Discrete molecular dynamics simulations of amyloid β-protein assembly

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Amyloid Diseases ⇔ Protein Misfolding / Aberrant Aggregation:

→ Alzheimer's disease (AD)	amyloid β-protein (Aβ)
→ Parkinson's disease	α-synuclein
→ cerebral amyloid angiopathy	amyloid β-protein (Aβ)
→ prion diseases (incl. "mad cow")	prions
→ Huntington's disease	huntingtin
→ type 2 diabetes mellitus	amylin

...

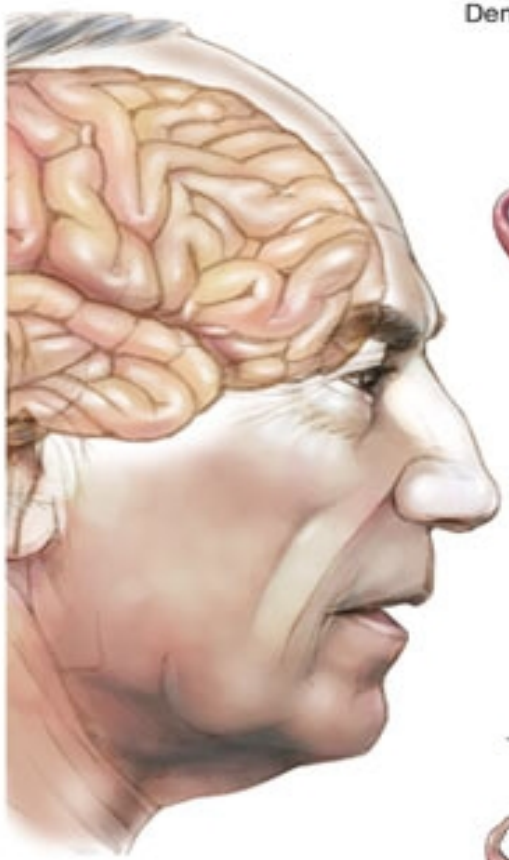
Characteristics:

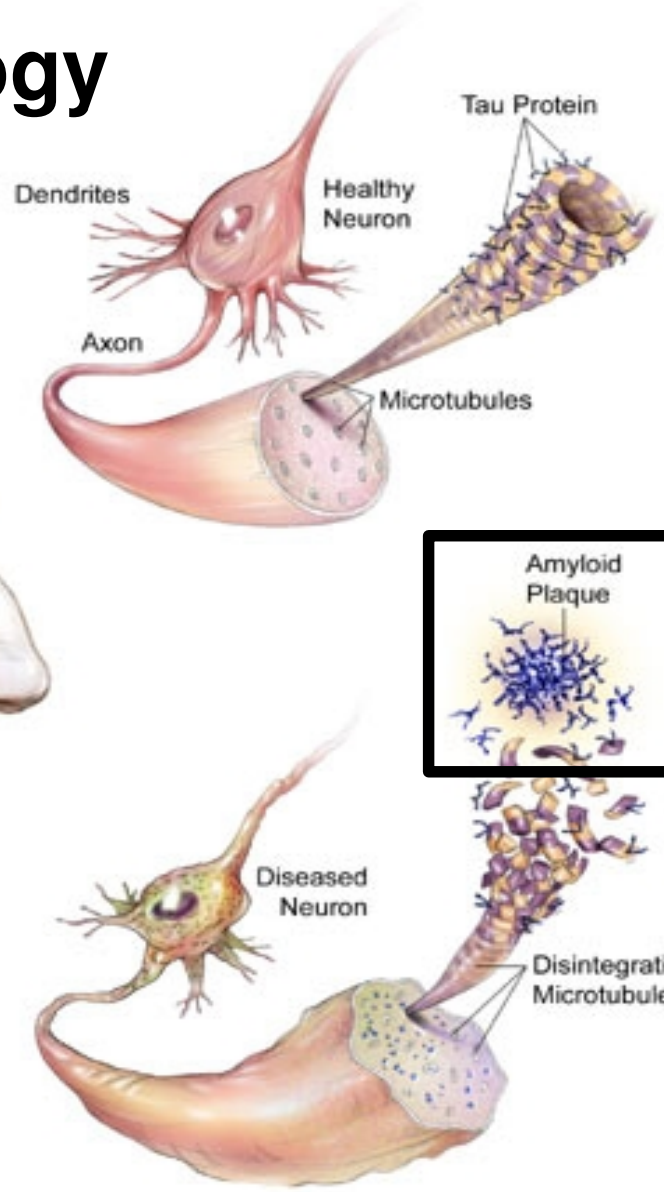
- → proteins with no common amino acid sequences
- → aggregation into fibrils with cross β-structure
- → emerging consensus: prefibrillar intermediates toxic

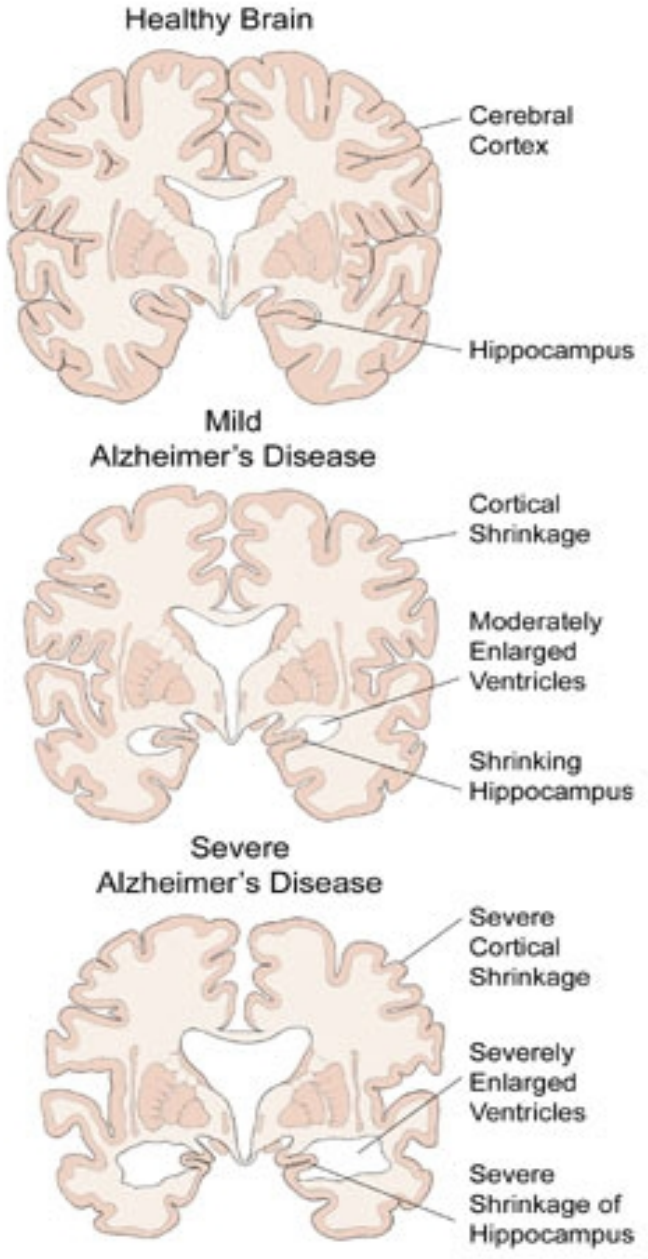
Concern:

in most sporadic forms, the age is a single common risk factor

AD Pathology





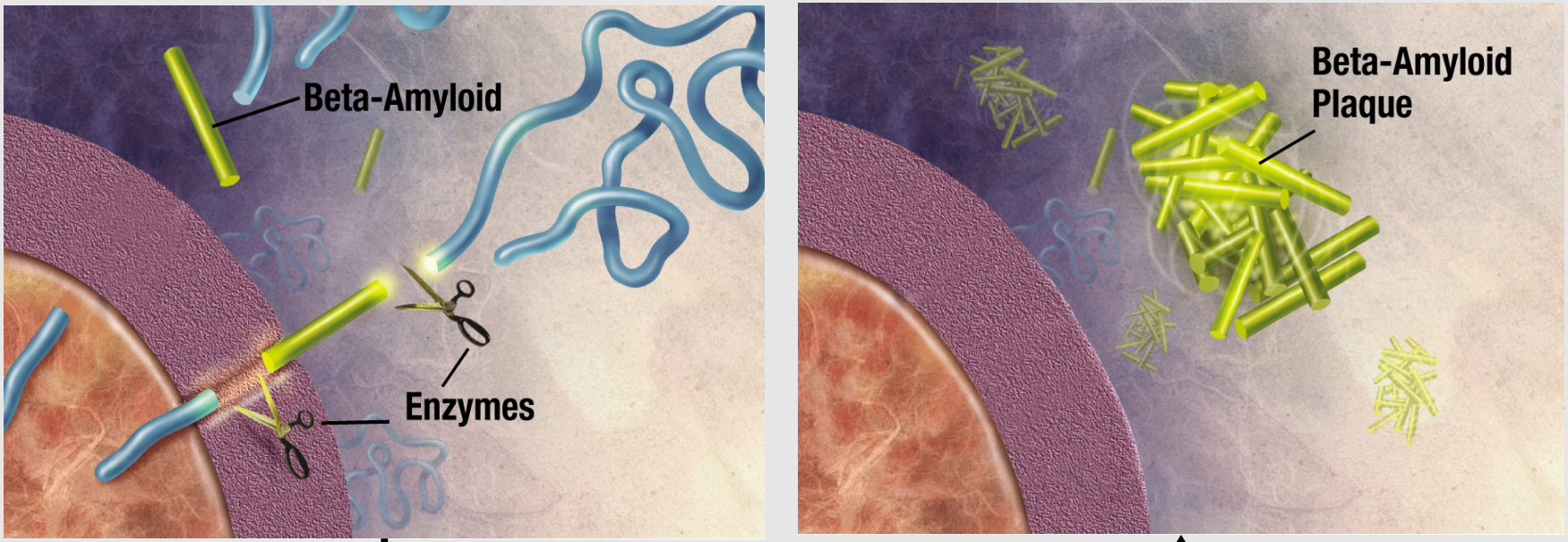


Amyloid Hypothesis Paradigm Shift:

Amyloid Plaques ⟶ Amyloid β-Protein (Aβ)

$A\beta_{1-40}$ and $A\beta_{1-42}$:

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV<u>IA</u>



PATHWAY?

$A\beta_{1-40}$ versus $A\beta_{1-42}$

- in the human body, 90% of all Aβ is $A\beta_{1-40}$ but $A\beta_{1-42}$ dominant in amyloid plaques
- *in vitro,* $A\beta_{1-42}$ forms more toxic fibrils, faster than $A\beta_{1-40}$
- of the two, $A\beta_{1-42}$ is genetically more strongly linked to AD
- $A\beta_{1-42}$ oligomers more toxic than $A\beta_{1-40}$ oligomers

(Dahlgren *et al.*, JBC, 2002).

