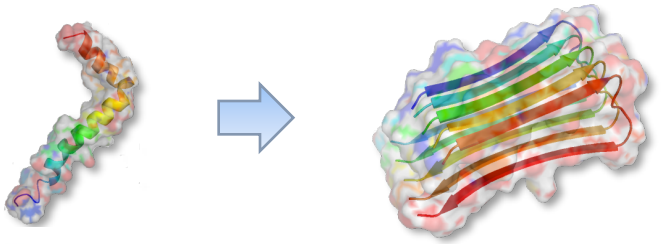
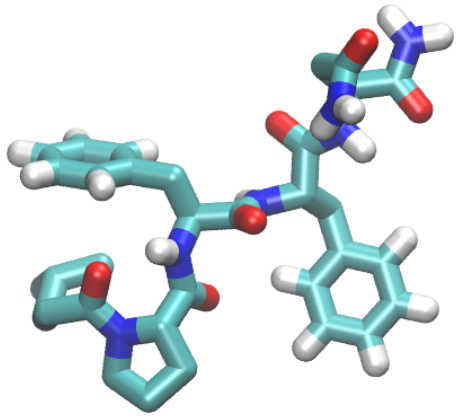
Outline

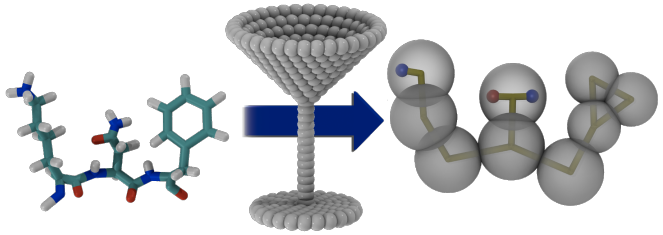
- Protein conformation diseases



- Alzheimer disease

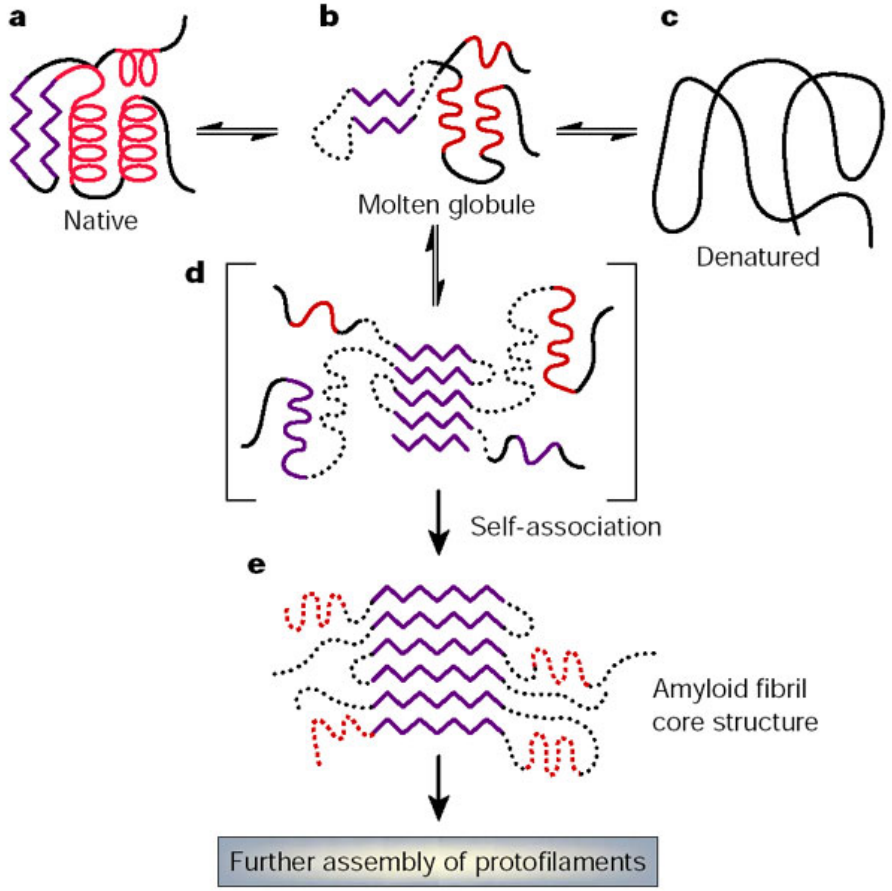


- β-sheet breakers
- Coarse Grained simulations
- Outlooks



Protein conformational disorders - Amyloidoses

Amyloidosis: a disorder in which insoluble protein fibrils are deposited in tissues and organs, impairing their function

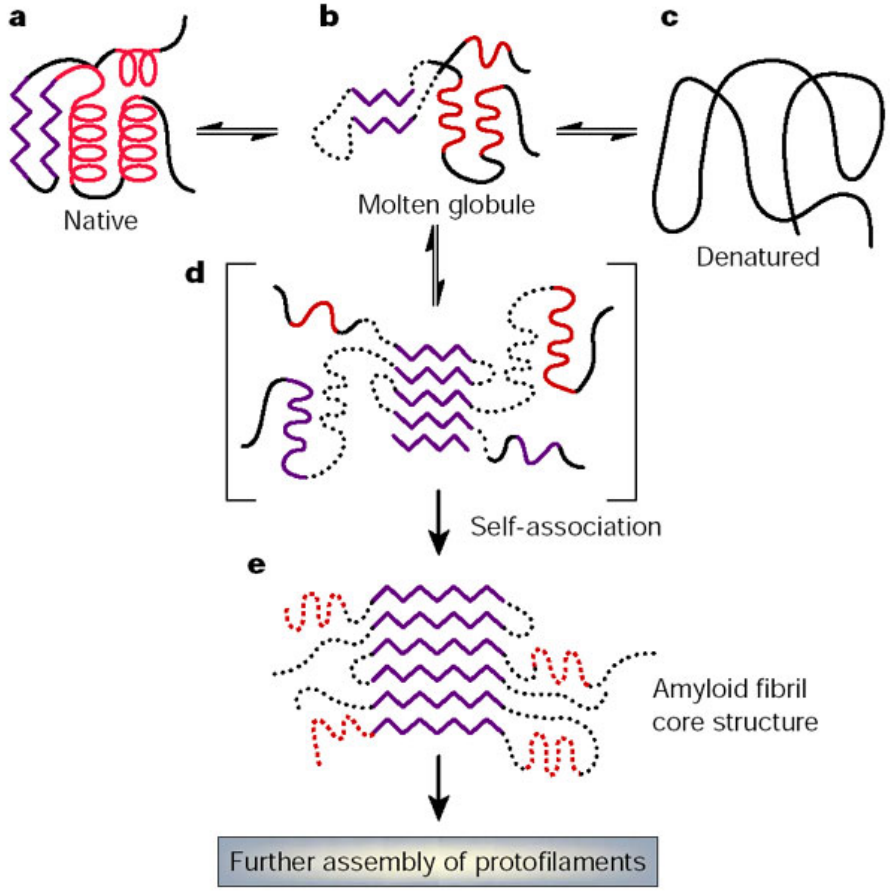


Protein aggregation characterises neuro-degenerative disorders

Protein Conformational Disorder	Fibril Subunit
Alzheimer's Disease	Aβ-peptide
Spongiform encephalopathies	Prion protein
Parkinson's disease	α-synuclein
Type II diabetes	Amylin
Thyroid carcinoma	Procalcitonin
Atrial amyloidosis	Atrial natriuretic factor
Amyotrophic lateral sclerosis	Superoxide dismutase
Huntington disease	Long Glutamine Stretches within proteins
Primary systemic amyloidosis	Ig light chains
Secondary systemic amyloidosis	Serum amyloid A
Senile systemic amyloidosis	Transthyretin (wild tipe)
Familial amyloidotic polyneuropathy I	Transthyretin (mutant)
Familial amyloidotic polyneuropathy II	Apolipoprotein A1
Familial Mediterranean fever	Serum amyloid A
Hemodialysis-related amyloidosis	b2-microglobulin
Finnish hereditary systemic amyloidosis	Gelsolin (mutant)
Lysozyme systemic amyloidosis	Lisozime
Insulin-related amyloidosis	Insulin
Fibrinogen systemic amyloidosis	Fibrinogen α chain

