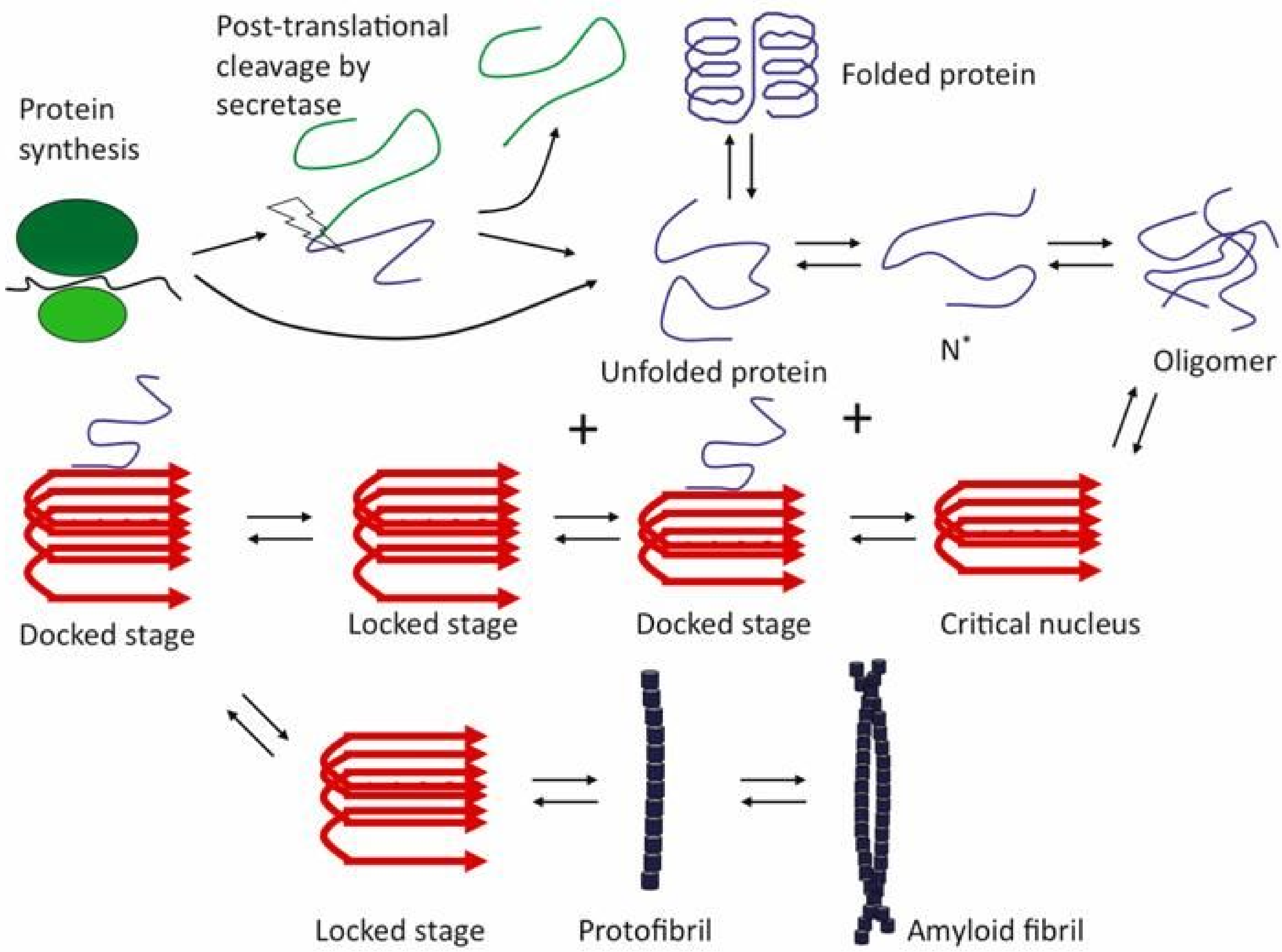
Growth mechanism of amyloid Fibrils and Oligomers

Govardhan Reddy
Ed O'Brien (Cambrdge,UK)
Mai Suan Li (Warsaw, Poland)
Bernard R. Brooks (NIH)
John E. Straub
(Boston University)

Phuong H. Nguyen (Germany)
Gerhard Stock (Germany)