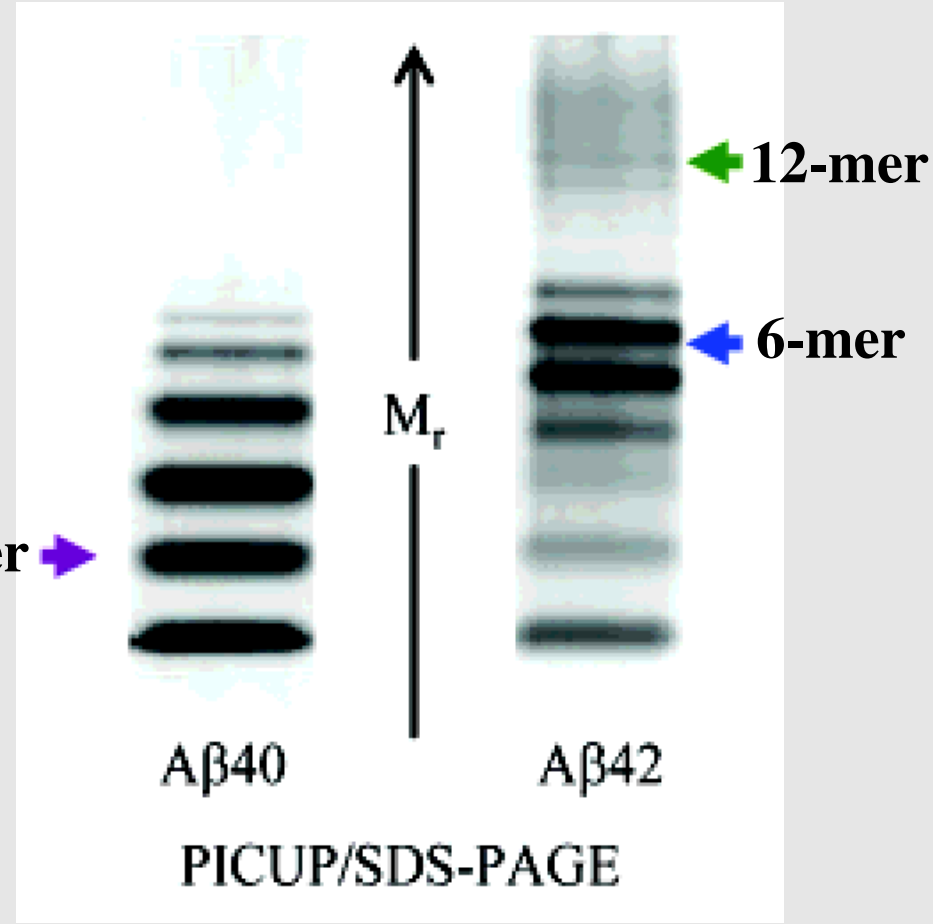
Why does a ~5% difference (I41 & A42) matter?

OLIGOMER FORMATION of Aβ40 & Aβ42 DIFFERS

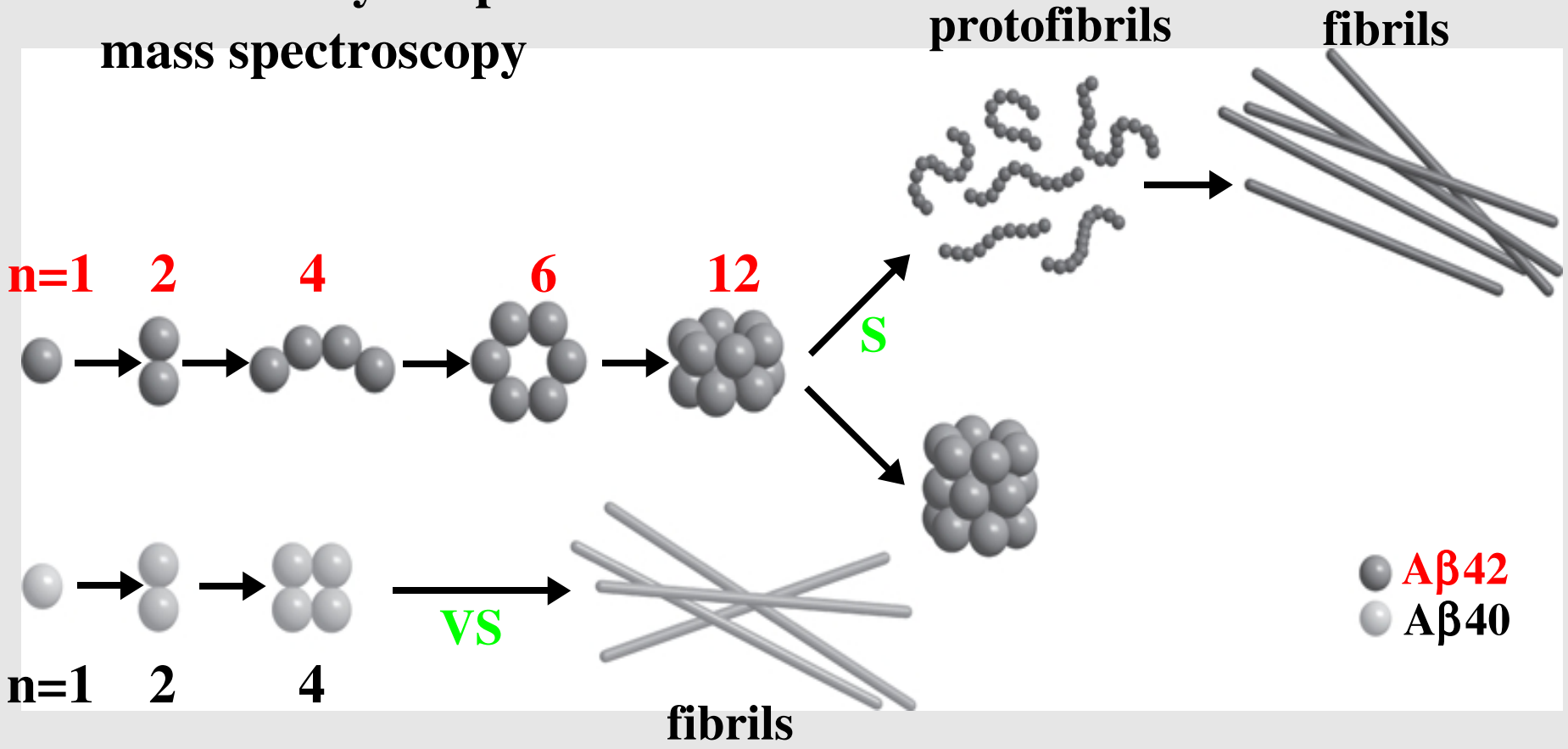
Experimental Evidence #1:

- **photo-induced cross-linking of unmodified proteins (PICUP)**
- **gel electrophoresis (SDS-PAGE)**

Bitan et al., PNAS, 2003.



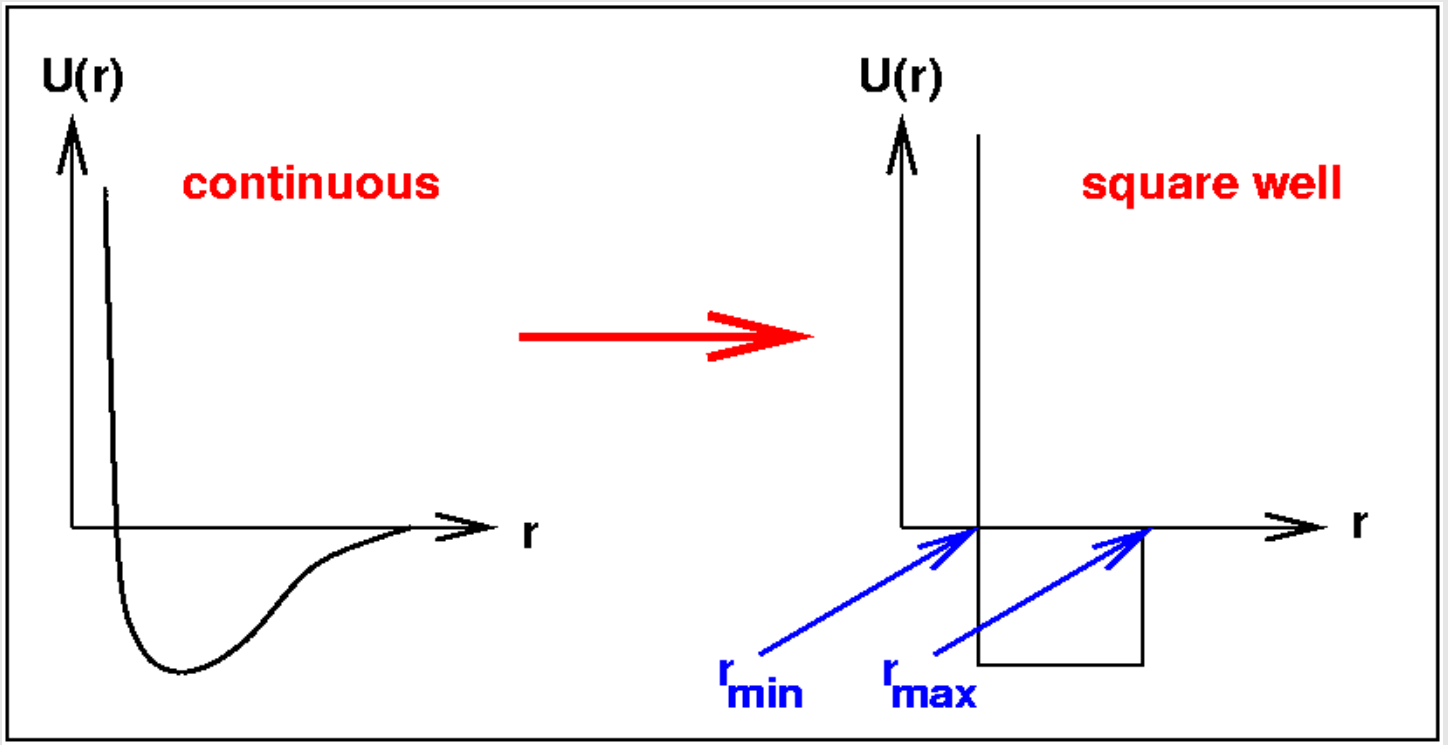
Experimental Evidence #2: ion mobility coupled with mass spectroscopy



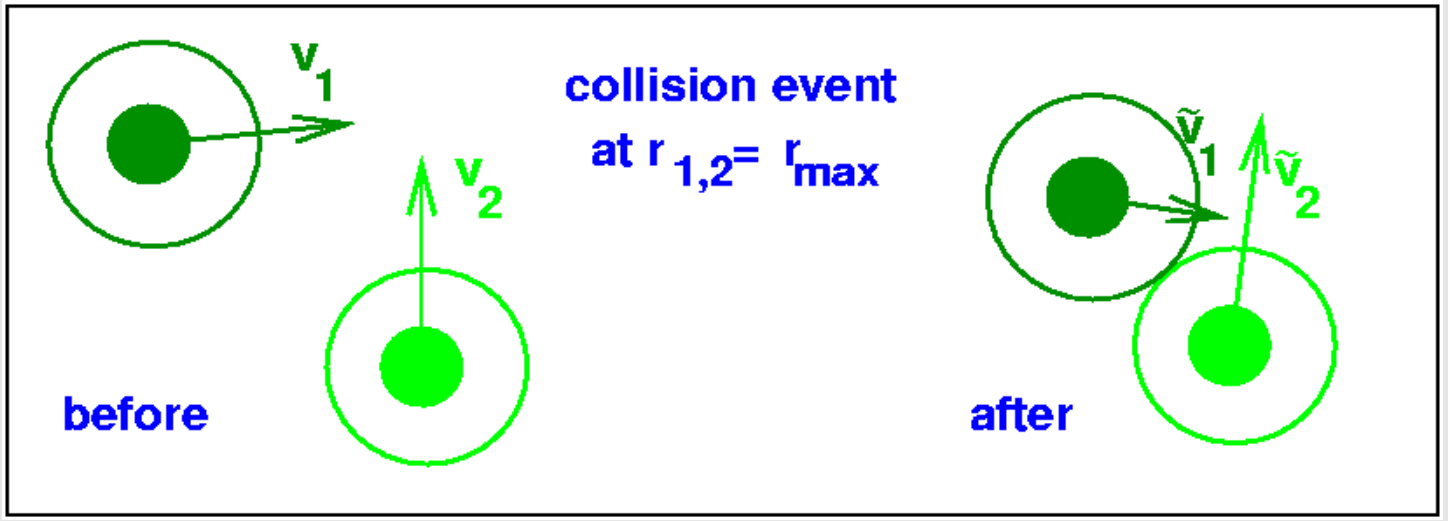
Bernstein *et al.*, Nat. Chem., 2009.