Protein conformational disorders - Amyloidoses

Amyloidosis: a disorder in which insoluble protein fibrils are deposited in tissues and organs, impairing their function

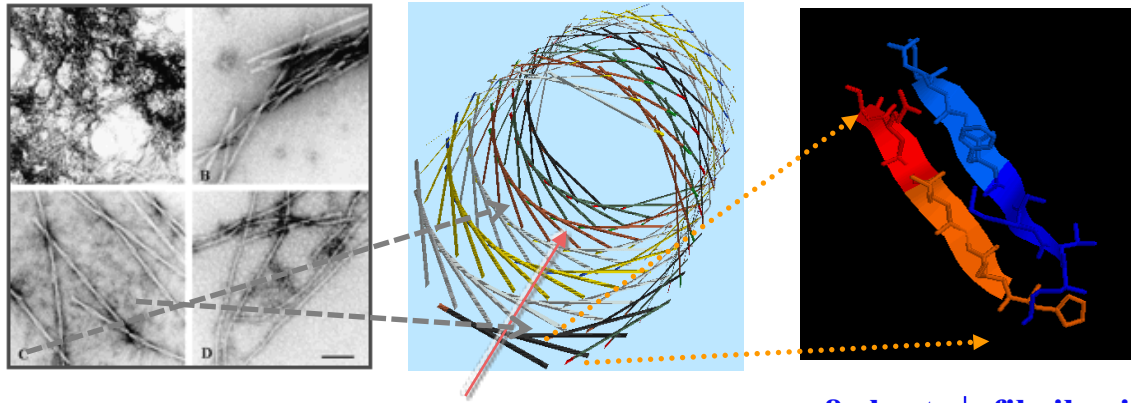


Protein aggregation characterises neuro-degenerative disorders

Though formed by proteins with unrelated proteins with homologies neither in sequence nor in structure

all amyloid fibrils have remarkably similar …

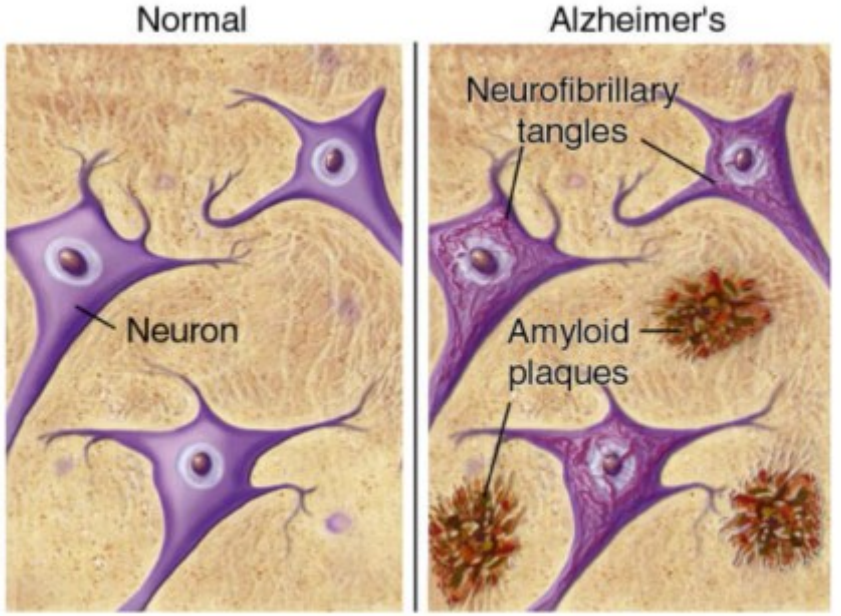
… histo-chemical properties & ultra-structural morphology



scale bar: 100 nm **cross-beta structure** **β-sheet ⊥ fibril axis**

Alzheimer's Disease (AD)

- **Neurodegenerative irreversible disease that affects the brain**
- **It causes memory loss, disorientation, behavioural issues**
- **An efficient treatment is still missing**

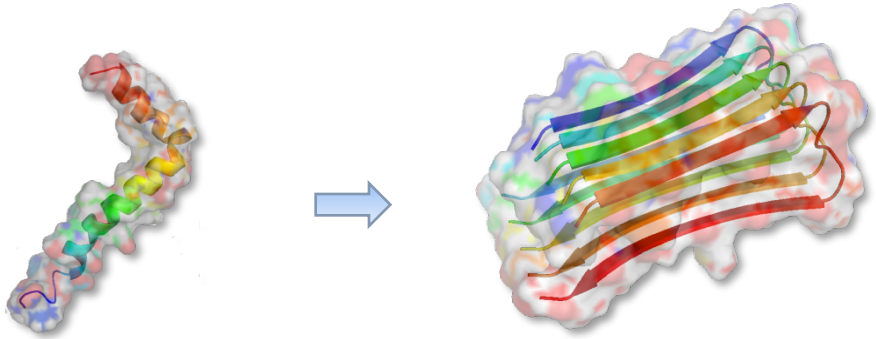


AD brains show two lesions

1- **Amyloid Plaques**:

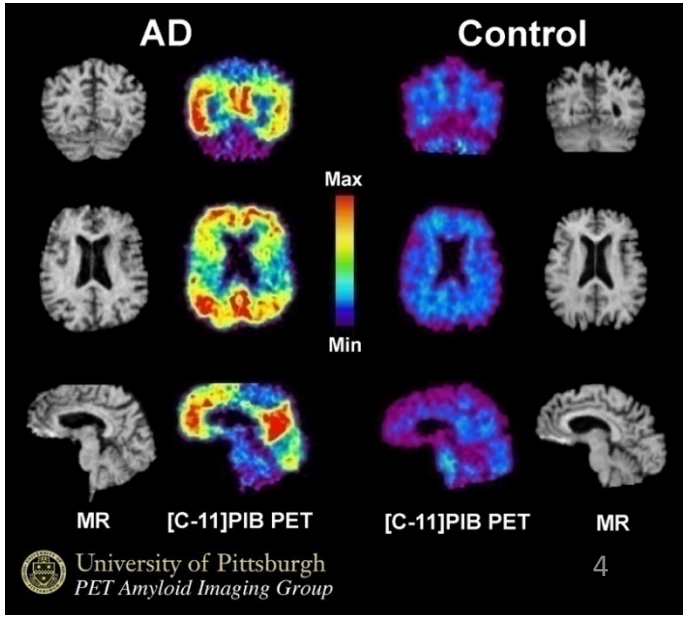
Extracellular deposits of Amyloid β (Aβ) peptide