Funding NIH

Fate of a Polypeptide Chain



“Post Evolutionary” Diseases

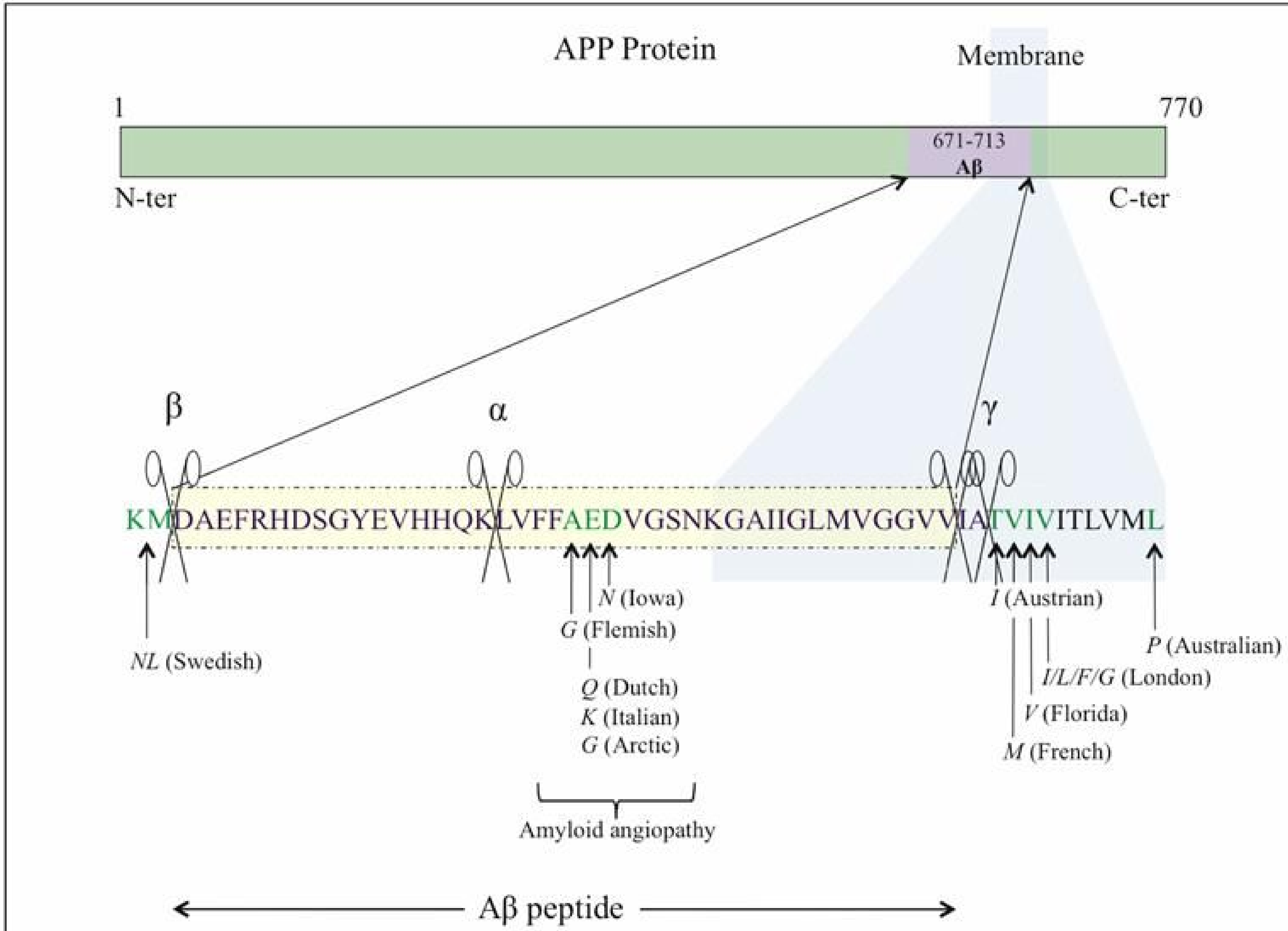
[Nature 418, 729-730 (2002)]

Clinical Syndrome	*Fibril subunit*
Alzheimer’s disease	1-40 or 1-42 fragment of Aβ protein
Transmissible spongiform encephalopathies	Full-length or fragments of prion protein
Reactive systemic amyloidosis	Fragment of amyloid A protein
Type II diabetes mellitus	Islet amyloid polypeptide (IAPP)
Senile systemic amyloidosis	Wild-type transthyretin
Hemodialysis-related amyloidosis	Full-length, wild-type β2-microglobulin