Discrete Molecular Dynamics (DMD)

Interparticle potential



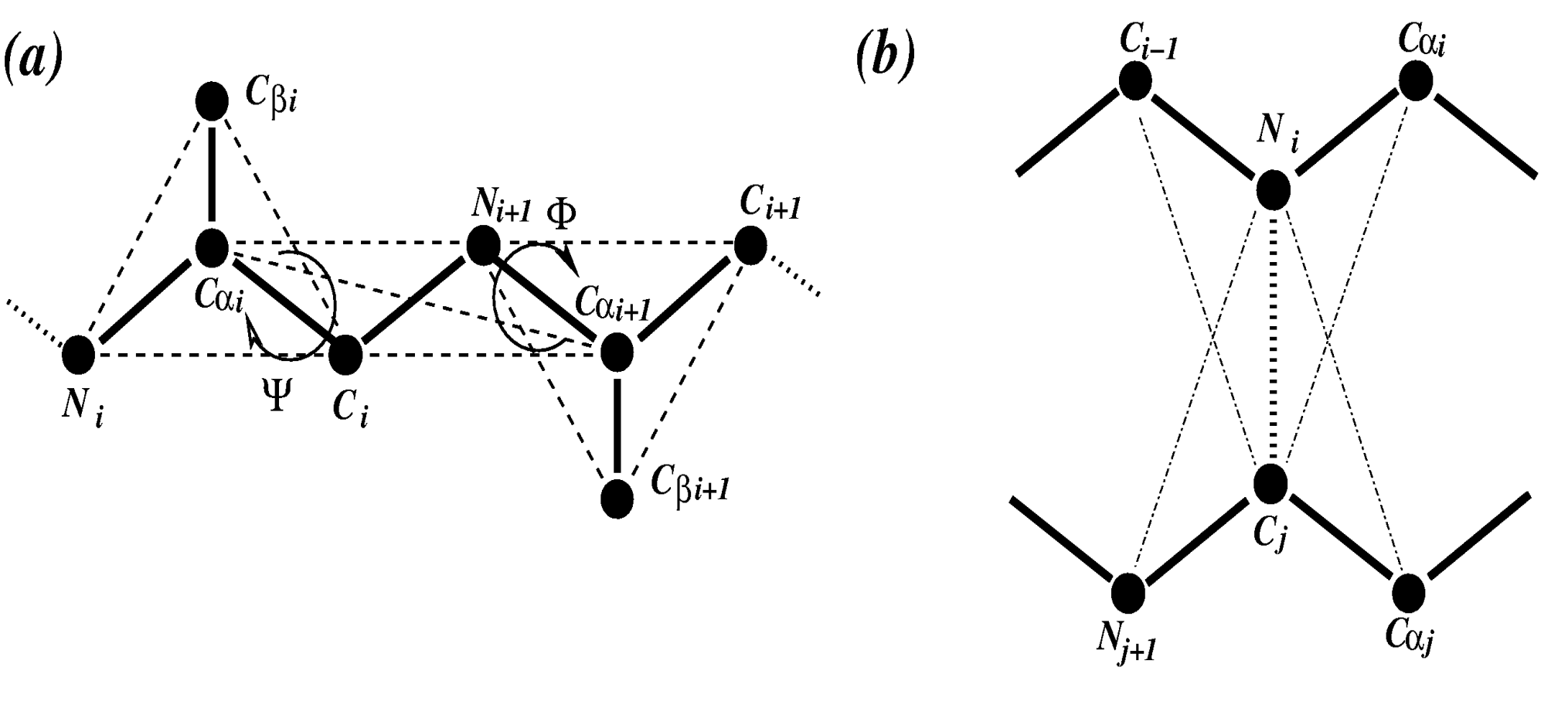
Collisions



Zhou et al., PRL, 1996; Zhou & Karplus, PNAS, 1997; Dokholyan et al., Fold. Des., 1998.

Four-bead protein model*

beads, bonds, & constraints **backbone hydrogen bond**



*Ding *et al.*, Proteins, 2003.

METHOD

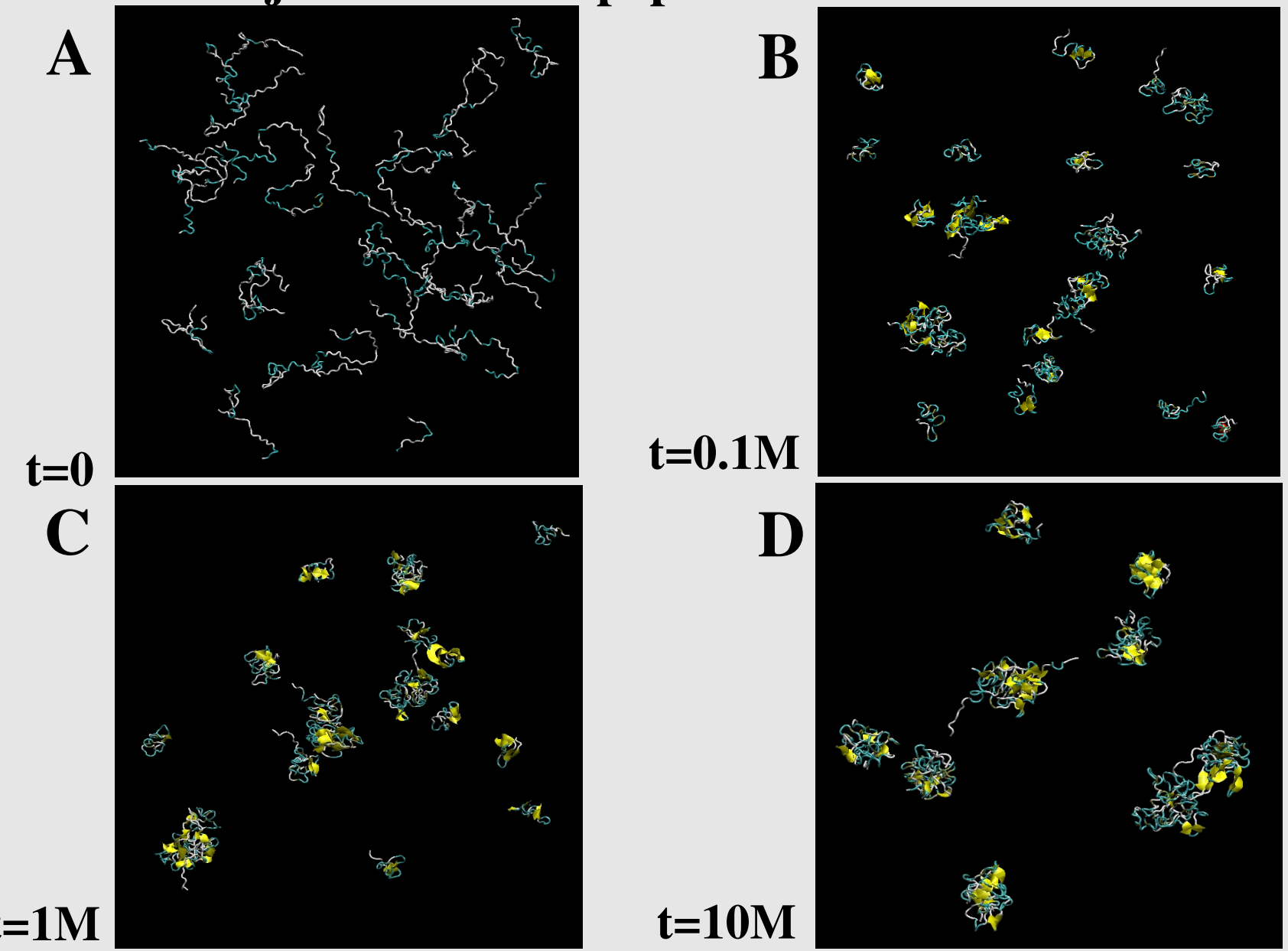
- discrete molecular dynamics (DMD) with the 4-bead protein model[1], backbone HBs[1]
- amino acid-specific interactions[2] due to:
  - hydrophobic/hydrophilic effect (E_{HP})

    Kyte-Doolittle hydropathy scale (JMB, 1982)
  - effective electrostatic interactions between charged side chain atoms (E_{CH})

E_{HP} and E_{CH}: implicit solvent parameters

[1]C.K. Hall *et al.*; Ding *et al.*, Proteins, 2003.
[2]Urbanc *et al.*, PNAS, 2004; Urbanc *et al.*, Meth. Enzym., 2006.

8 trajectories of 32 peptides in a cubic box each



Q1. Which Interactions Drive Aβ Assembly?

E_{HP}=0.3

E_{CH}=0

OLD:

-10 M time steps

-T=0.15

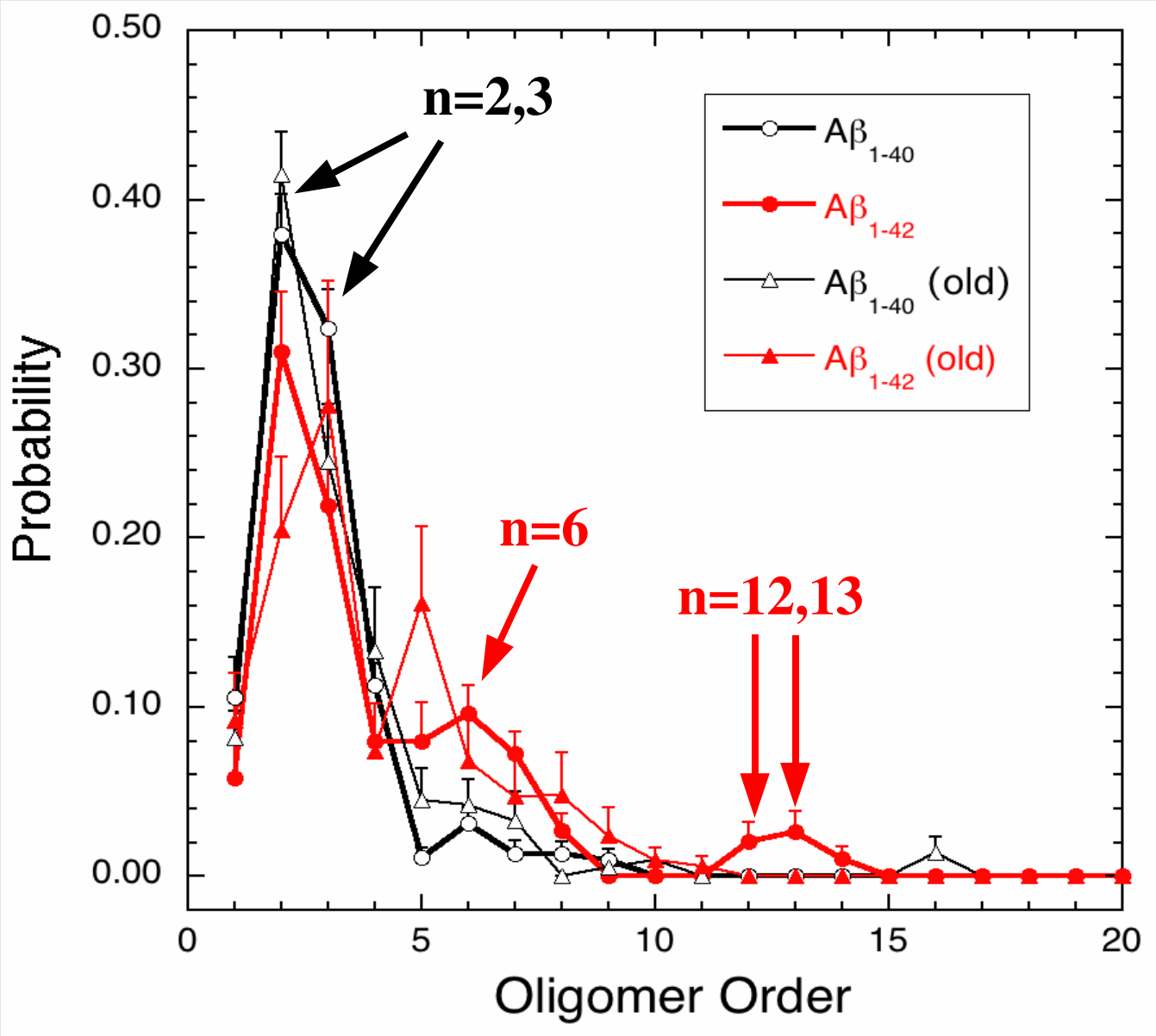
(Urbanc *et al.*, PNAS, 2004)