Almost spherical with a 10-100 mm diameter



2- **Neurofibrillar Tangles:**

Intracellular anormal elicoidal fibers mainly composed by *tau* protein



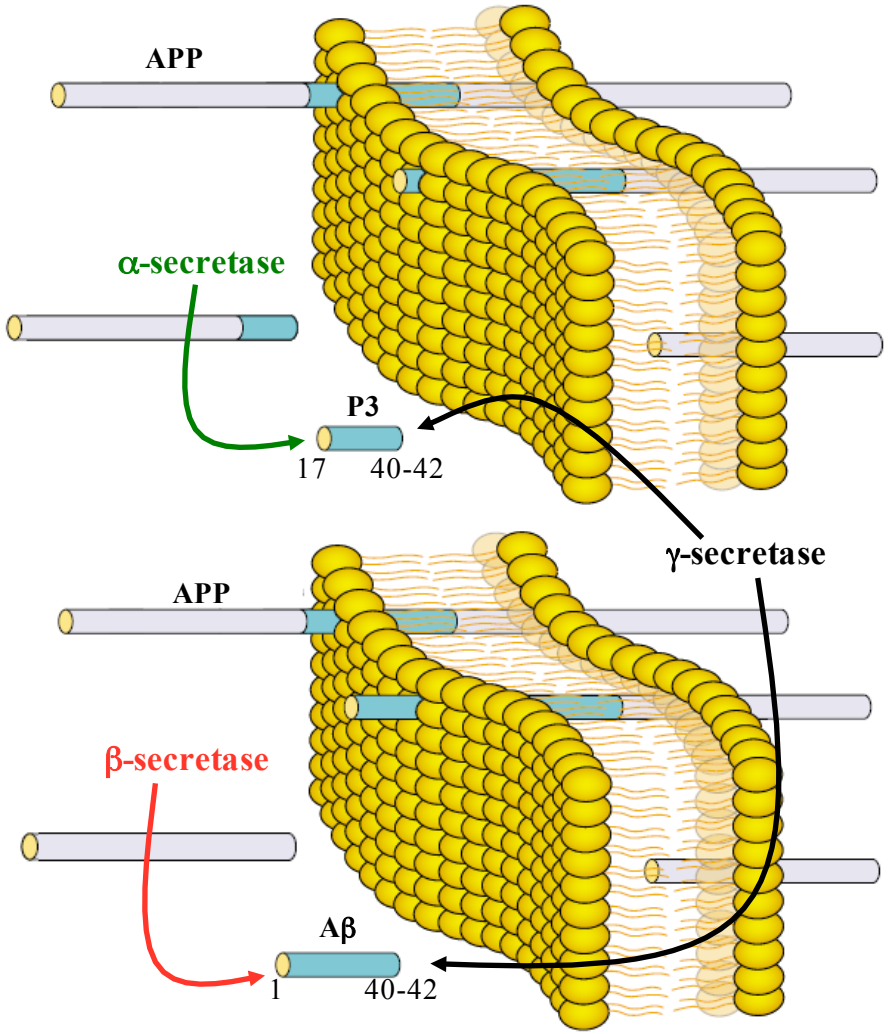
Amyloid β-peptide (Aβ)

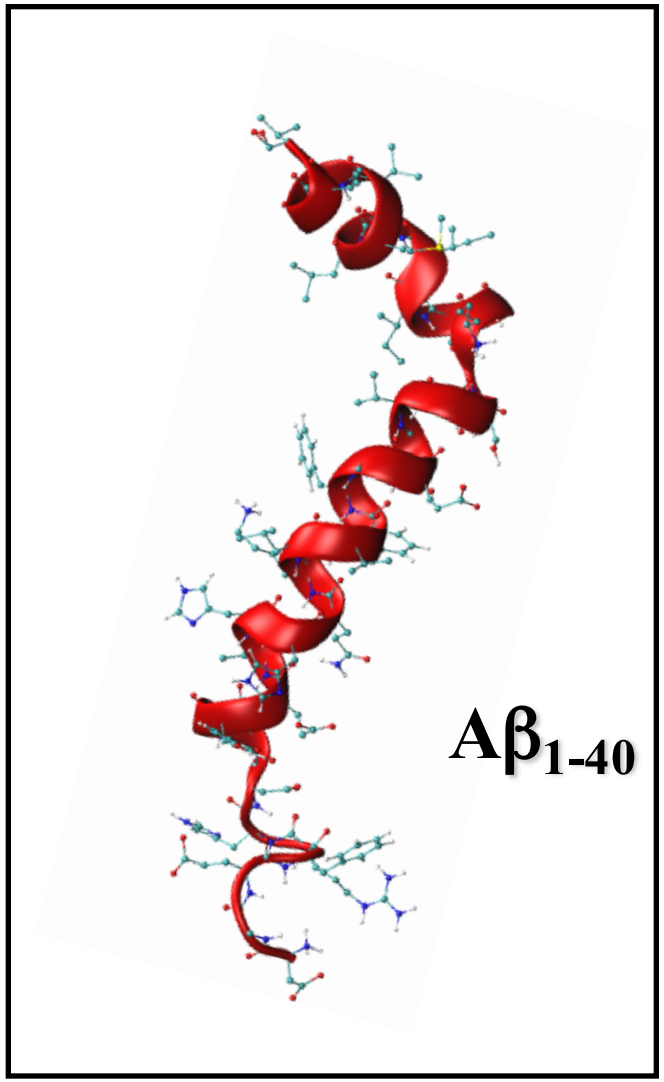
•α- & γ-secretases cleavage ⇒ **non-pathological** peptide P3

• Aβ is derived from proteolitic cleavage of APP protein (Amyloid Precursor Protein).

•APP: 770 trans-membrane protein coded in chromosome 21

•β- & γ-secretases cleavage ⇒ **pathological** peptides Aβ1-40, Aβ1-42

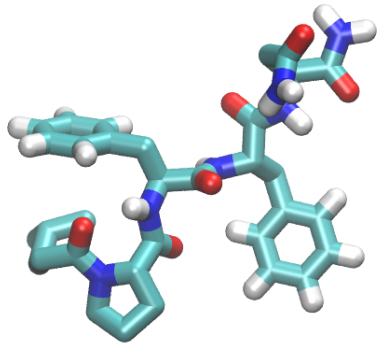




β-sheet breakers (BsB) – State of the art

Small peptides, called β-sheet breaker peptides (BSBp's), are able to inhibit or delay the conformational transition of Aβ peptide from α-helix to β-sheet

