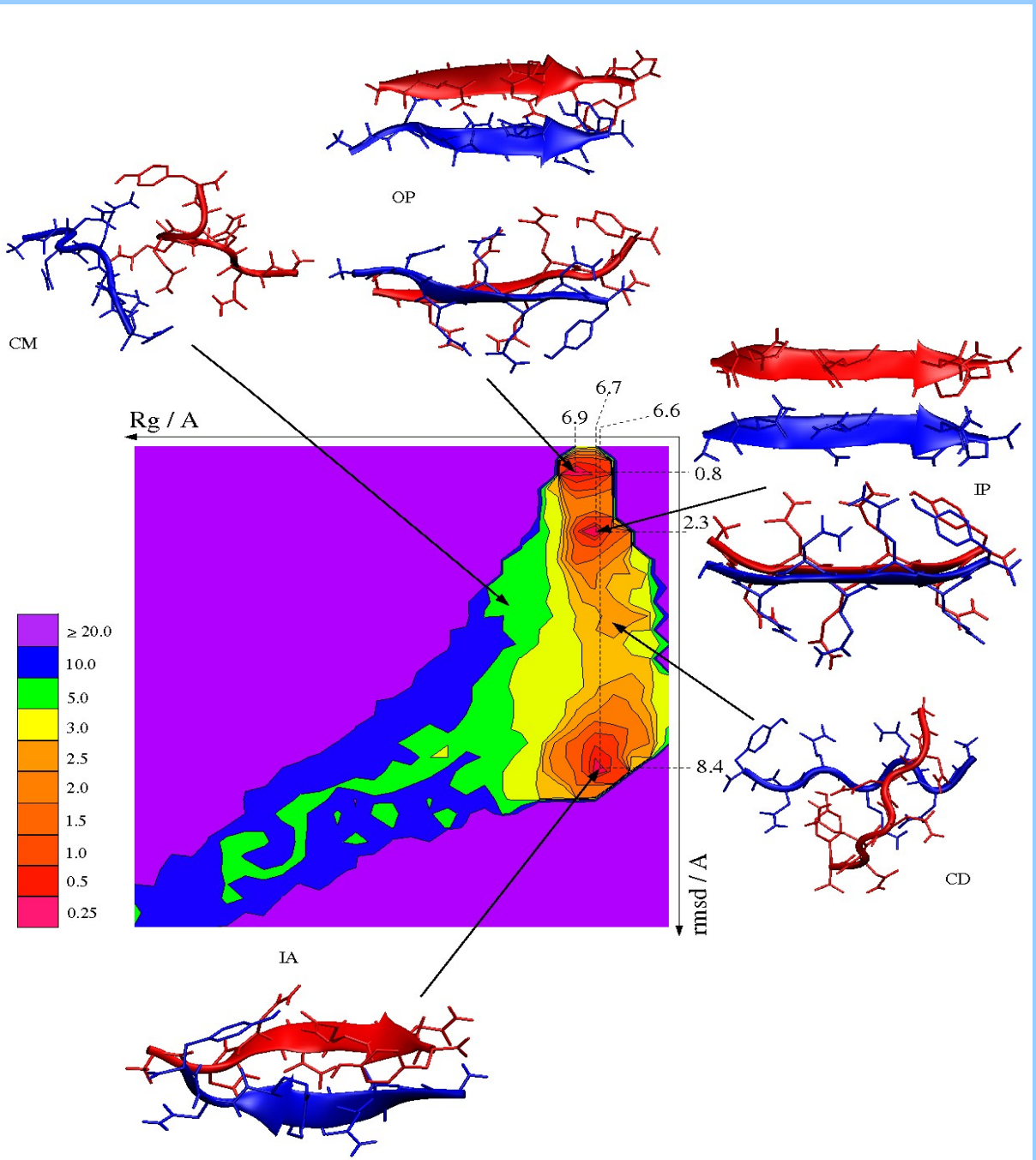


The GNNQQNY monomer



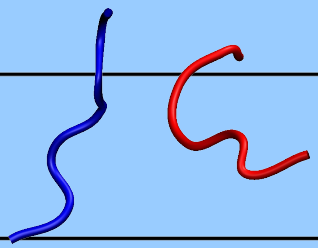
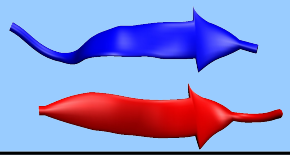
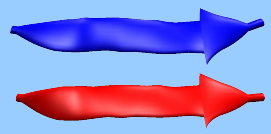
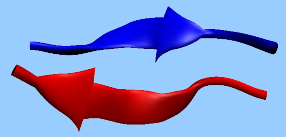
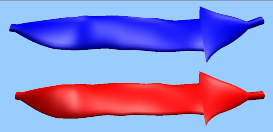
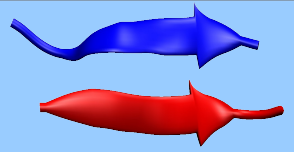
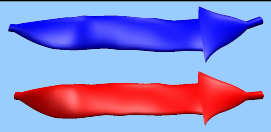
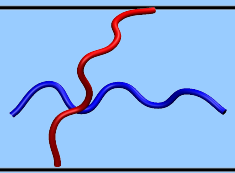
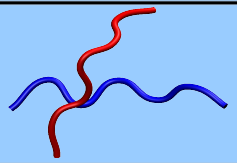
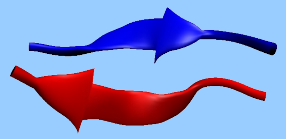
- CHARMM19 + EEF1
- 100-ns REMD with 8 replicas between 250 and 400 K
- FES for T=298 K, order parameters:
  i) radius of gyration
  ii) C_α-RMSD from the β-strand