NEW:

-20 M time steps

-T=0.13 (more physiological T)

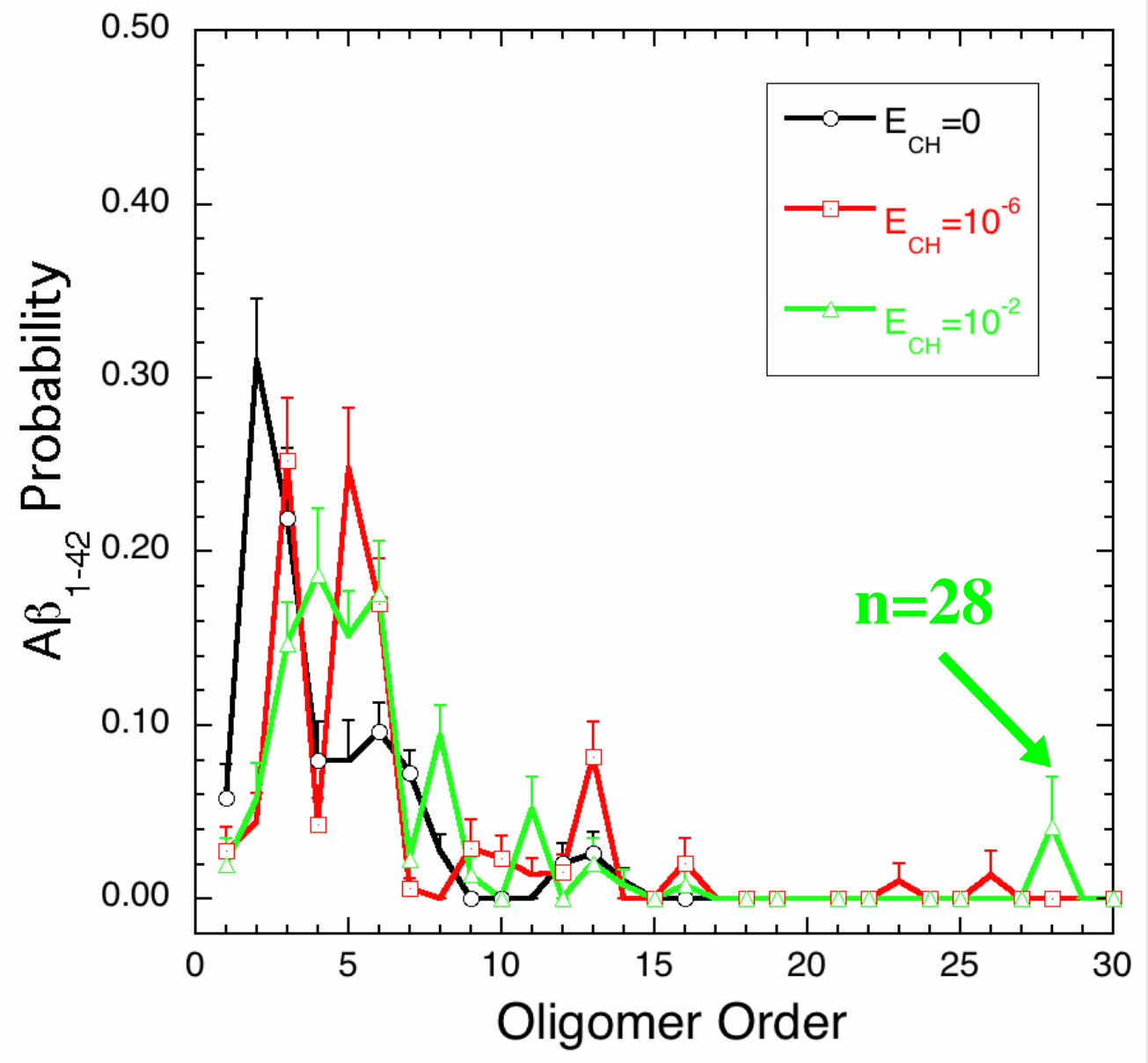
Lam *et al.*, JACS, 2008.



Urbanc *et al.*, JACS, in revision.

Charged Polar Residues:

- hydrophilic repulsion
- electrostatic INT among charged residues

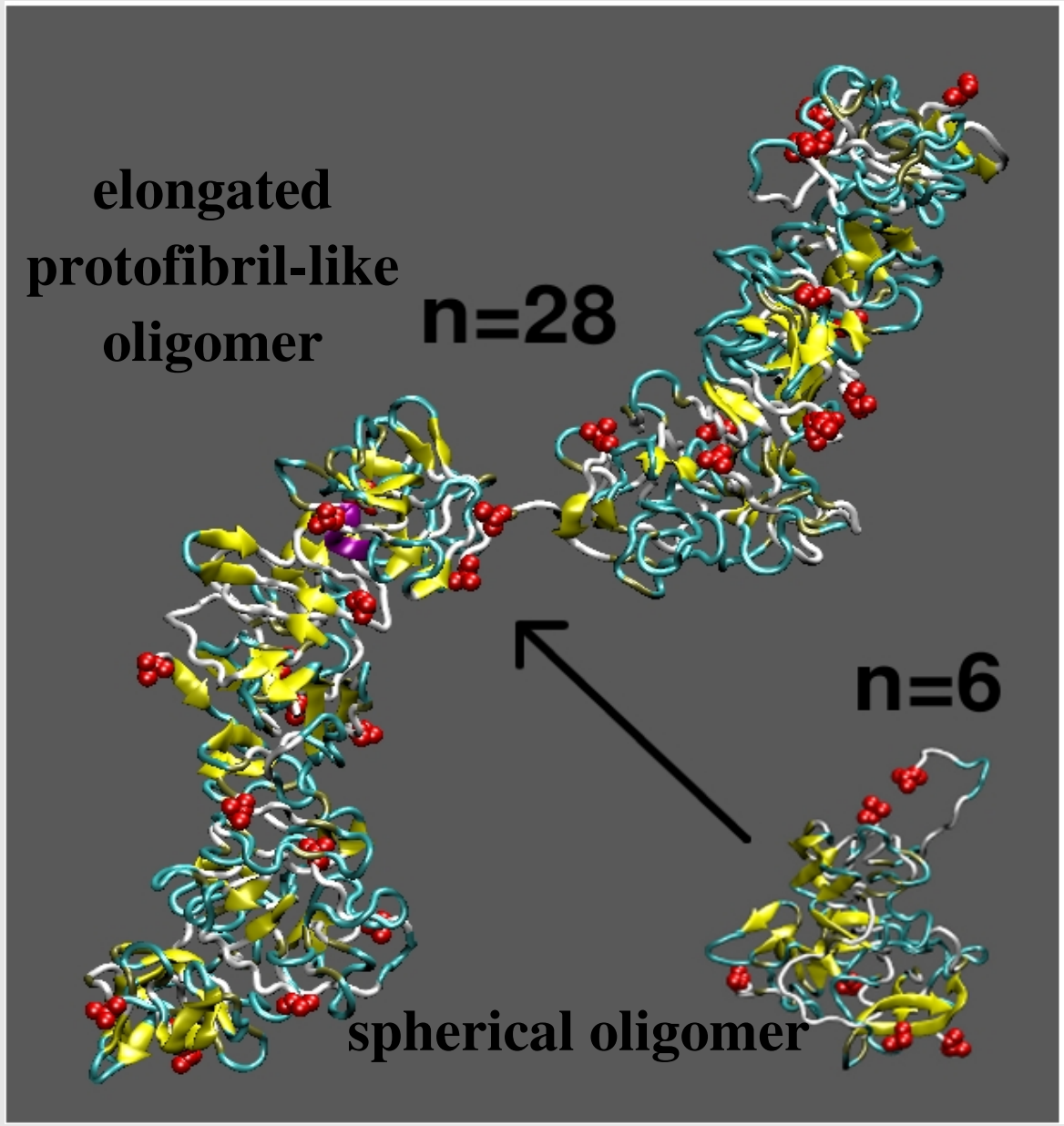


Urbanc *et al*., JACS, in revision.

Electrostatic interactions

⇒

spherical → elongated protofibril-like oligomers.



Urbanc *et al*., JACS, in revision.

Q1. Which Interactions Drive Aβ Assembly?

A1.1 **Oligomer formation driven by hydrophobic collapse during which charged groups are completely solvated ($E_{CH}=0$).**