They are designed to mimic the central part of Aβ peptides

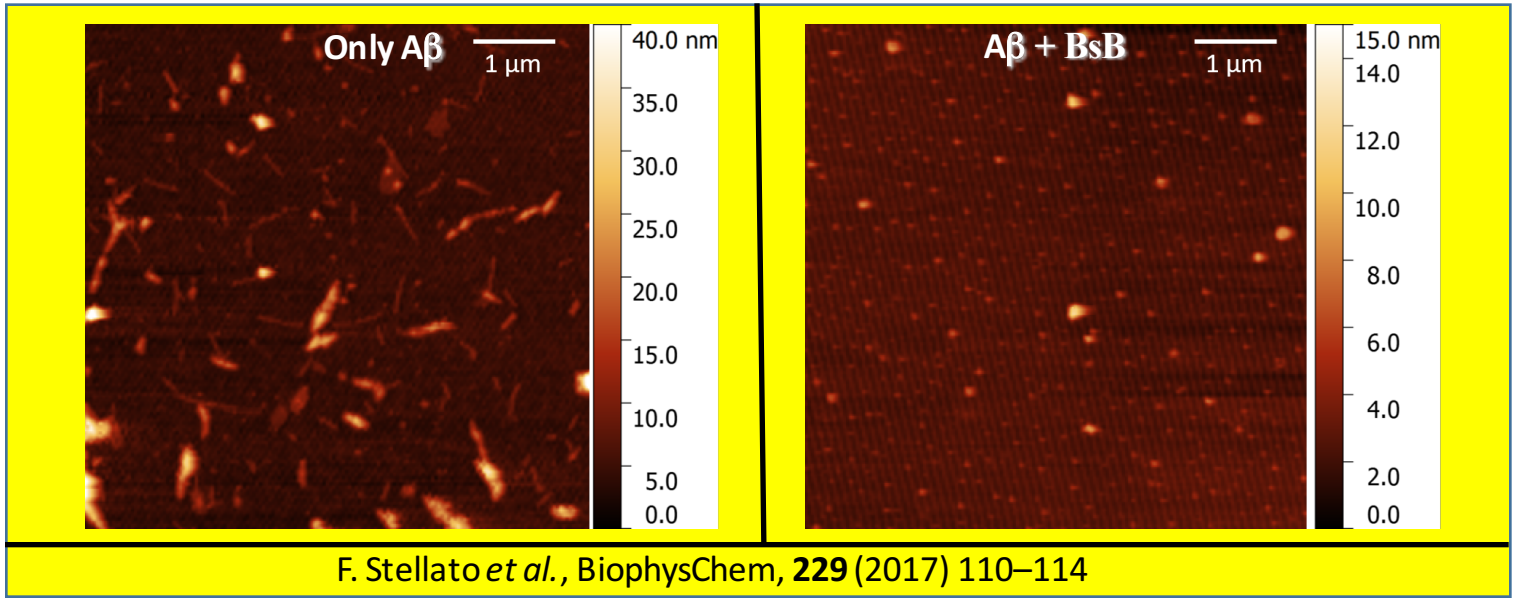


17 21

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Aβ

breaker Ac-LPFFN-NH_2



F. Stellato *et al.*, BiophysChem, **229** (2017) 110–114

β-sheet breakers (BsB) – State of the art

Computational and Experimental Studies on β-Sheet Breakers Targeting $A\beta_{1-40}$ Fibrils

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BsB studied

- Soto breaker = Ac-LPFFD-NH_2
- Soto breaker + taurine = Tau-LPFFD-NH_2
- Modified Soto = Ac-LPFFN-NH_2

(designed by Tor Vergata biphysics group)

Techniques employed:

Thioflavin T fluorescence, Circular dichroism, Mass spectrometry, Classical all atom MD

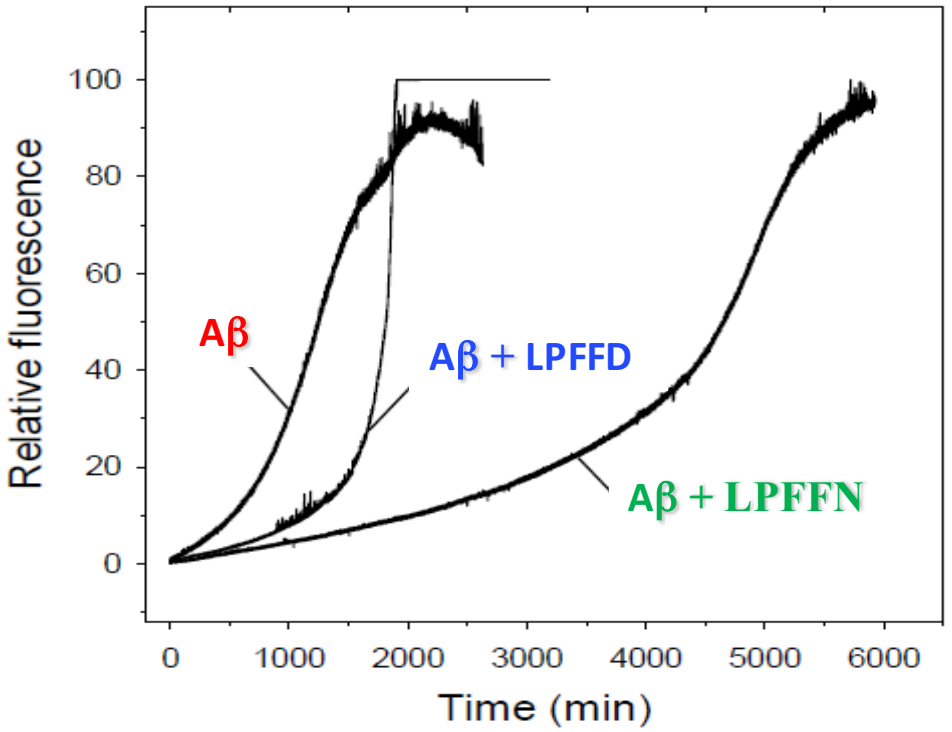
β-sheet breakers (BsB) – State of the art

From *in vitro* experiments we obtained that:

• As shown from the longer lag phase in ThT Fluorescence spectra, **in the presence of Ac-LPFFN-NH_2 fibrils formation is delayed**;

• CD measurements have shown that **Ac-LPFFN-NH_2 stabilizes $A\beta_{1-40}$ secondary structure** thus reducing its propensity to form β-sheets;

• Mass Spectrometry shows that Ac-LPFFN-NH_2 is the BSB that interacts more strongly with $A\beta_{1-40}$ and especially with 17-21 region.



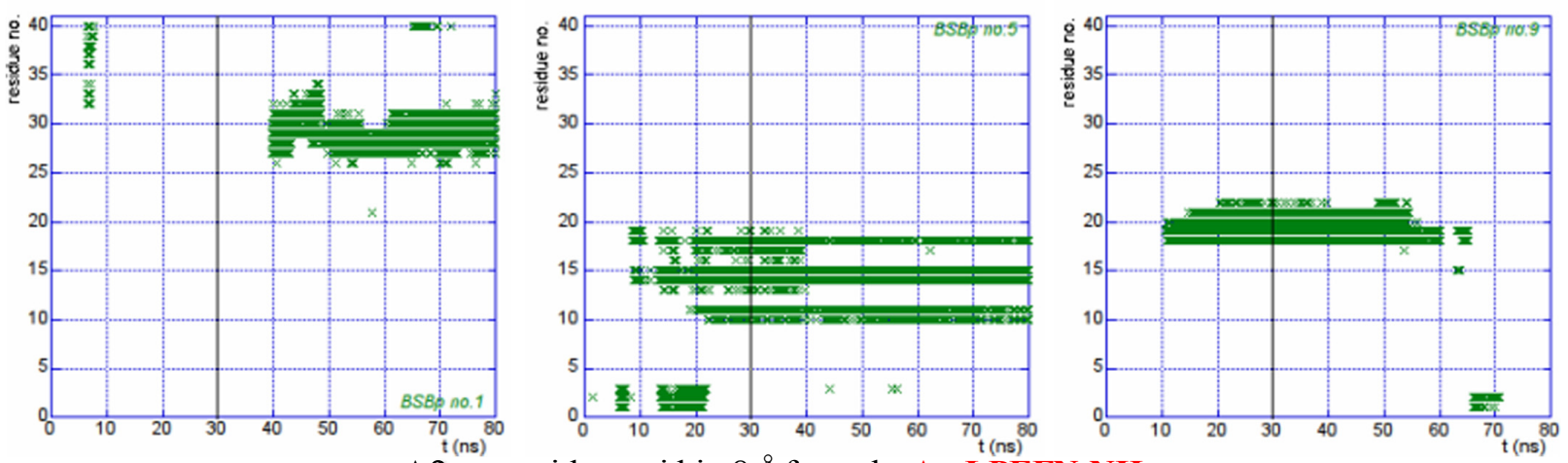
β-sheet breakers (BsB) – State of the art