The FES of the GNNQQNY dimer

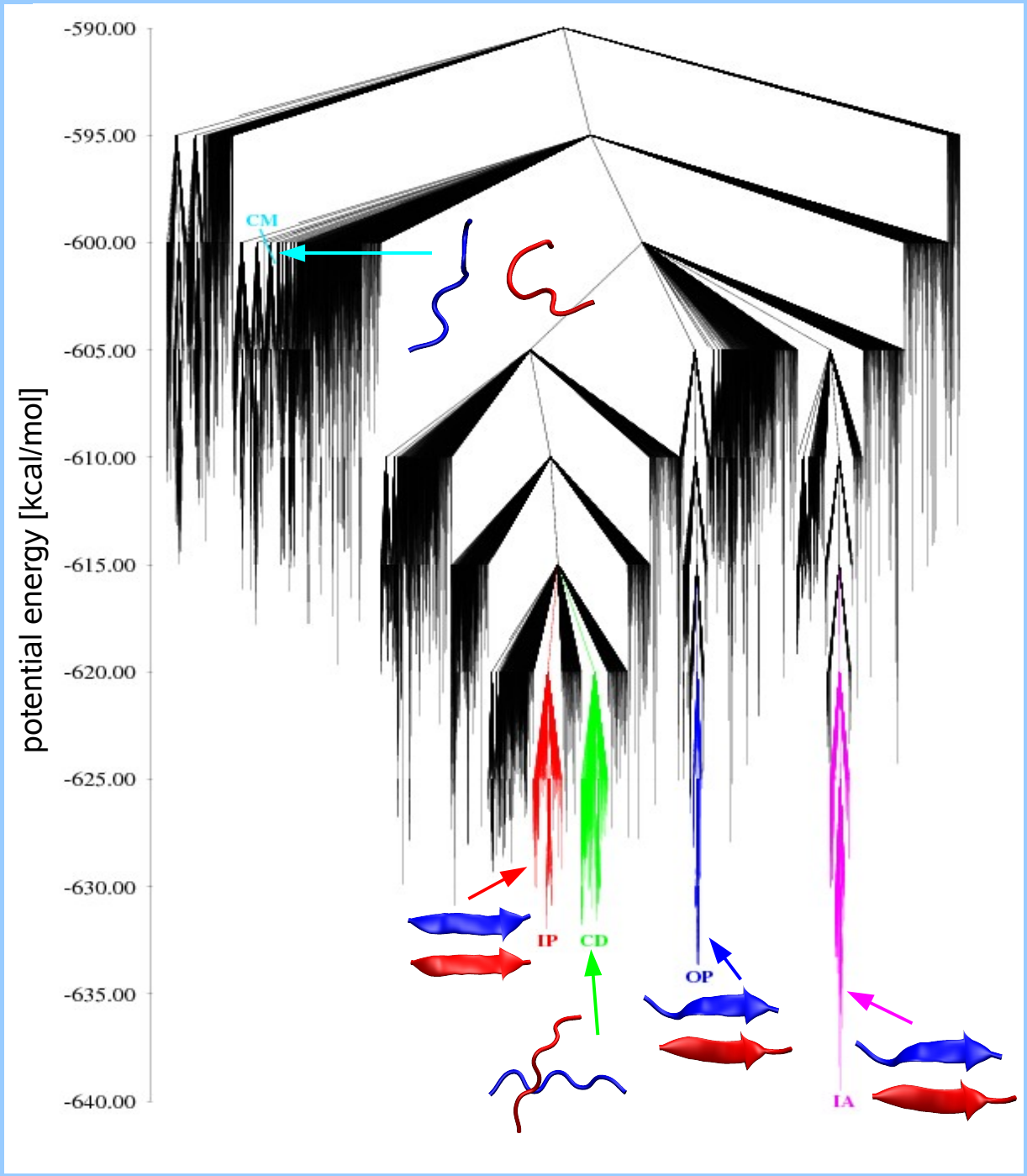


- CHARMM19 + EEF1
- 100-ns REMD with 16 replicas between 220 and 600 K
- concentration of 10 mM
- FES for T=298 K, order parameters:
  i) radius of gyration
  ii) C_α-RMSD from the off-register, parallel dimer (OP)
  (found in a global optimization run)

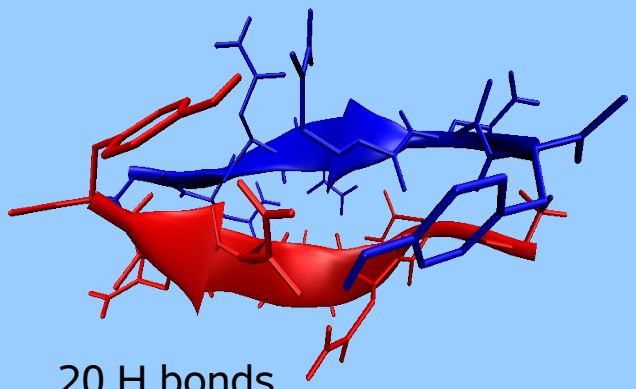
DPS for the GNNQQNY dimer

A	B	$k^{\mathrm{GT}}_{B\leftarrow A}/\mathrm{s}^{-1}$	$k^{\mathrm{GT}}_{A\leftarrow B}/\mathrm{s}^{-1}$
		10^{+4}	10^{-8}
		10^{-1}	10^{-11}
		10^{-2}	10^{-11}
		10^{-7}	10^{-6}
		10^{-2}	10^{+4}
		10^{-4}	10^{-6}

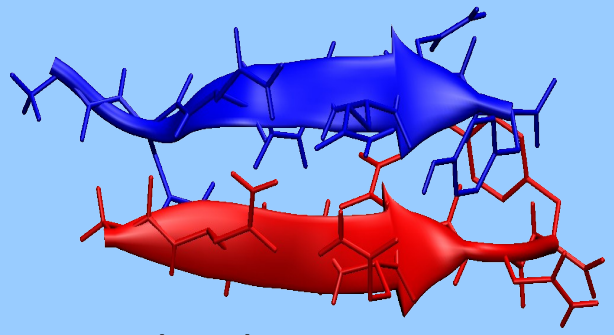
Disconnectivity graph for the GNNQQNY dimer



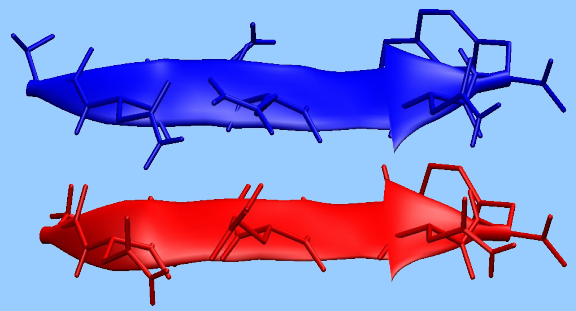
Interaction energy for the GNNQQNY dimer