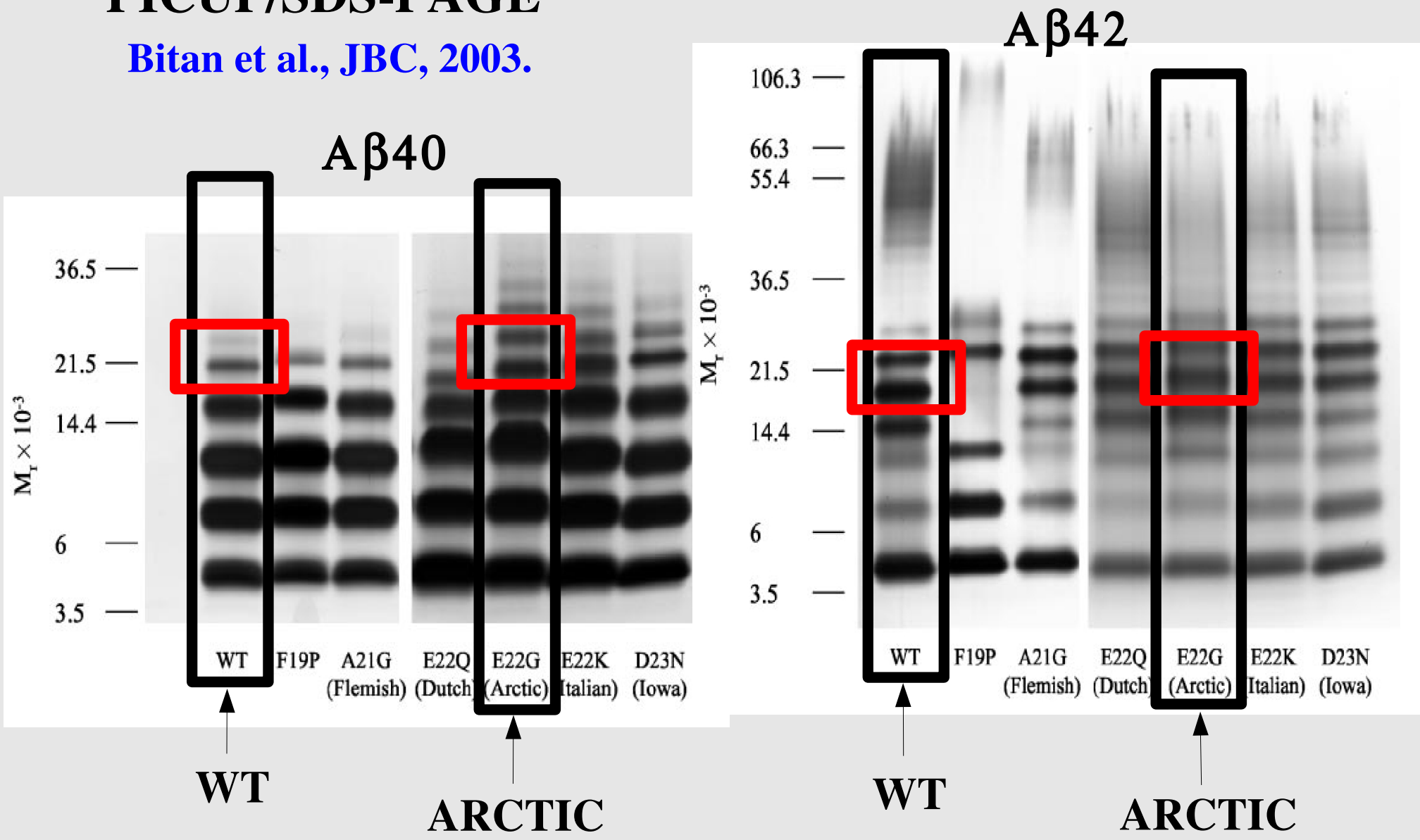
A1.2 **Partial desolvation of charged a.a. groups ($E_{CH}>0$) enhances the assembly pathway from spherical oligomers to protofibrils.**

Q2. Does the Same DMD Approach Work for the Arctic Mutants (E22G)*?

*associated with a familial form of AD

PICUP/SDS-PAGE

Bitan et al., JBC, 2003.

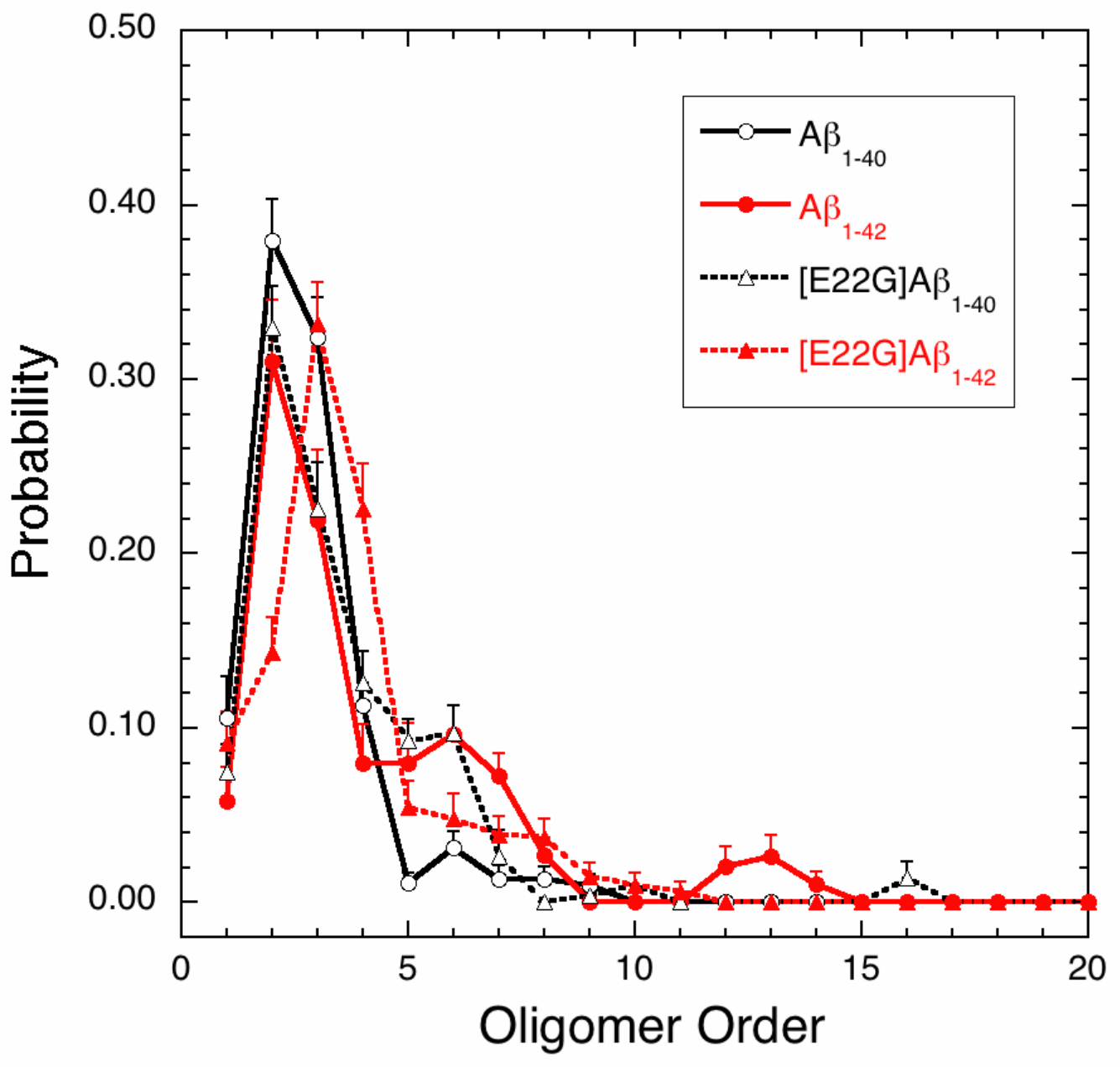


A2. YES:

→ Arctic $A\beta_{1-40}$ pentamers & hexamers

→ Arctic $A\beta_{1-42}$ NO pentamers & hexamers

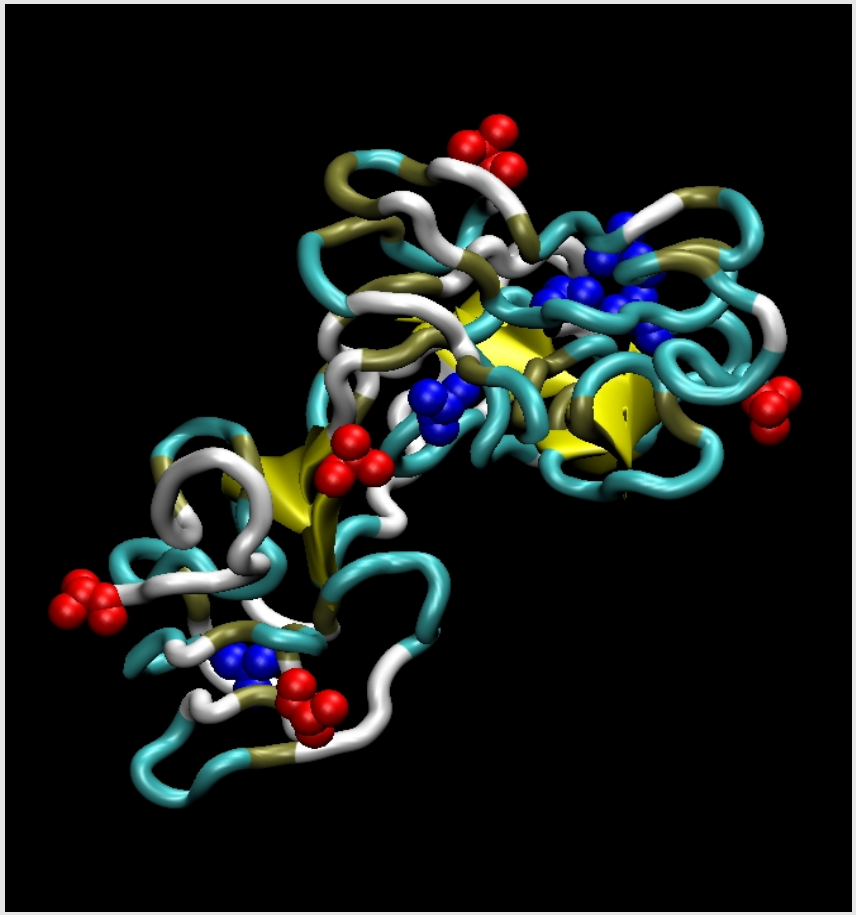
→ NO higher-order oligomers



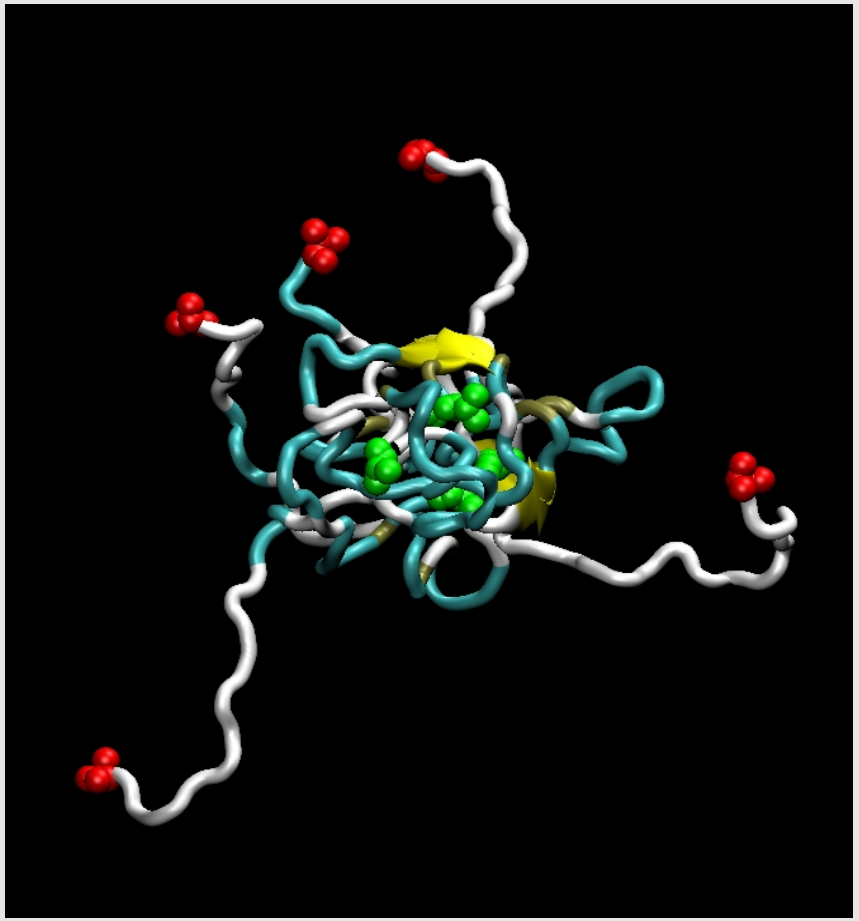
Urbanc *et al.*, JACS, in revision.

Q3. Are Oligomers of $A\beta_{1-40}$, $A\beta_{1-42}$, $[E22G]A\beta_{1-40}$ and $[E22G]A\beta_{1-42}$ Structurally Different?