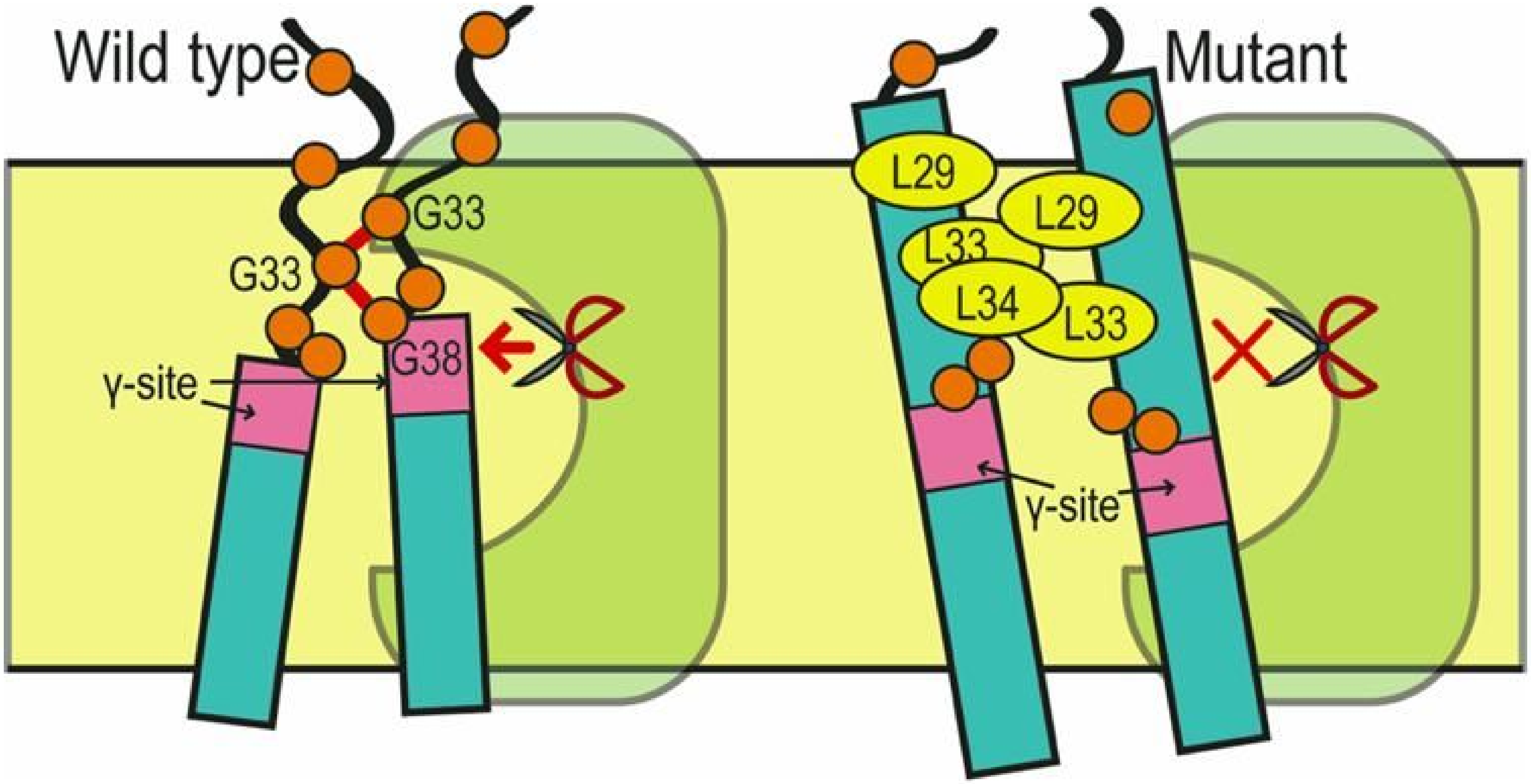
What is the common colecular growth mechanism, if any?

Current Problems of Interest (to us)

Role of secretases (Drug Targets) in metabolic processing of APP into Aβ



Cleavage & products



Once cleaved how does Aβ associate?