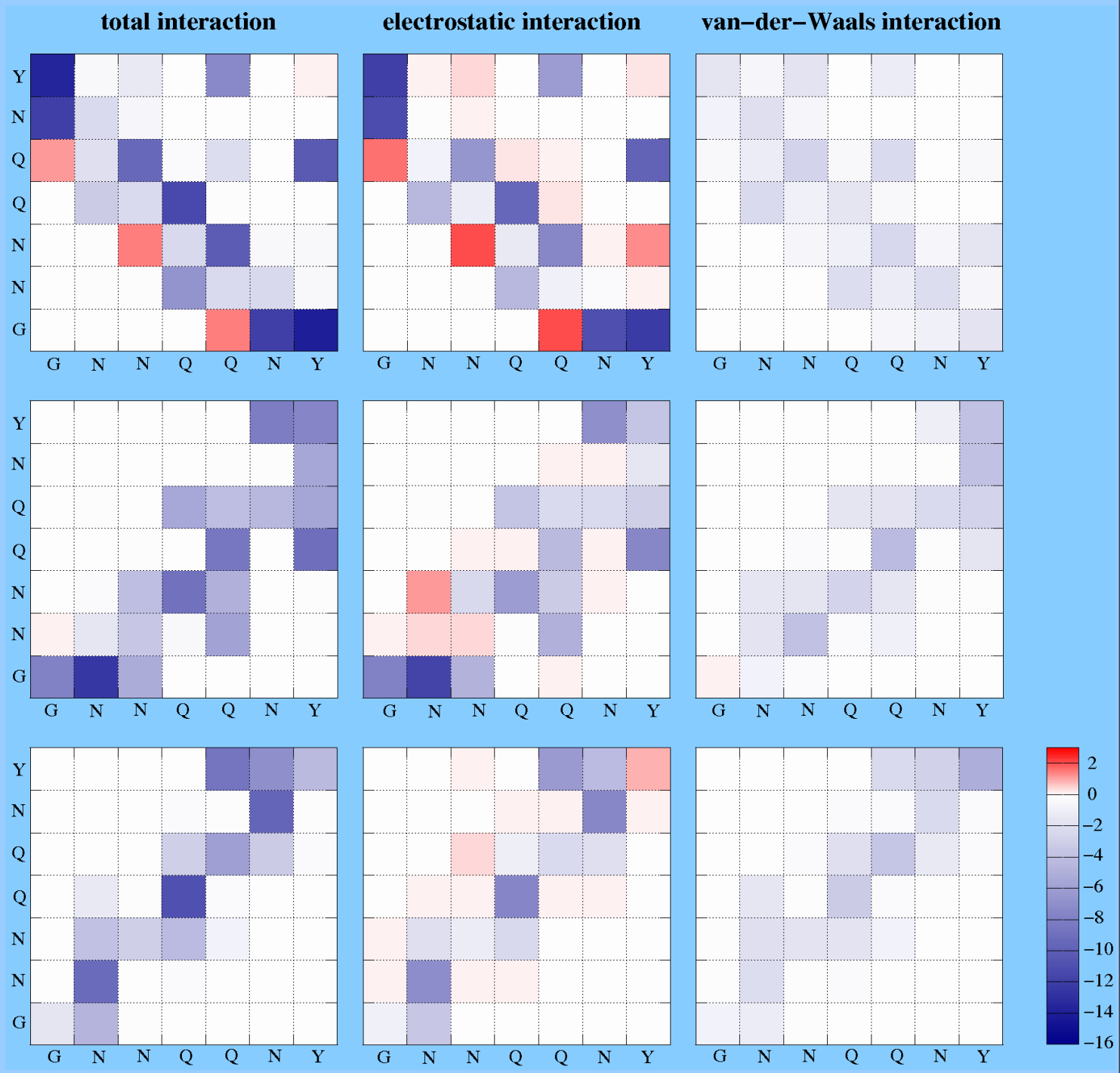
20 H bonds



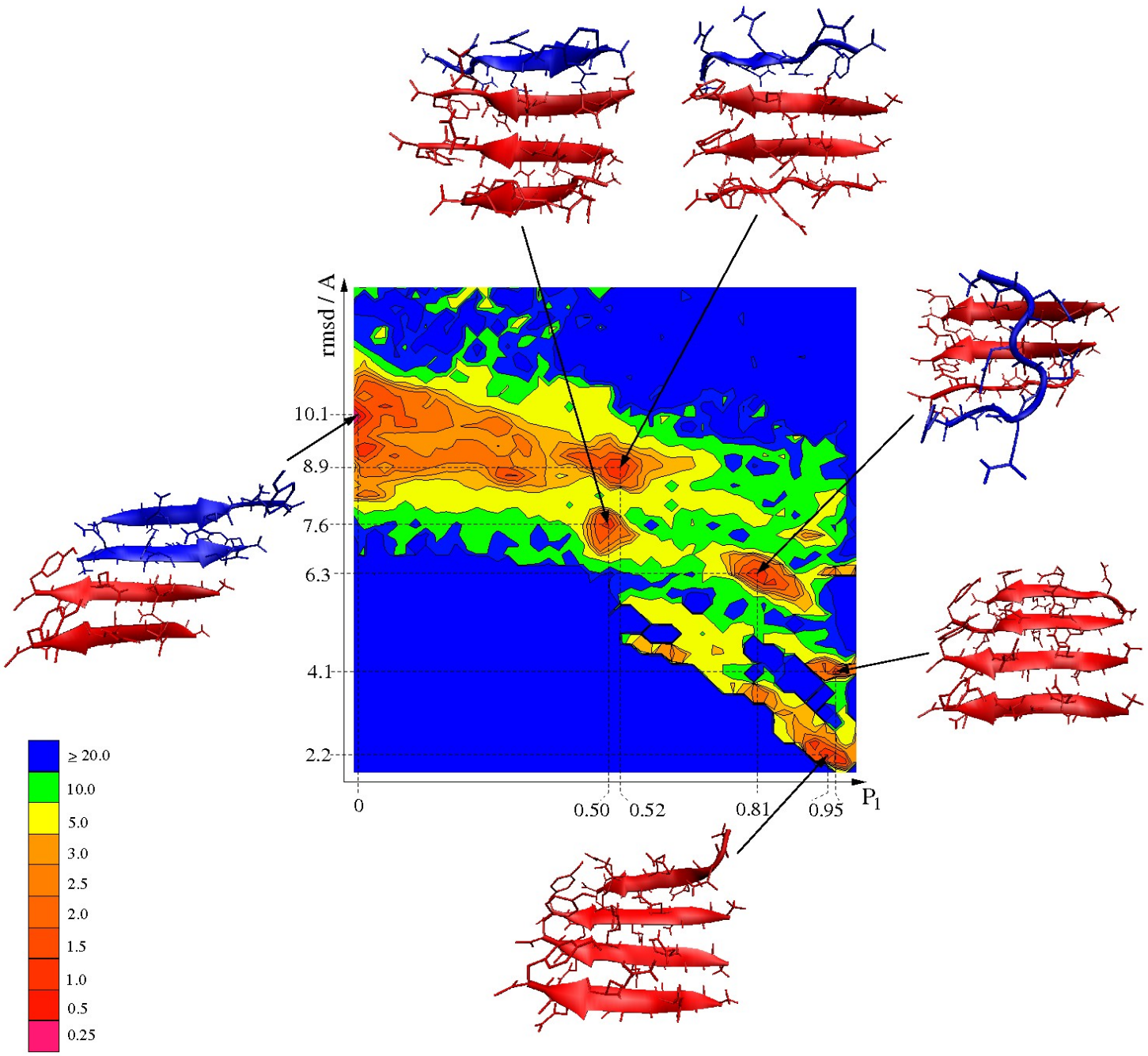
15 H bonds



14 H bonds



The GNNQQNY tetramer



- CHARMM19 + EEF1
- 100-ns REMD with 16 replicas from 220 to 600 K
- concentration of 10 mM
- FES for T=298 K, order parameters:

  i) C_α-RMSD from the β-sheet tetramer (PDB structure)

  ii) polar order parameter

$$\langle P_1 \rangle = \frac{1}{N} \sum_{i=1}^{N} \mathbf{z}_i \cdot \mathbf{d}$$