From MD simulations we obtained that

→ All **BsB are able to interact with $A\beta_{1\text{-}40}$** and to reduce its residues mobility (Ac-LPFFN-NH_2 being the most effective)

→ All BsB come close to 25-35 hydrophobic region but **Ac-LPFFN-NH_2 interacts also with 17-21 region**, which conserves its α-helix content

→ Ac-LPFFN-NH_2 works by stabilizing the starting α-helix secondary structure of $A\beta_{1\text{-}40}$ peptide

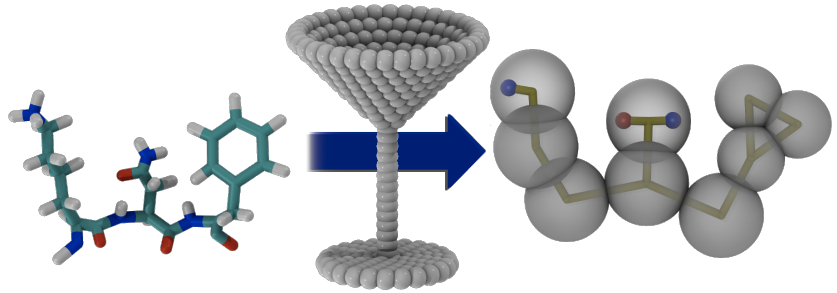


$A\beta_{1-40}$ residues within 8 Å from the Ac-LPFFN-NH_2

Ac-LPFFN-NH_2 can thus be considered as a lead compound to prevent and/or destabilize (delay) $A\beta_{1\text{-}40}$ fibril formation and aggregation

Coarse Grained Molecular Dynamics – Martini force field

Coarse grained (CG): suitably chosen groups of atoms are treated as a elementary objects (**beads**) of the systems

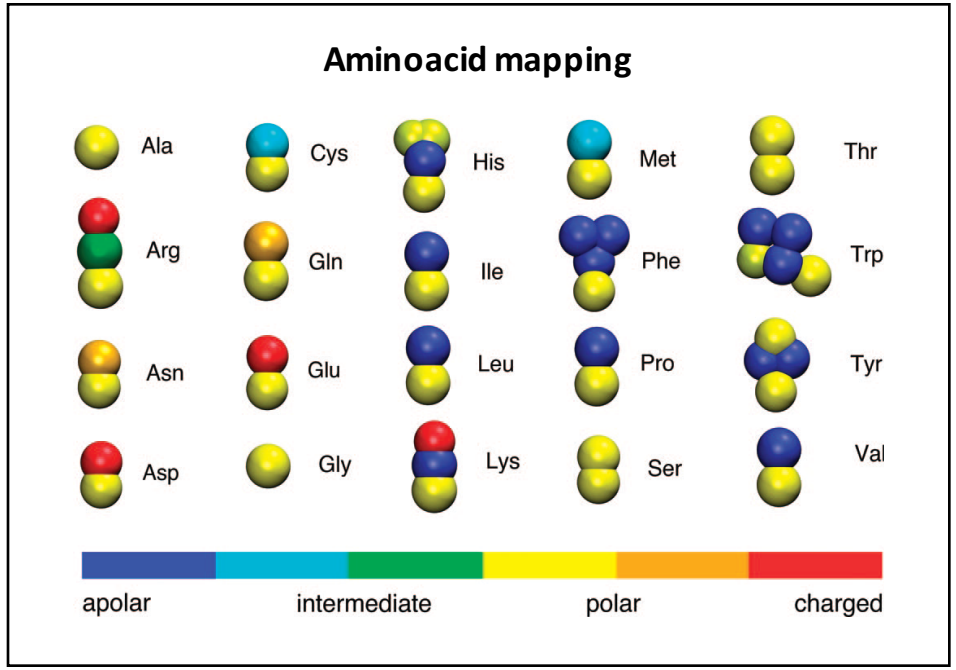


The force field has been parameterized in a systematic way, **combining top-down and bottum-up strategies**

Non bonded interaction: reproduction of experimental partitioning free energy between polar and apolar phases

Bonded interactions: derived from reference all-atom simulations

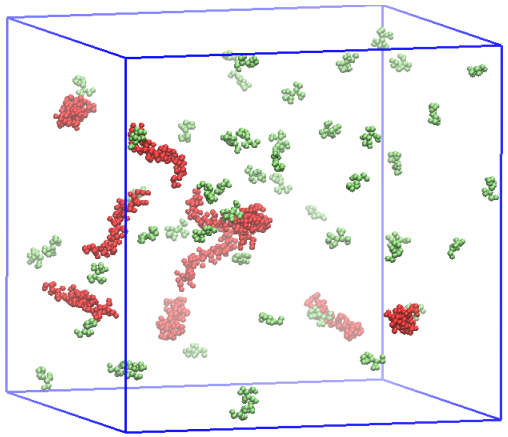
The model uses a **four-to-one mapping**, i.e. on average four heavy atoms and associated hydrogens are represented by a single interaction center. In order to keep the model simple, only four main types of interaction sites are defined: polar, non-polar, apolar, and charged



Marrink *et al.*, JPhysChemB (2007) **111:**7812-7824
Monticelli *et al.*, JCTC (2008) **4**:819-834
Poma, *et al.*, JCTC (2017) **13**:1366–1374

Aβ + BsB – More molecular dynamics simulations... Coarse Grained approach

It could be interesting to perform **MD simulations** to study the effect of the presence of the BsB on the aggregation process on systems made by **several Aβ and dozens of LPFFN**