3D structure of $A\beta_{1-40}$ and $A\beta_{1-42}$ pentamers



$A\beta_{1-40}$



$A\beta_{1-42}$

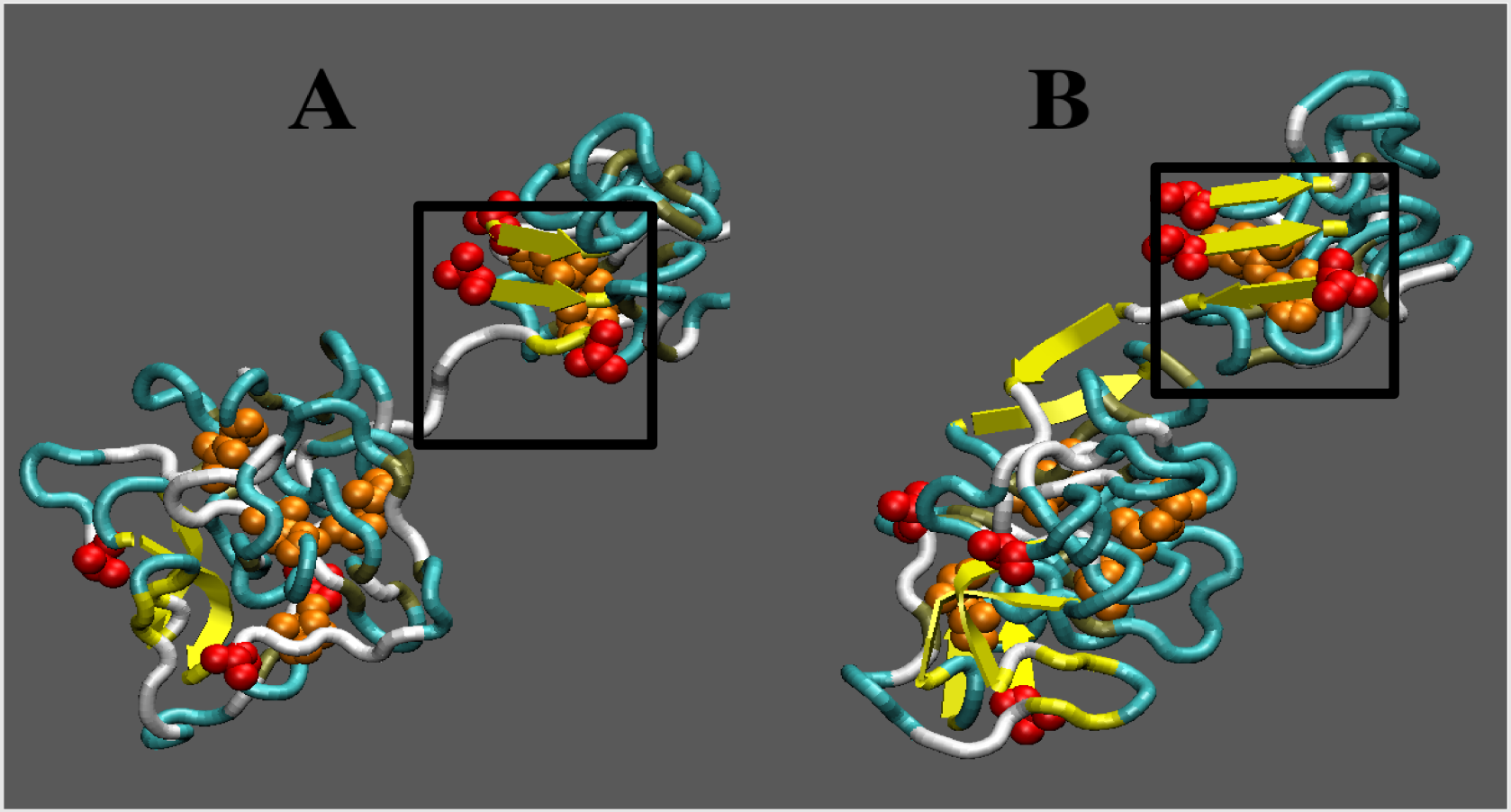
N-termini: D1 (Aβ40 & Aβ42); C-termini: V40 (Aβ40) & A42 (Aβ42)

Urbanc *et al.*, PNAS, 2004.

$A\beta_{1\text{-}40}$ forms a hexamer via intermolecular A2-F4 HBs

(a tetramer and a dimer)

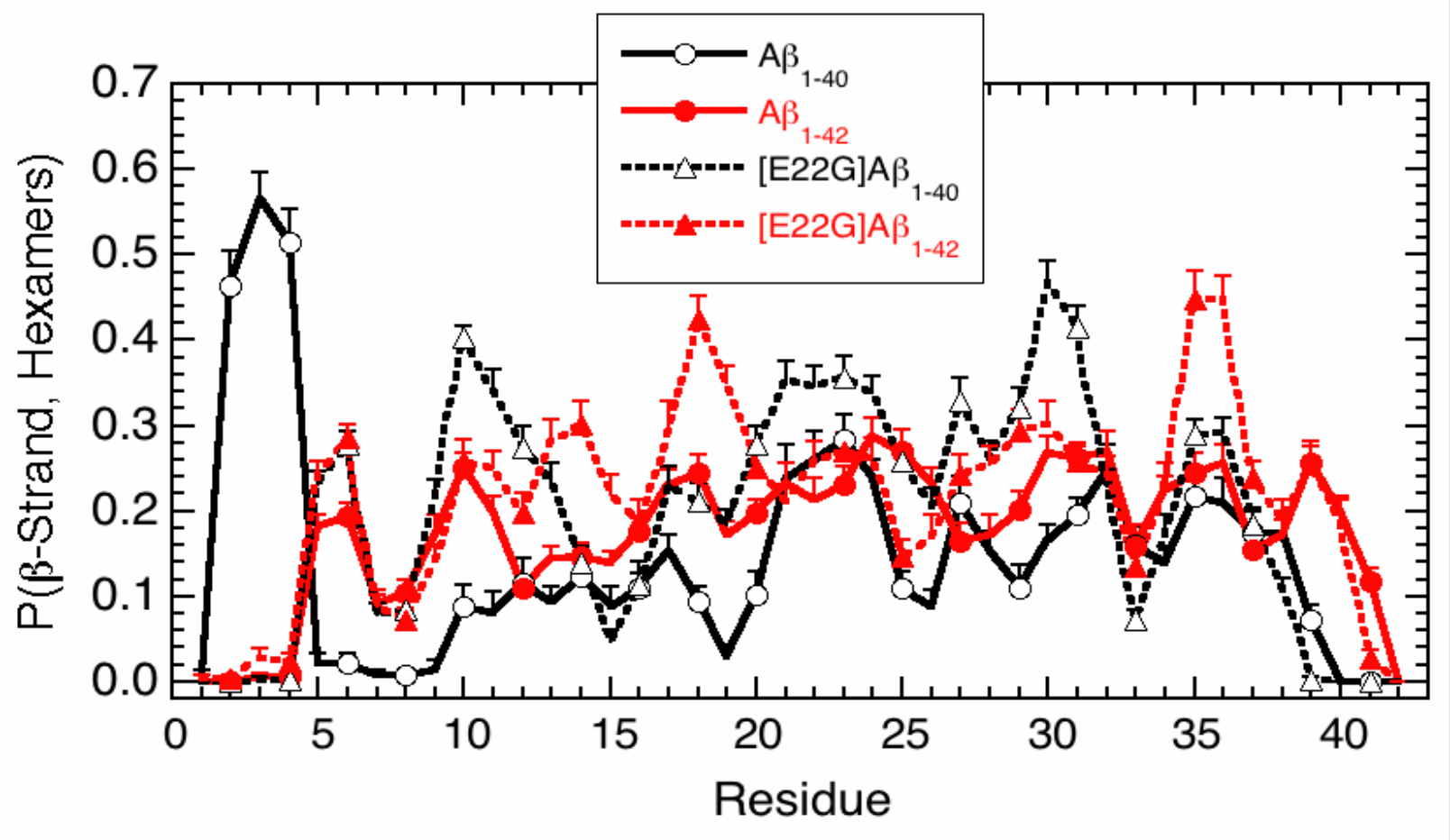
A: docking **B: locking**



Urbanc *et al.*, JACS, in revision.

→ $A\beta_{1\text{-}40}$ distinct A2-F4 β-strand

→ the Arctic oligomers resemble $A\beta_{1\text{-}42}$ but more β-structure

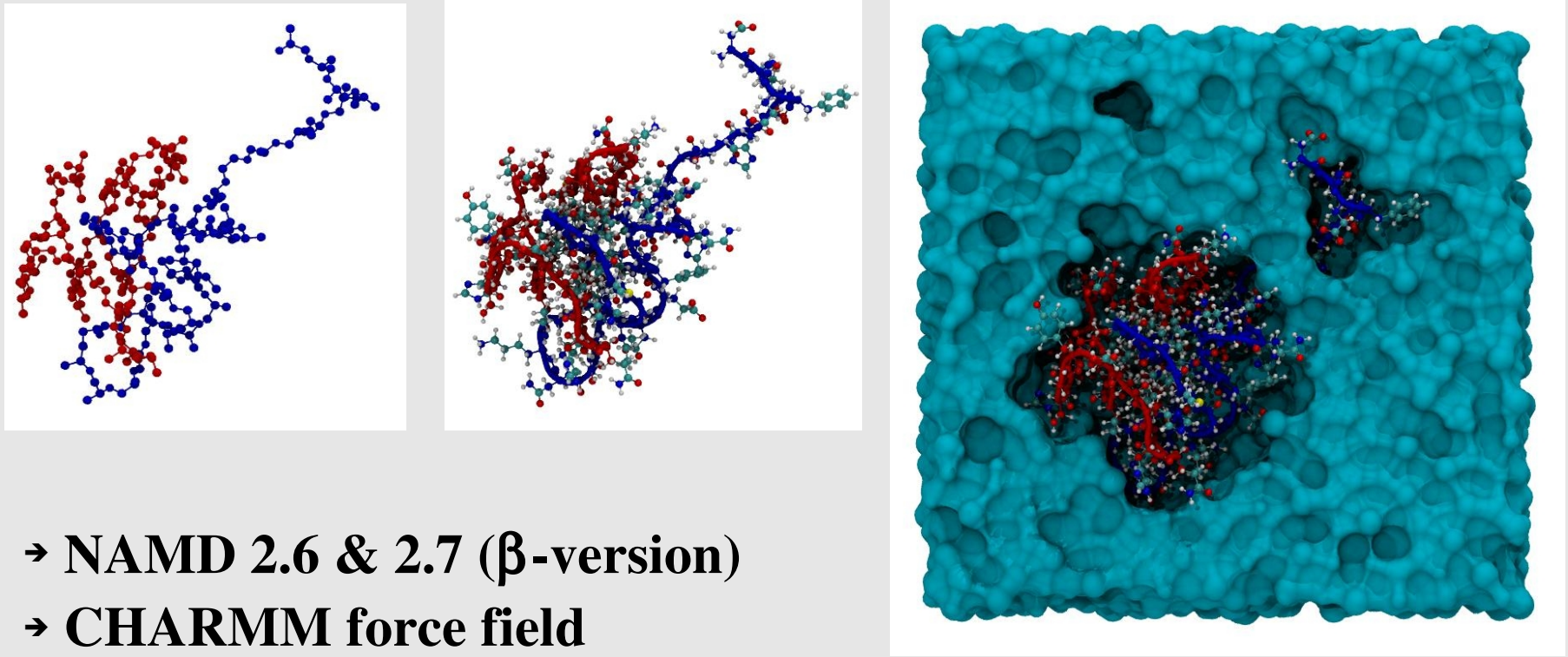


Urbanc *et al.*, JACS, in revision.