$\mathbf{d}$ director

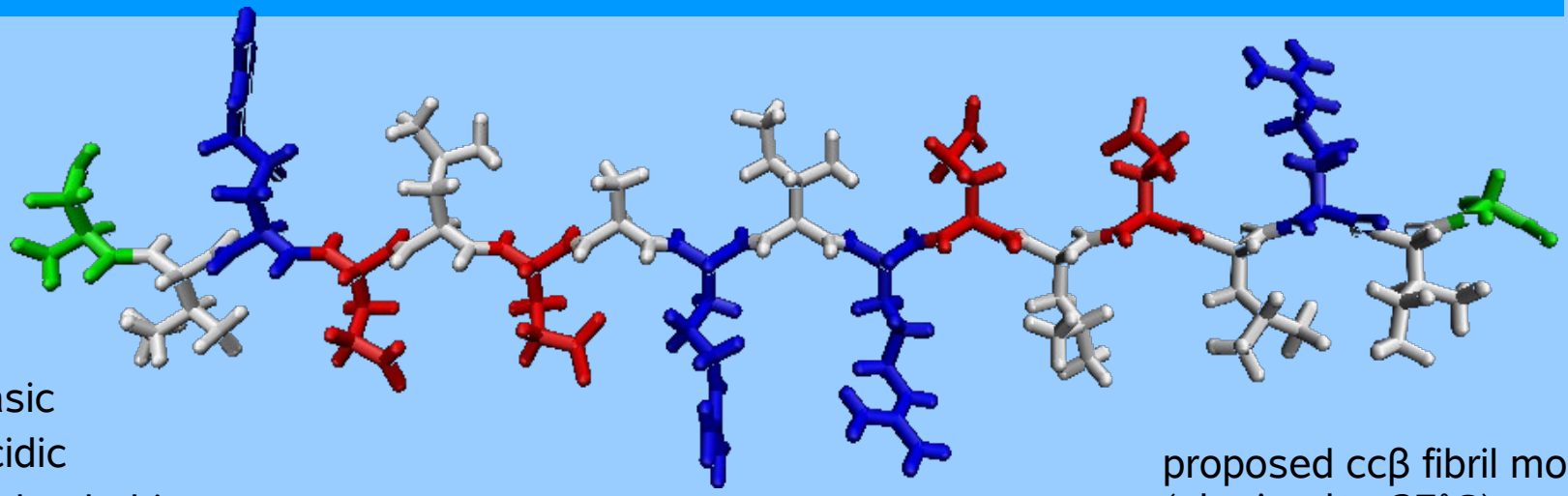
$\mathbf{z}_i$ end-to-end vector of peptide i

Example III :

ccβ

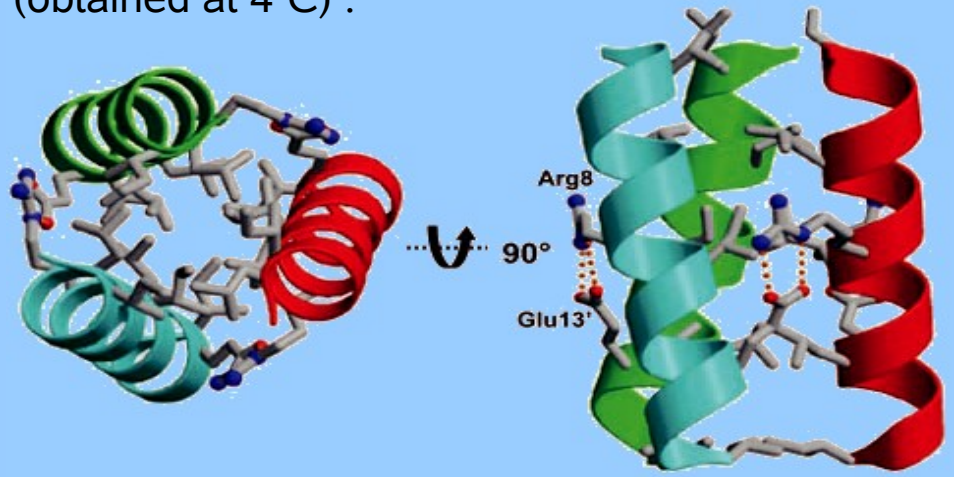
Amyloid formation by ccβ

SER-ILE-ARG-GLU-LEU-GLU-ALA-ARG-ILE-ARG-GLU-LEU-GLU-LEU-ARG-ILE-GLY

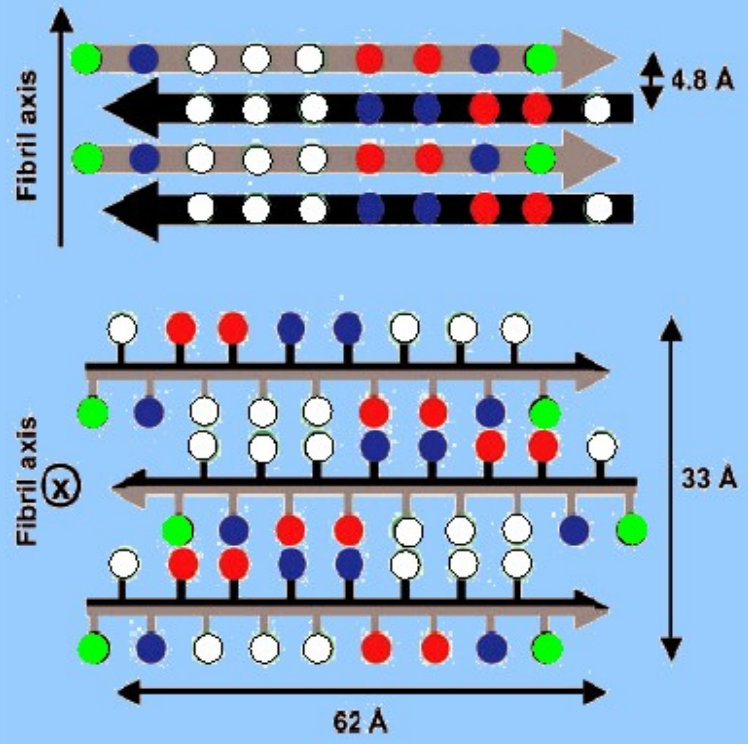


basic
acidic
hydrophobic
polar

crystal structure of ccβ
(obtained at 4˚C) :