10 Aβ and 50 LPFFN + solvent
~ 24nm of box length
Number of entities:
CG: 180K beads
AA: 1.3M atoms

Reducing system DOF

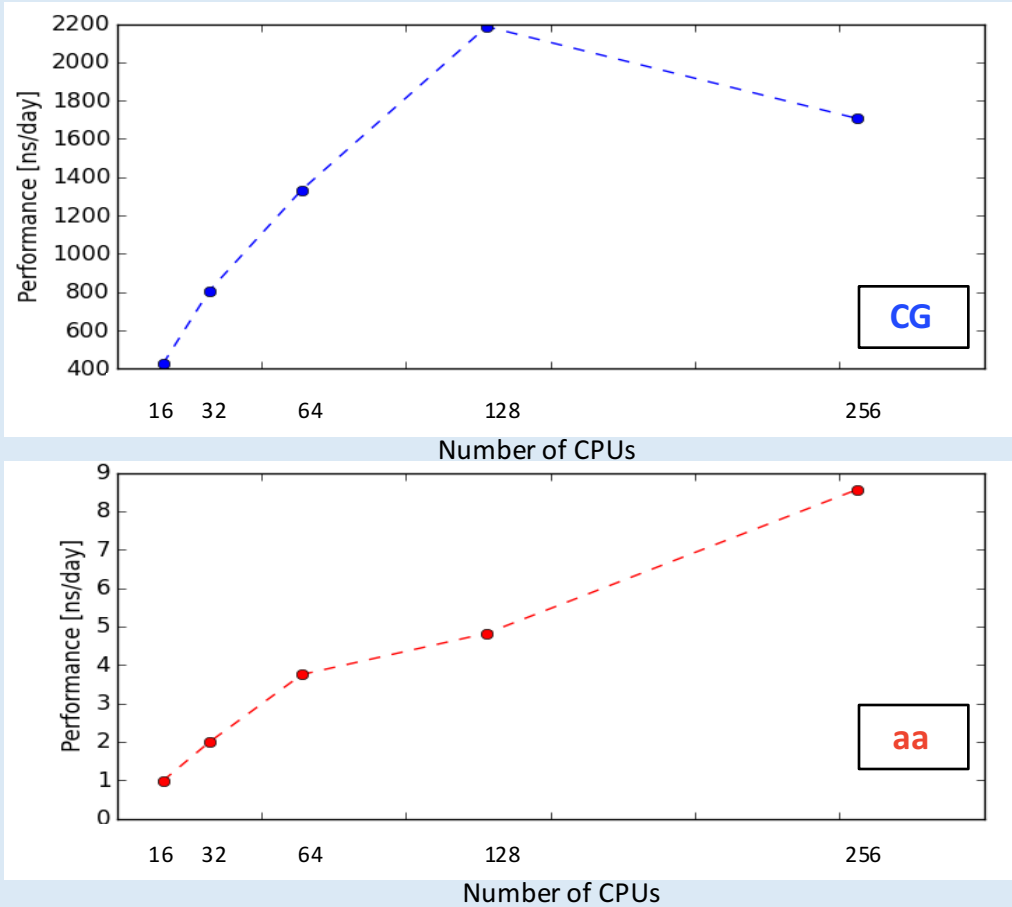
+

Increasing the simulations timestep (dt)

=

Gaining performance (simulation can approach the *ms* timescale)

Performance vs number of CPUs



$$\frac{\text{Performance}_{CG}}{\text{Performance}_{AA}} \approx 400$$

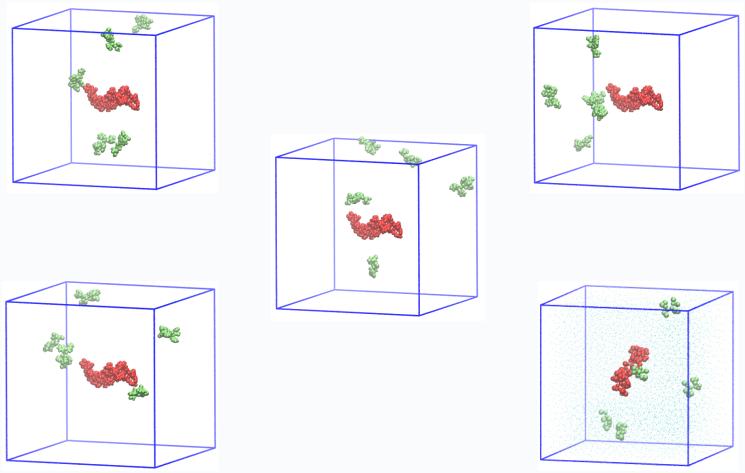
Simulations are performed on **Intel Xeon E5-2697 v4 (Broadwell) at 2.30 GHz** with GROMACS 5.1.2 version

Assess the reliability of CG: all atoms vs Martini ff

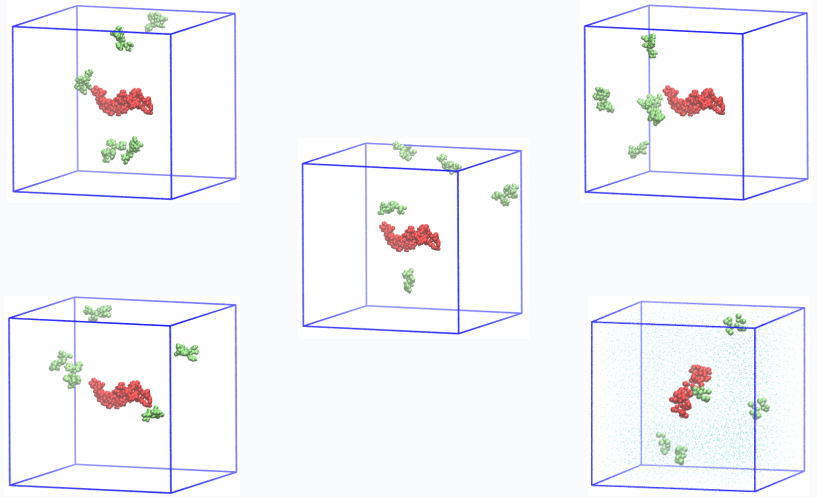
To asses the reliability of the CG force field we performed MD simulation both in aa and in CG representation on solvated systems composed of **1 Aβ + 5 LPFFN**

To have enough statistics, we simulated, in **NpT ensemble**, 5 systems in which the initial relative positions of **Aβ** and **LPFFN** are different

All atoms (**Amber99[1] & gromos53a6[2]**)



Coarse Grained (**Martini**)

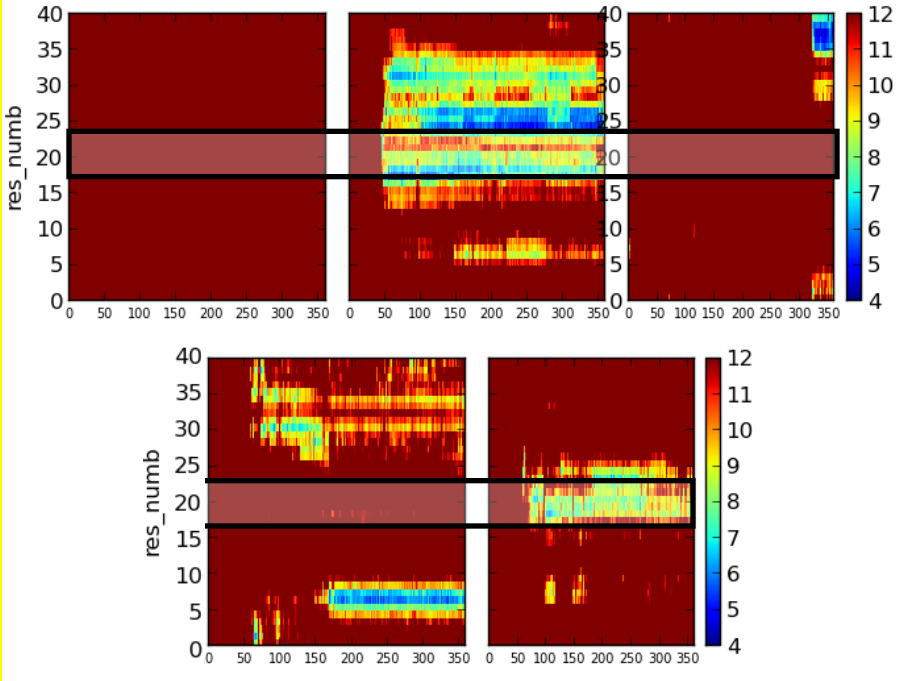


[1] Wang *et al.*, JCompChem (2000) **21**:1049-1074
[2] Oostenbrink *et al.*,JCompChem (2004) **25.13**:1656-1676