$A\beta_{1\text{-}42}$ Dimer: From 4-Bead to All-Atom in Water

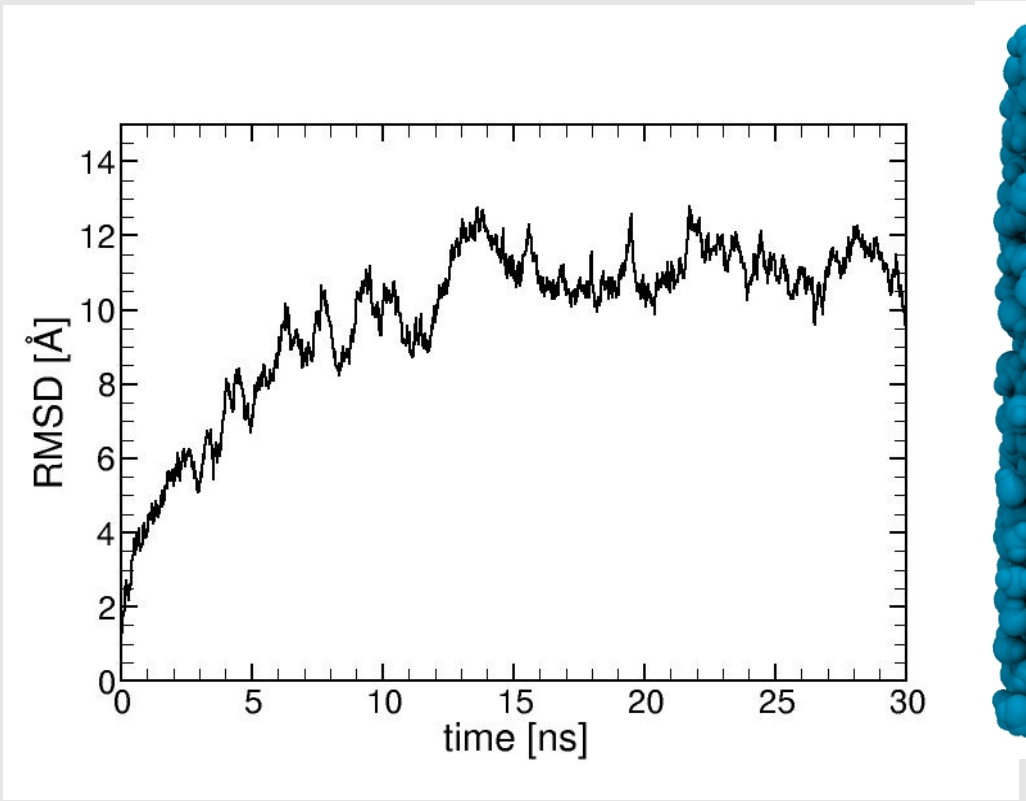
Stability Analysis

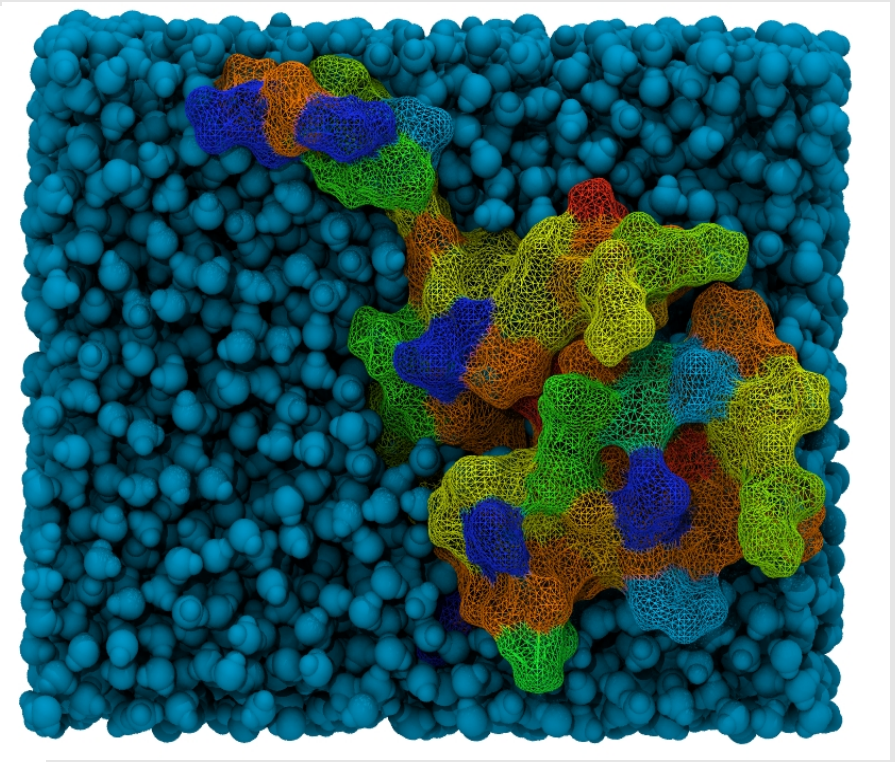
4-bead **all-atom** **water**



- NAMD 2.6 & 2.7 (β-version)
- CHARMM force field
- TIP3P water model

Barz *et al.*, in preparation.





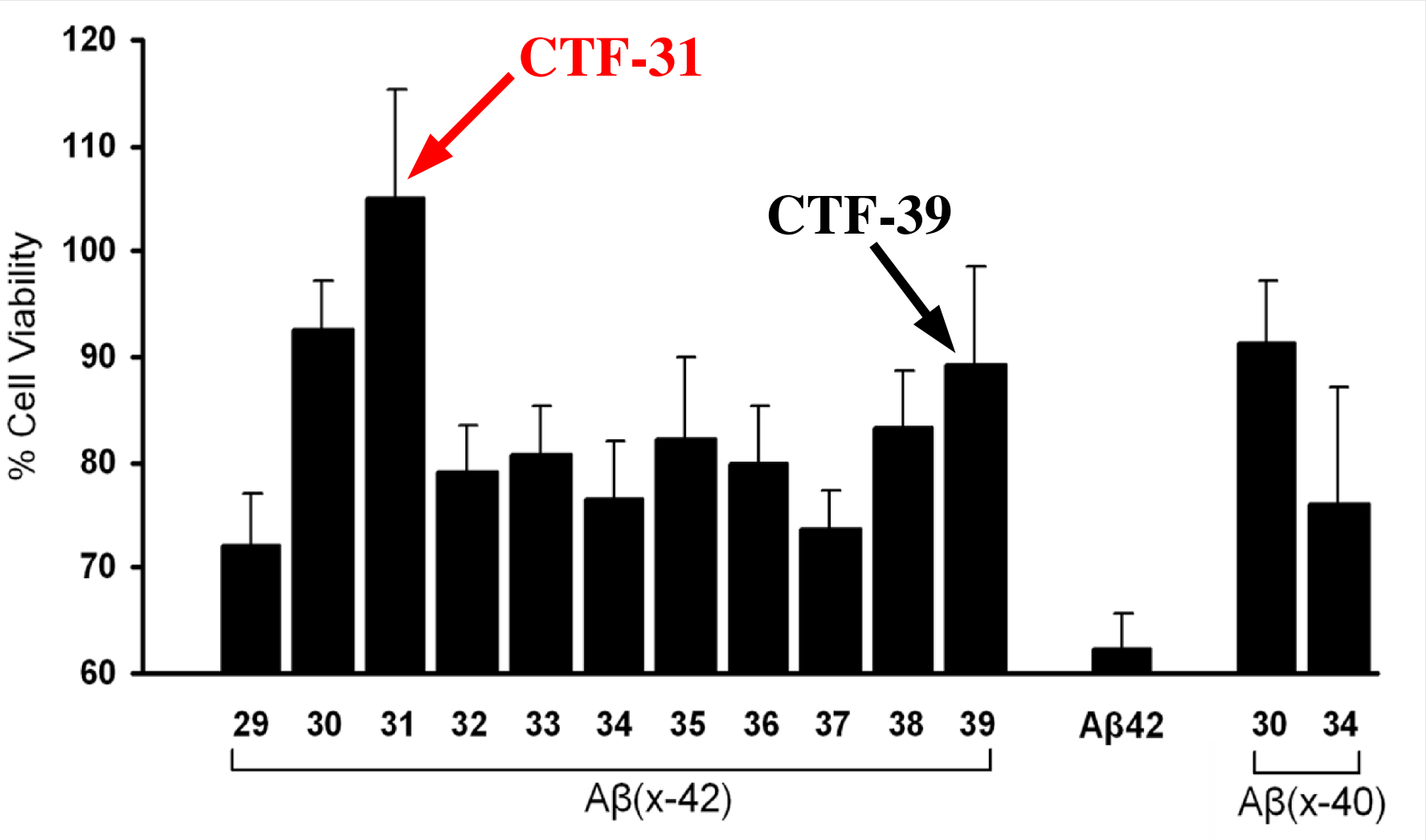
0 hbonds 2 hbonds 4 hbonds

Q4. Can Structural Information Derived By DMD Be Used to Study $A\beta_{1-42}$ Toxicity Inhibition?

C-Terminal Fragments of $A\beta_{1\text{-}42}$ (CTFs)

$A\beta_{1\text{-}42}$ Toxicity Inhibitors

CTFs Reduce $A\beta_{1\text{-}42}$ Toxicity in Cell Cultures

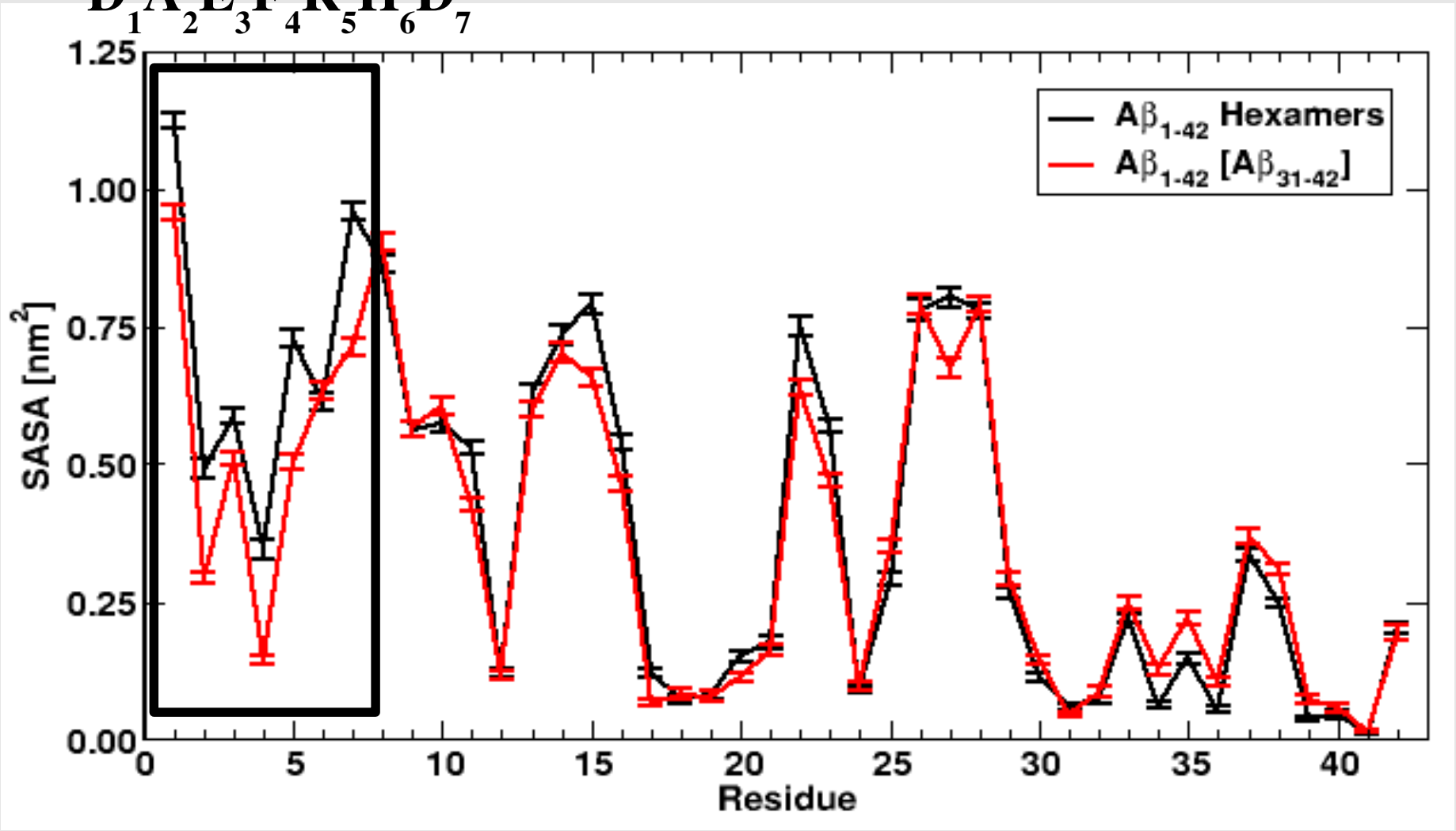


Fradinger *et al.*, PNAS, 2008.