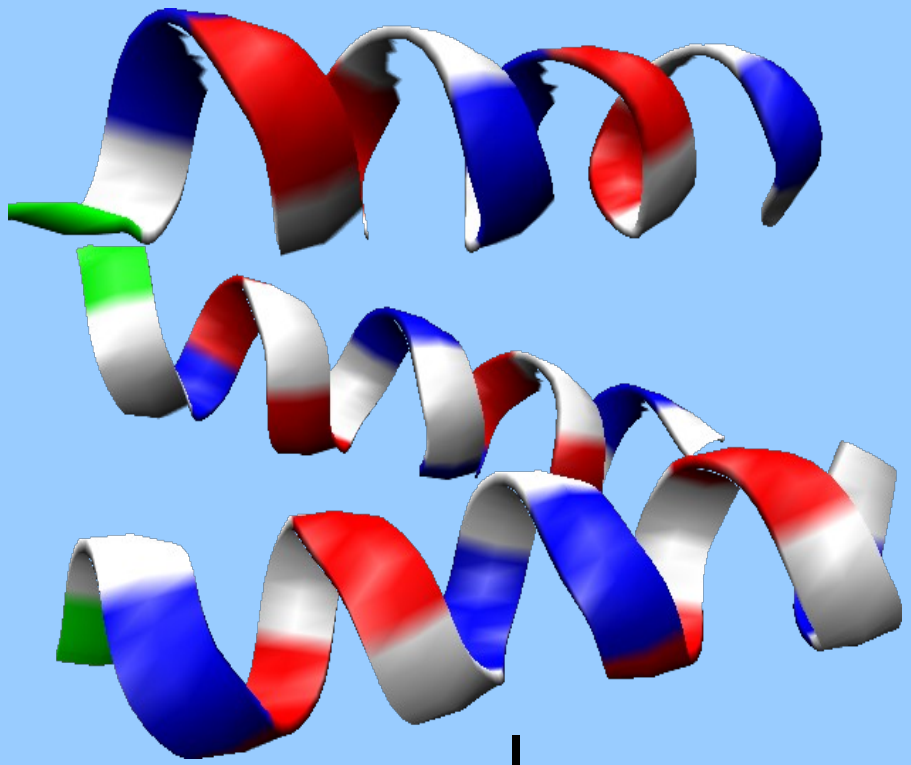


proposed ccβ fibril model
(obtained at 37˚C) :



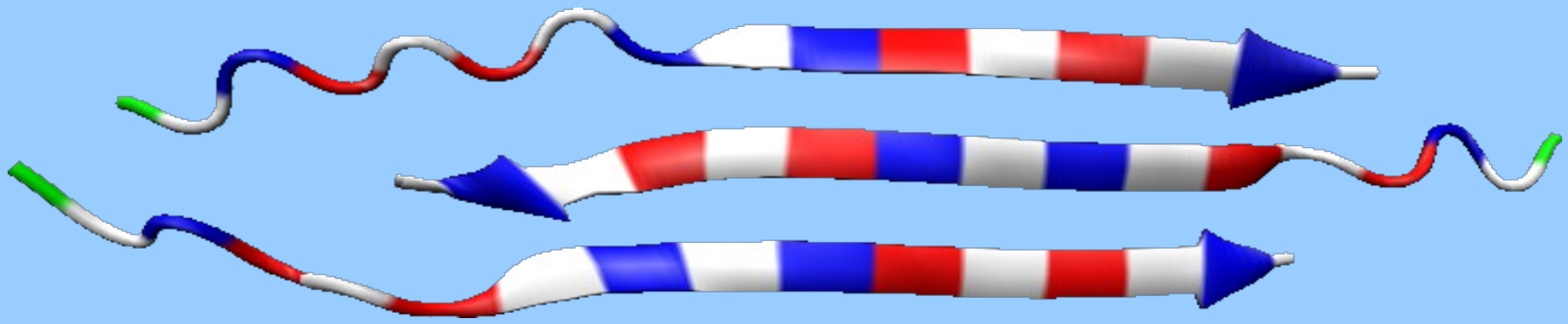
Kammerer, R. A. *et al.* , PNAS 101, 4435-4450 (2004)

Amyloid formation by ccβ



pathway ?

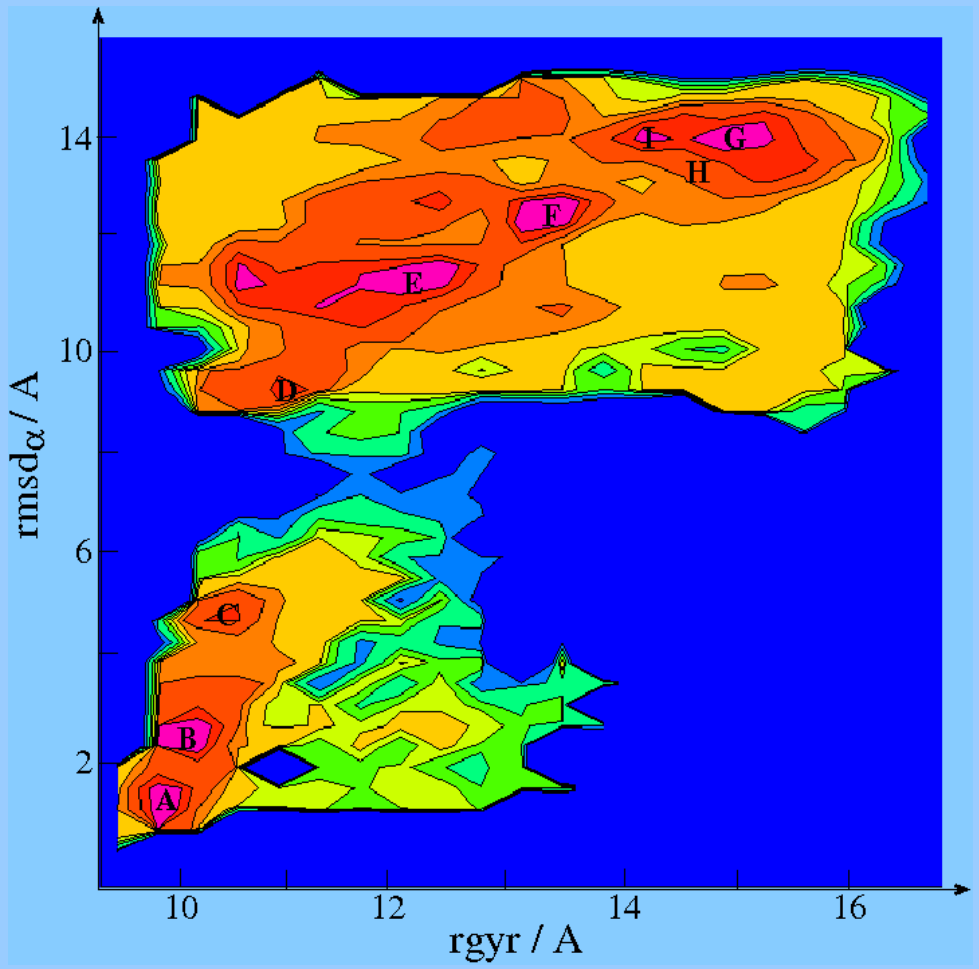
β-sheet constructed with CNSsolve,
fulfills NMR data from Kammerer *et al.*:

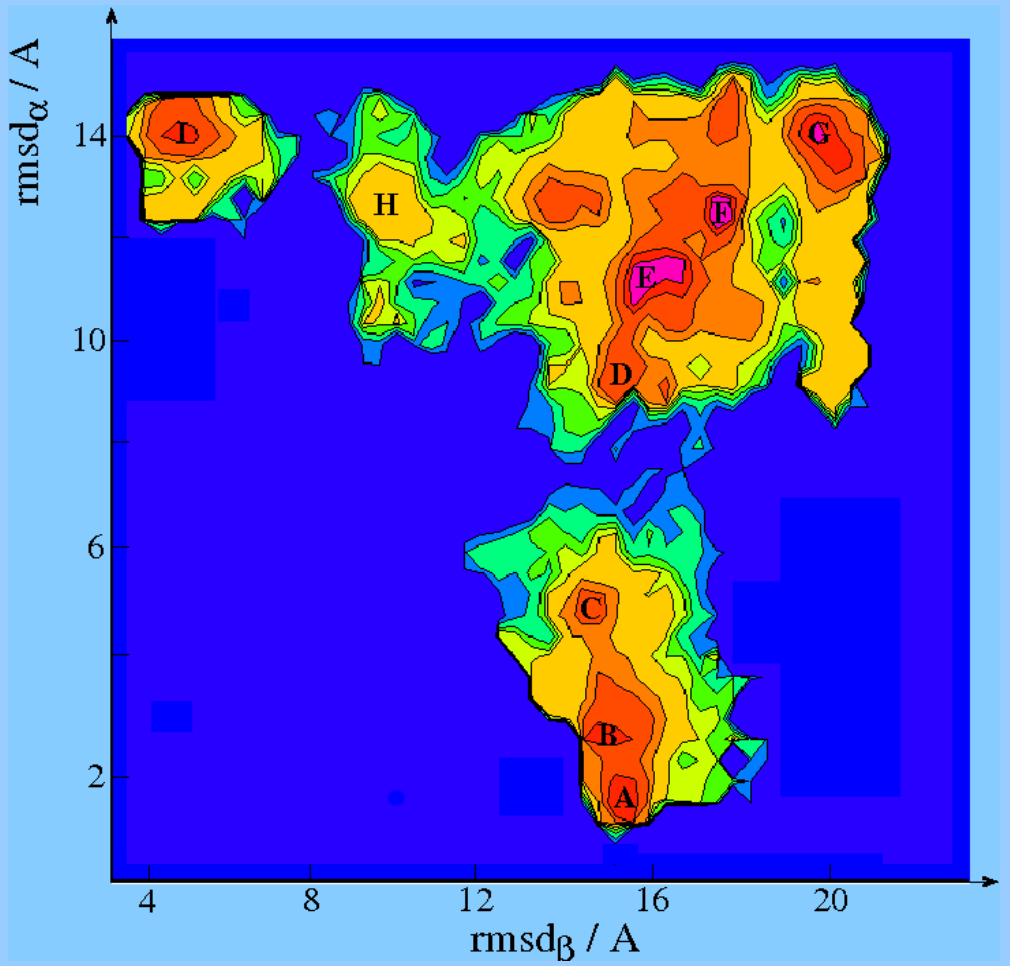


REMD for the ccβ trimer

WHAM free energy surfaces at 310 K :

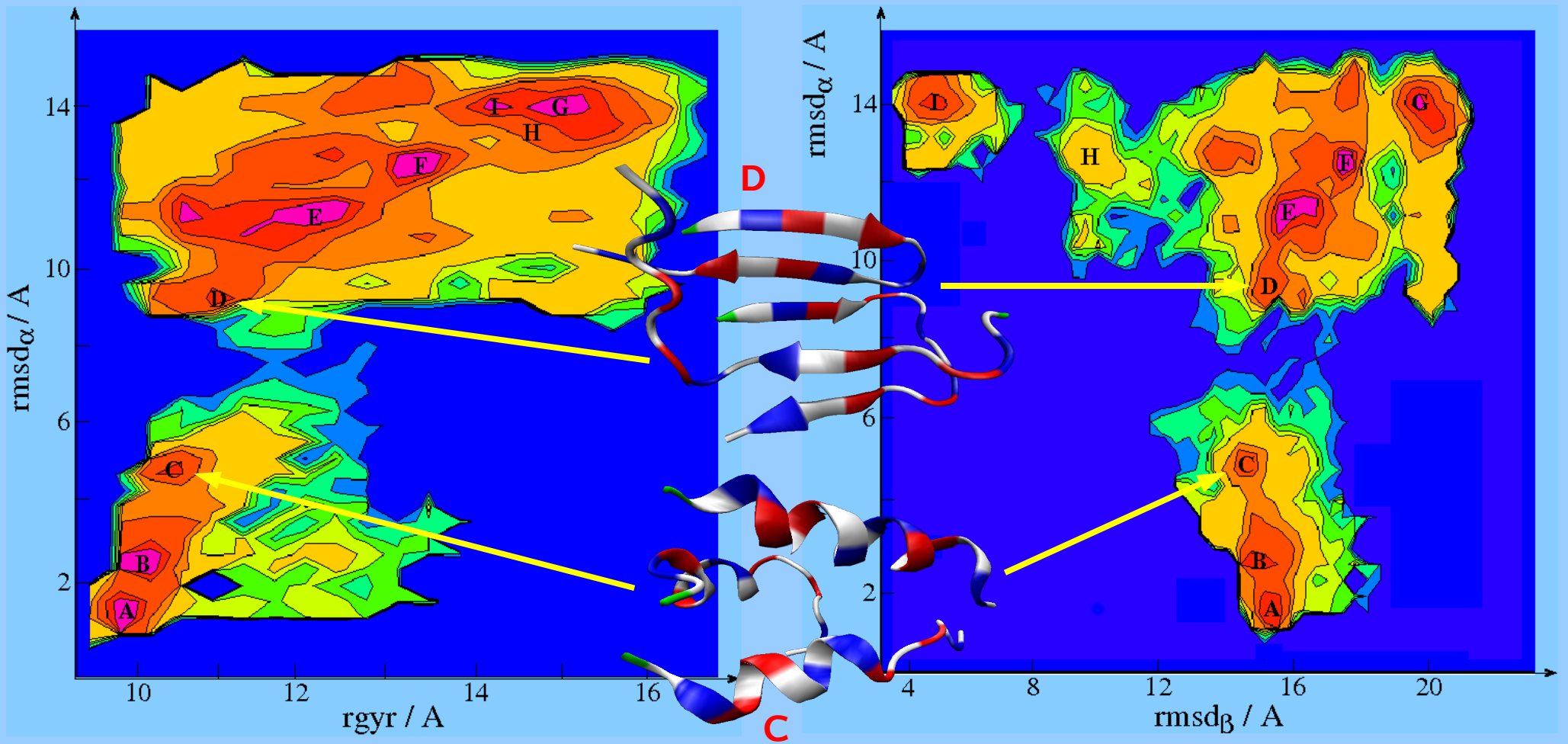
- CHARMM19 + EEF1
- 100 ns REMD with 16 replicas between 220 and 600 K
- concentration of 10 mM
- order parameters: RMSD from α-helix trimer, RMSD from β-sheet and radius of gyration (rgyr)