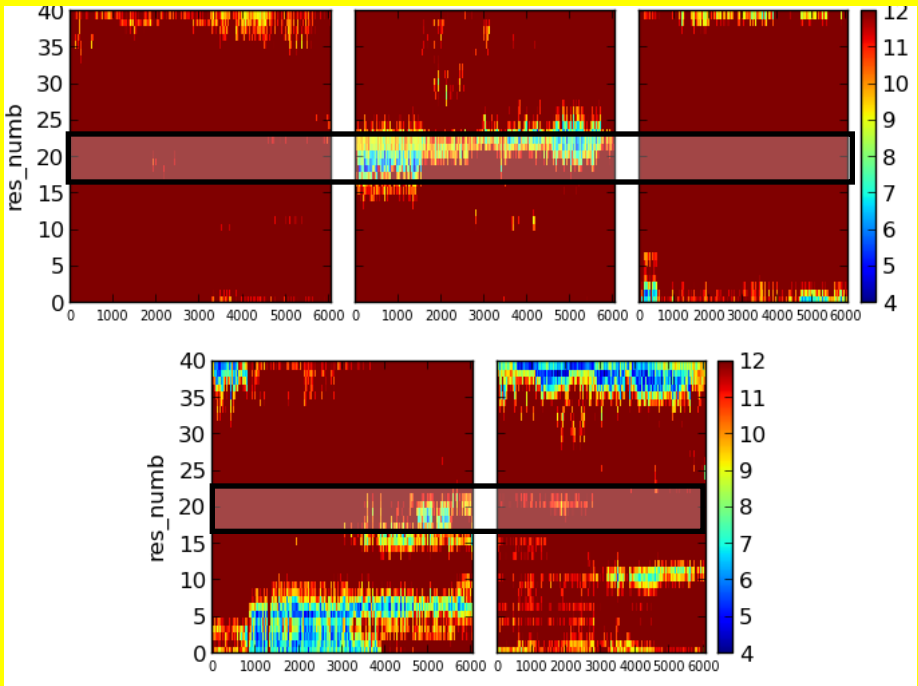
Assess the reliability of CG: all atoms vs Martini ff

- **Ac-LPFFN-NH_2** go in contact with the **Aβ**
- **Ac-LPFFN-NH_2** bind to the **17-21 region**

All atoms representation (gromos 53a6 ff)



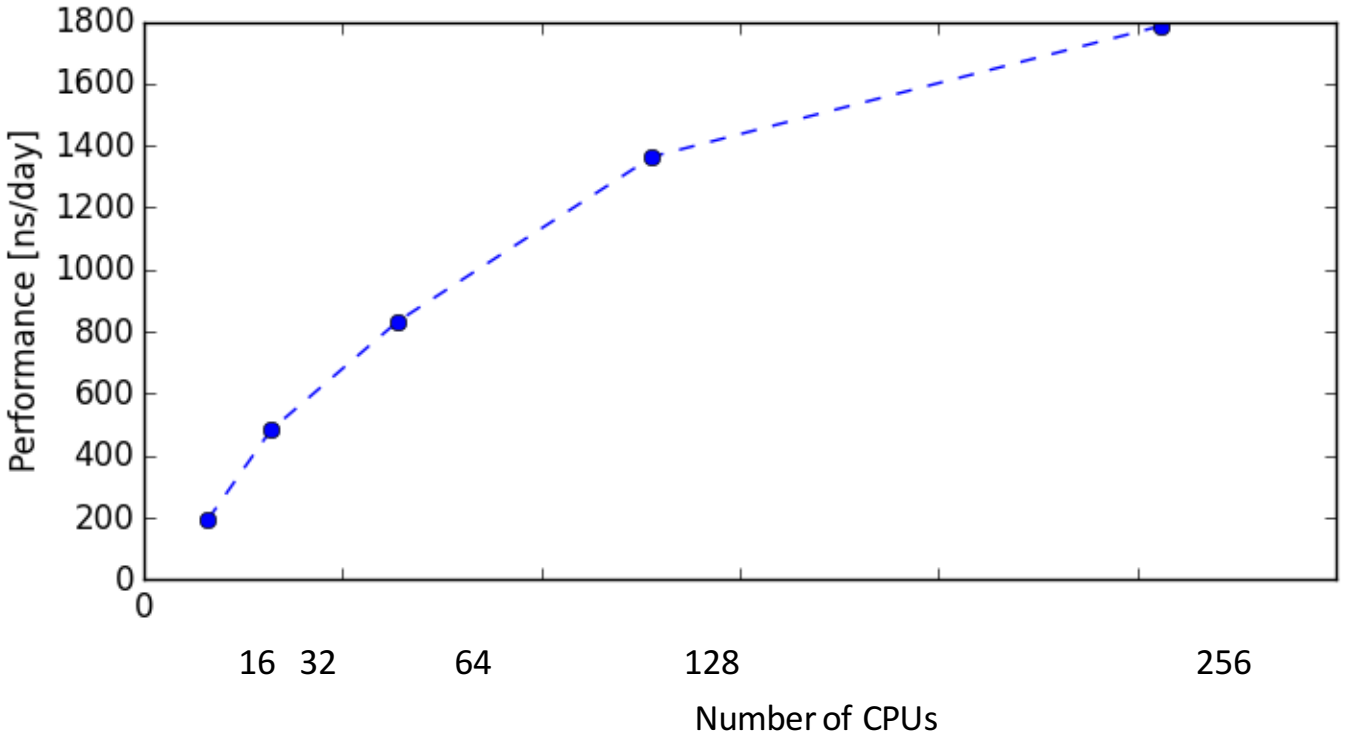
Coarse Grained representation (Martini ff)

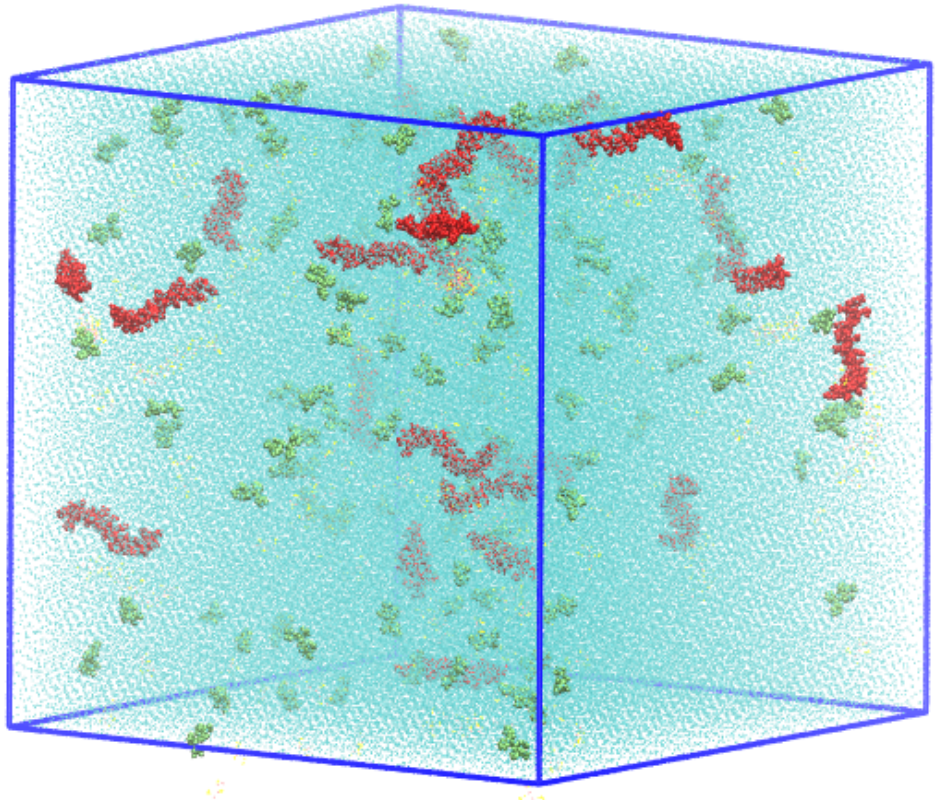


There is a good agreement between the two representations!!!