FUTURE:

Is the lack of structure at D1-D7 linked to toxicity?

$D_1 A_2 E_3 F_4 R_5 H_6 D_7$



CONCLUSIONS

- **two implicit solvent parameters of the DMD model tuned to the *in vitro* Aβ oligomer size distribution**

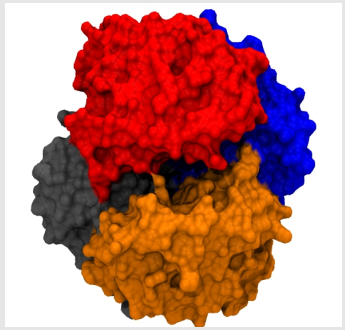
- **the DMD approach captures folding and oligomer formation differences between the two Aβ alloforms & their Arctic mutants**

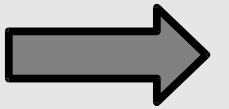
- **charged residues ⇒ elongated protofibril-like oligomers**

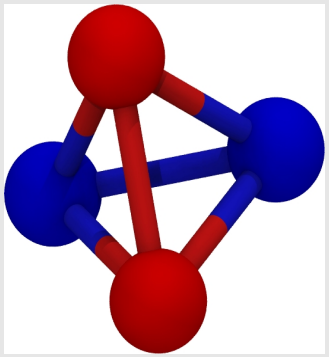
- **[E22G]$A\beta_{1-40}$ and $A\beta_{1-42}$ oligomers structurally alike: Arctic form of AD caused by [E22G]$A\beta_{1-40}$ toxicity?**

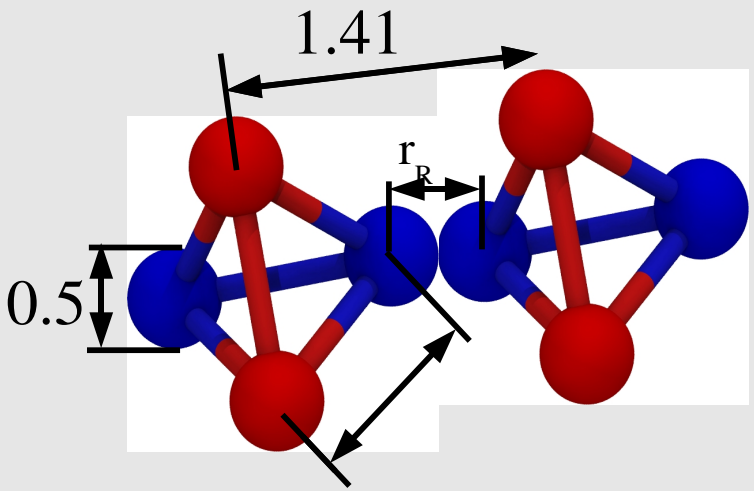
- **DMD approach: screening of $A\beta_{1-42}$ toxicity inhibitors**

Assembly of hemoglobin: relevance to sickle-cell disease



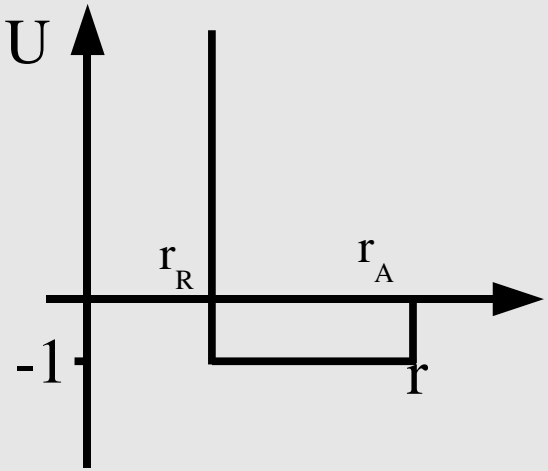


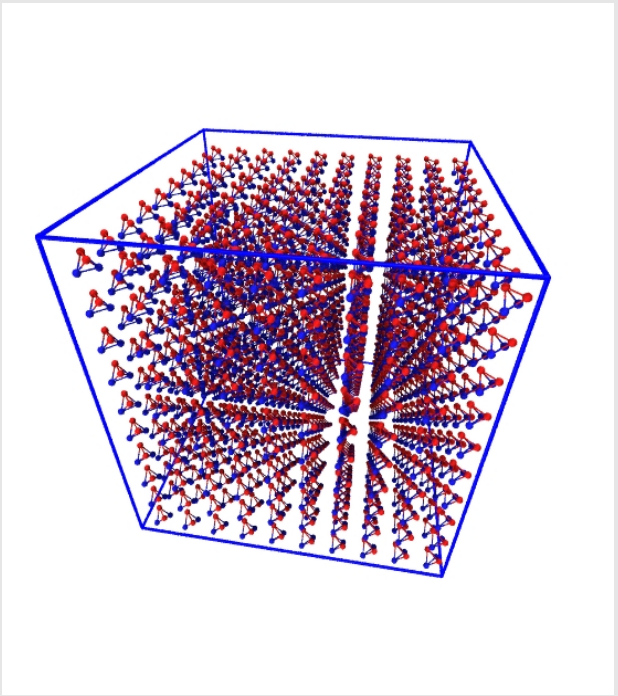




All-atom hemoglobin

4-bead hemoglobin

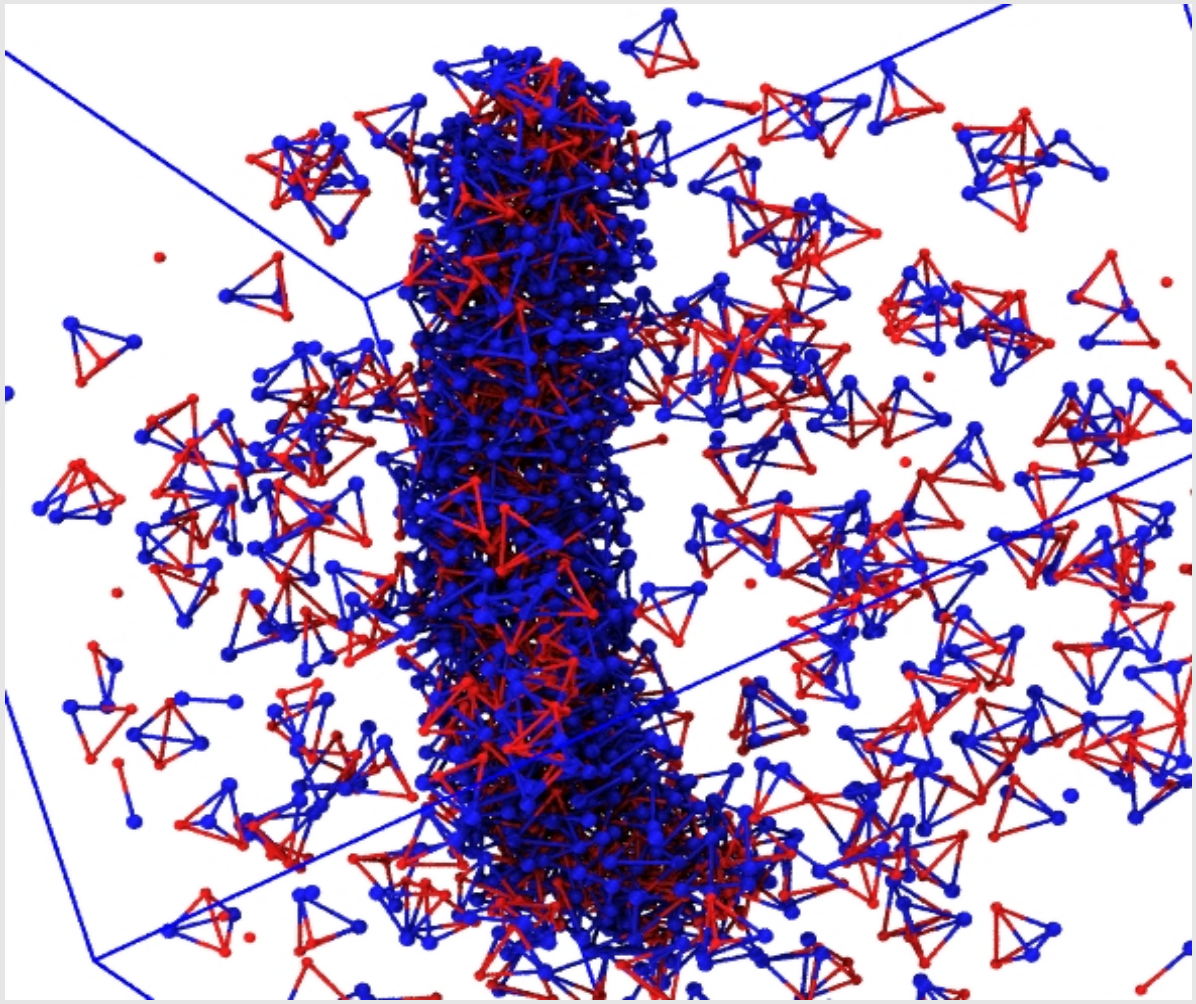




Temperature = 0.98 E

Density = 0.11 tetra/L^3

r_A = 1.5 L, r_R = 0.5 L



Barz *et al.*, in preparation.

Current Graduate & Postdoctoral Collaborators:

Bogdan Barz (Aβ oligomer stability by all-atom MD, hemoglobin)

Mark Betnel (Aβ versus α-synuclein folding & assembly)

Derya Meral (Aβ folding)

Yuriy V. Sereda (united atom protein model)

Former:

Alfonso Lam (Aβ folding), Beckman Research Institute

Other Collaborators:

➔ Drexel Univeristy:

Luis Cruz (all-atom MD of $A\beta_{21-30}$, protsView)

Frank Ferrone (hemoglobin)

➔ UCLA:

Gal Bitan (Aβ toxicity inhibition & PICUP)

David B. Teplow (Aβ folding, oligomerization & inhibition)

2000-2006

DMD Code and Protein Model Development:

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Gerry Paul (Boston Univeristy)
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FUTURE DIRECTIONS