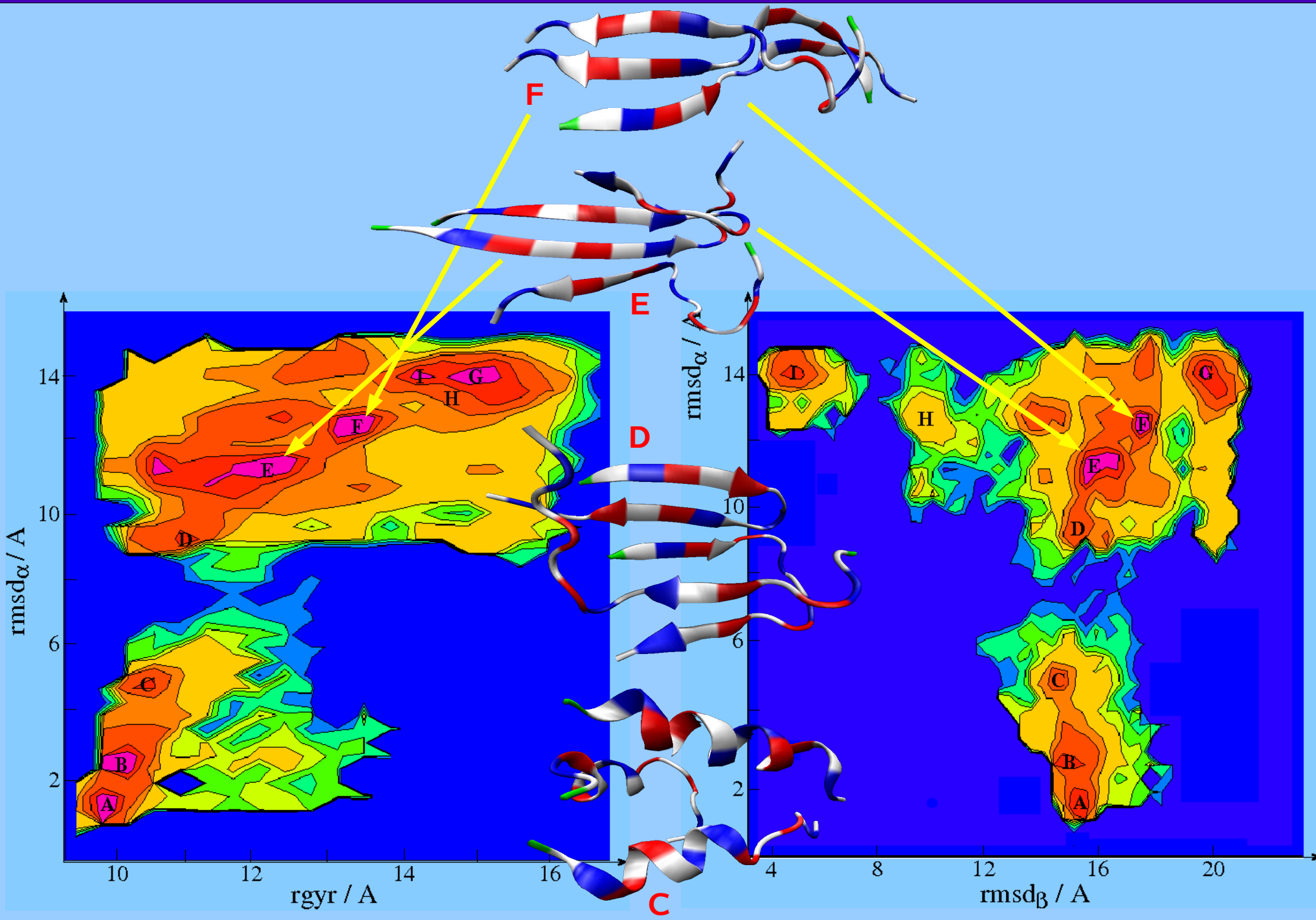


What structures dominate the free energy surface ?