Ongoing on Martini…

- We are now running simulations on 10 systems composed of **25 Aβ + 125 LPFFN**

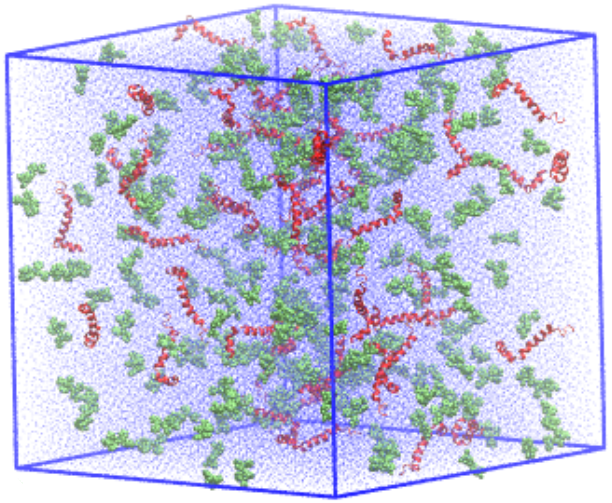




Evaluation of the binding energy as function of the number of the assembled molecules by pulling one–by-one from the aggregates

Aβ :	2288 beads
LPFFN:	1750 beads
Solvent :	274907 beads

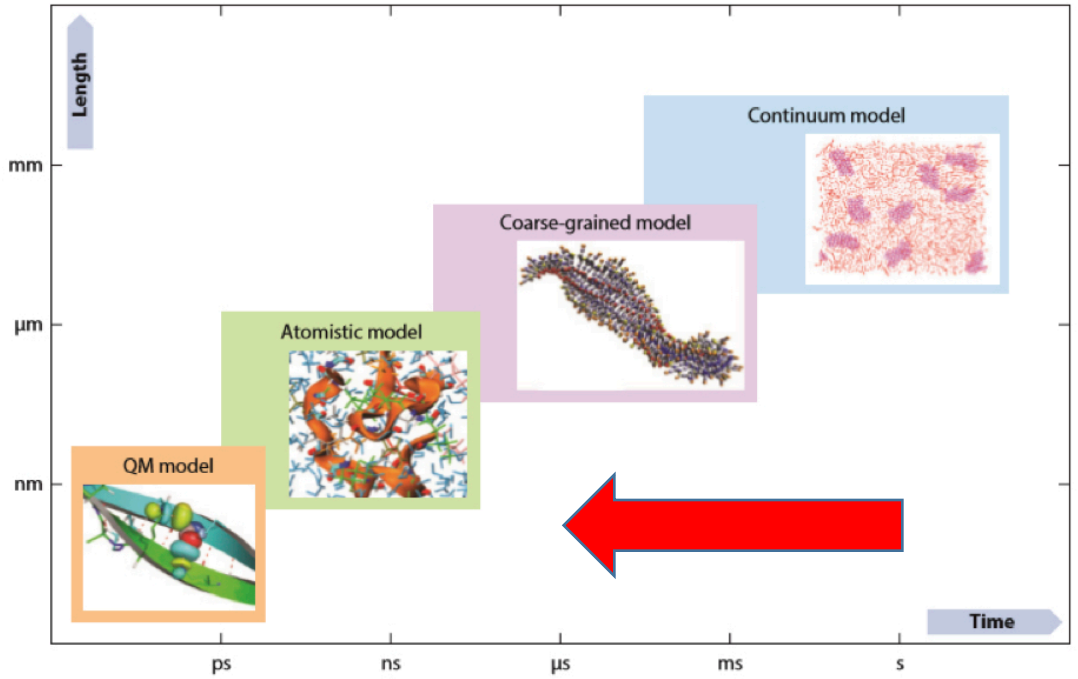
Outlooks



- CG allows to study even bigger systems

  (~ 100 Aβ + 500 LPFFN + solvent)

- Exploiting the so-called Multiscale approach:
  - Backmap to **AA** portion of the resulting aggregates (<10^5 atoms) and perform more **AA simulations**
  - Backmap to quantum mechanical description, inclusion of metal ions and perform ***ab initio*** calculation on small subsystems (200 atoms)

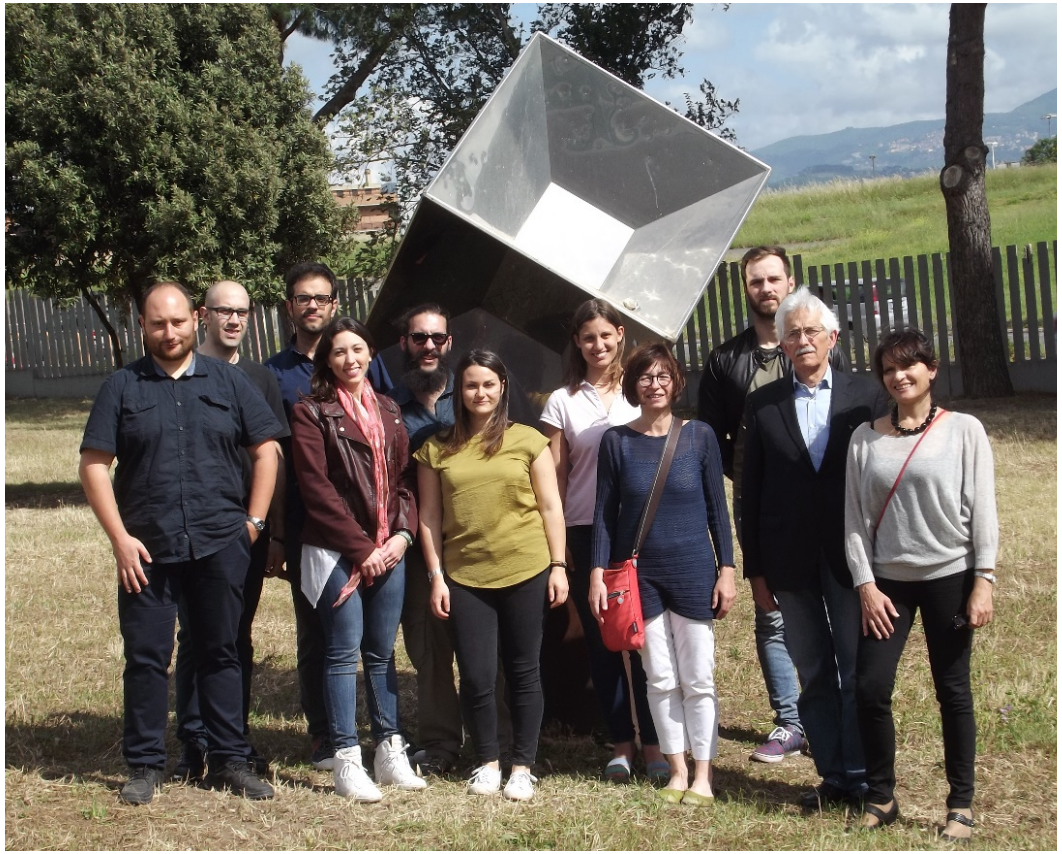


A. Morriss-Andrews *et al.*, *Annu. Rev. Phys. Chem.* (2015) **66**:643-666

Acknowledgement

The Biophysics Group in Tor Vergata

http://biophys.roma2.infn.it



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Universiteit Leiden

F Buda, A Sevink

Further collaborators:

G La Penna (CNR); O Proux (ESRF); G Millhauser (UCSC); S Saxena (U Pittsburgh); A Spevacek (UCSC); V Consalvi (RM1)

Thank you for the attention…