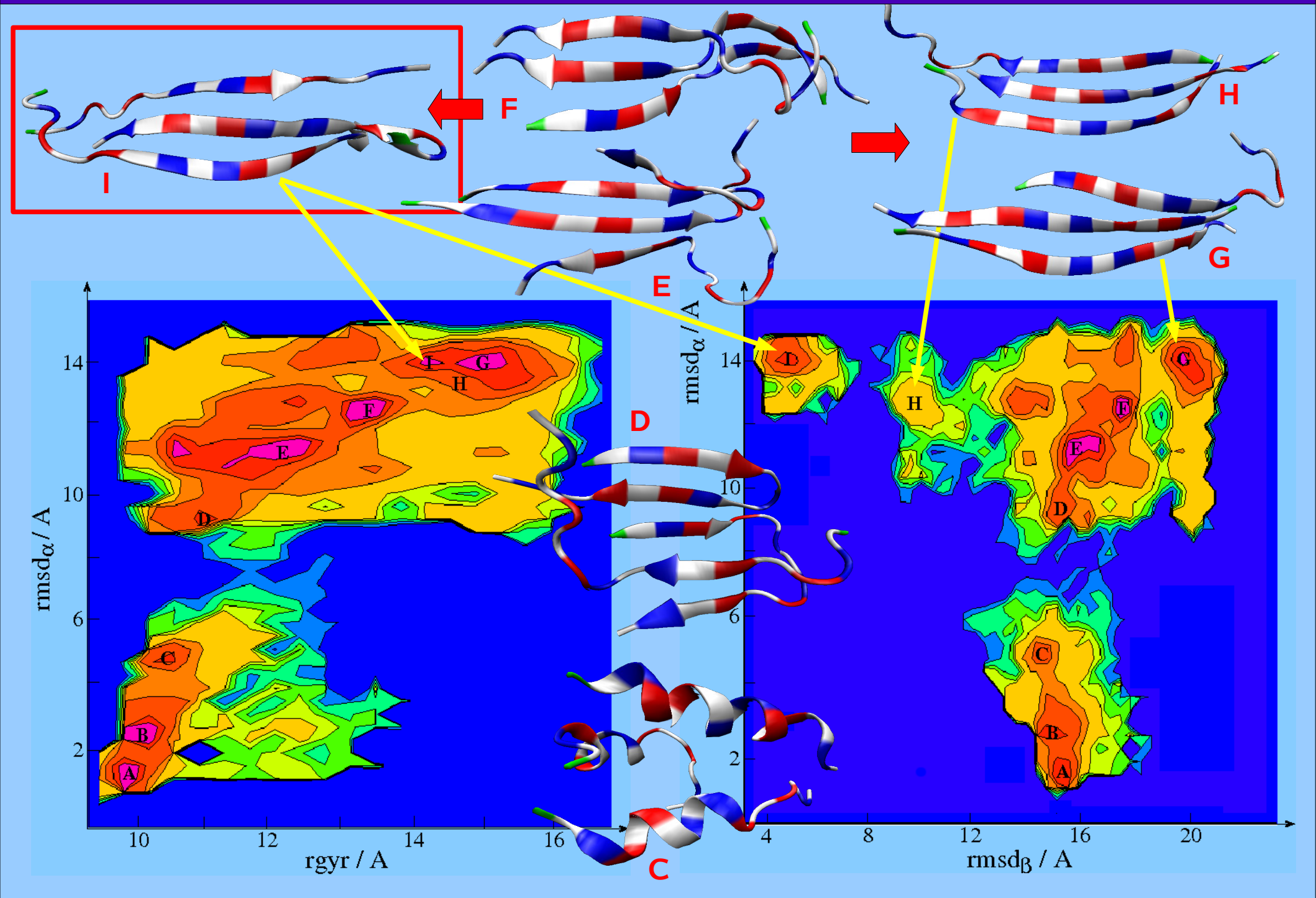
FES for the ccβ trimer



FES for the ccβ trimer



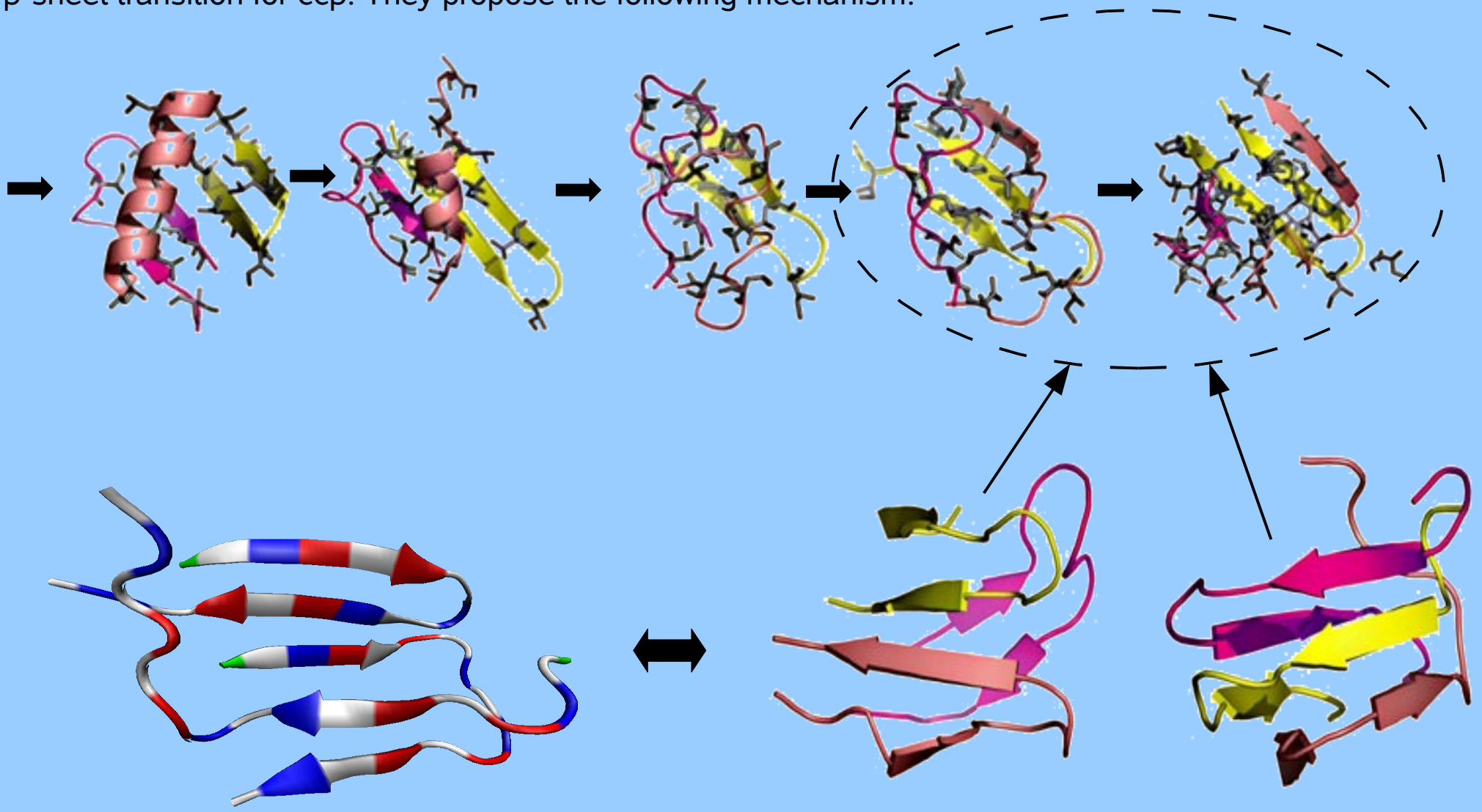
FES for the ccβ trimer



Amyloid formation by ccβ

β-hairpin aggregate as intermediate ?

N. V. Dokholyan and co-workers did an MD study with a coarse-grained potential to study the α-helix to β-sheet transition for ccβ. They propose the following mechanism:



Ding, F. *et al.*, J. Biol. Chem. 280, 40235 (2005)

Future projects

Future Projects

KFFE : Free energy surfaces for the KFFE dimer using different potentials and solvent models.
Origin of the structural differences.

GNNQQNY : Finishing the tetramer study.
Studying the octamer.

ccβ : Finding a pathway with $k \geq 10^{-8}$ 1/s.

Comparison of self-assembly pathways for amyloidogenic hepta- and octapeptides with different hydrophobicity.

Methodological developments to improve Basin Hopping and Discrete Path Sampling for the exploration of amyloid formation.

Acknowledgments

Thanks to

David Wales for support and methodological developments.

Chris Whittleston for doing most of the GNNQQNY calculations.

Michele Vendruscolo for helpful discussions.

Paul Strodel for the DFT calculations for the KFFE dimer.

Anthony Fitzpatrick for providing the β-sheet structure for ccβ.

BBSRC for financial support.

Thank you for listening !