Kinetics of oligomer formation, Growth of fibrils

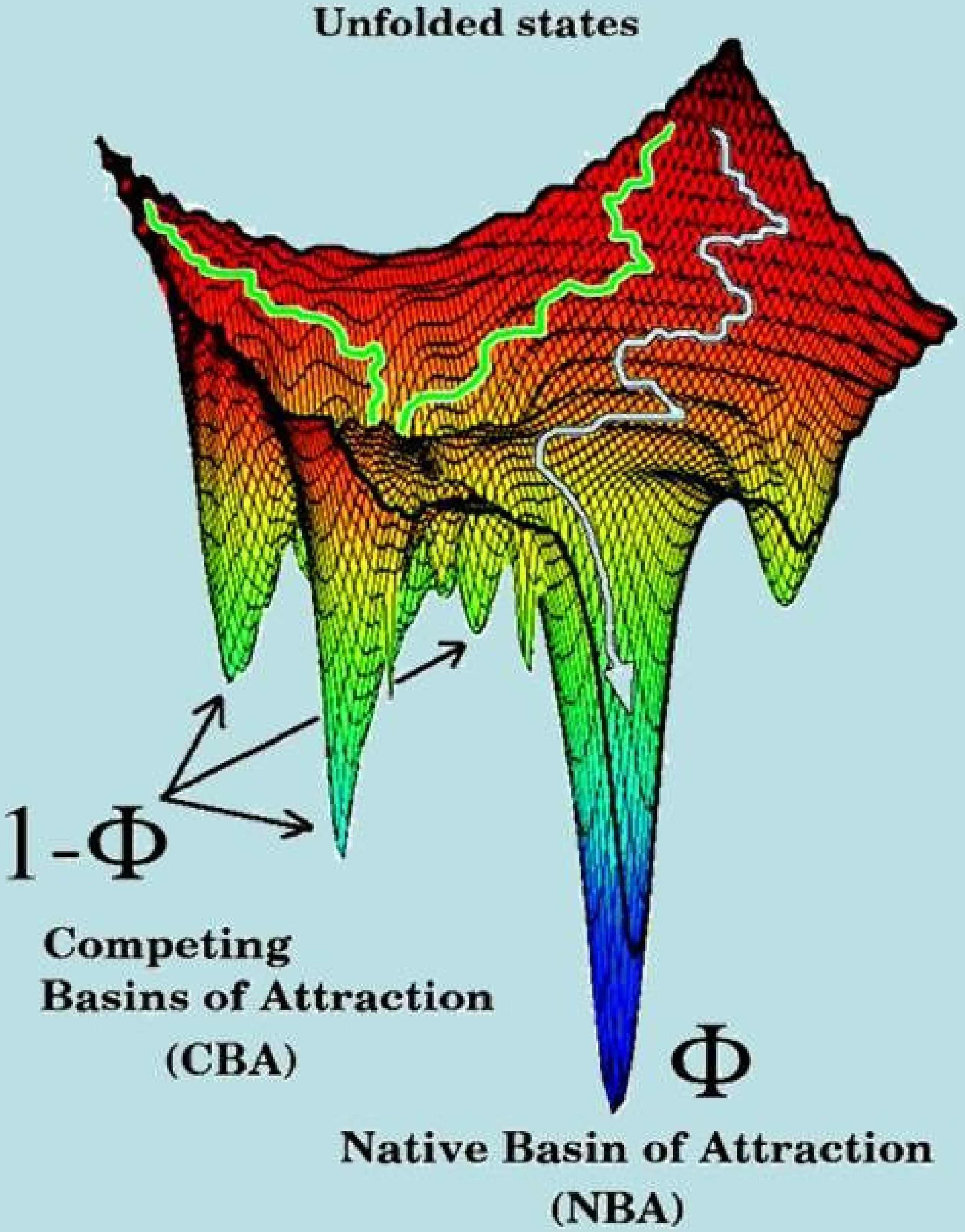
Toy model + Aβ + Sup35 (Yeast prions)

Outline of the Talk

- Fluctuations in the Monomers & Implications
- Growth of monomers and Nucleation (?)
- Molecular events in the addition of monomers to fibrils (role of water Key)

Protein Misfolding Consequences

Relation to monomeric folding: Comfortational Diseases



Hyeon and dt
Biochemistry (05)

Monomer can misfold to multiple conformations

Structural variations in the CBAs imprinted in oligomers and fibrils

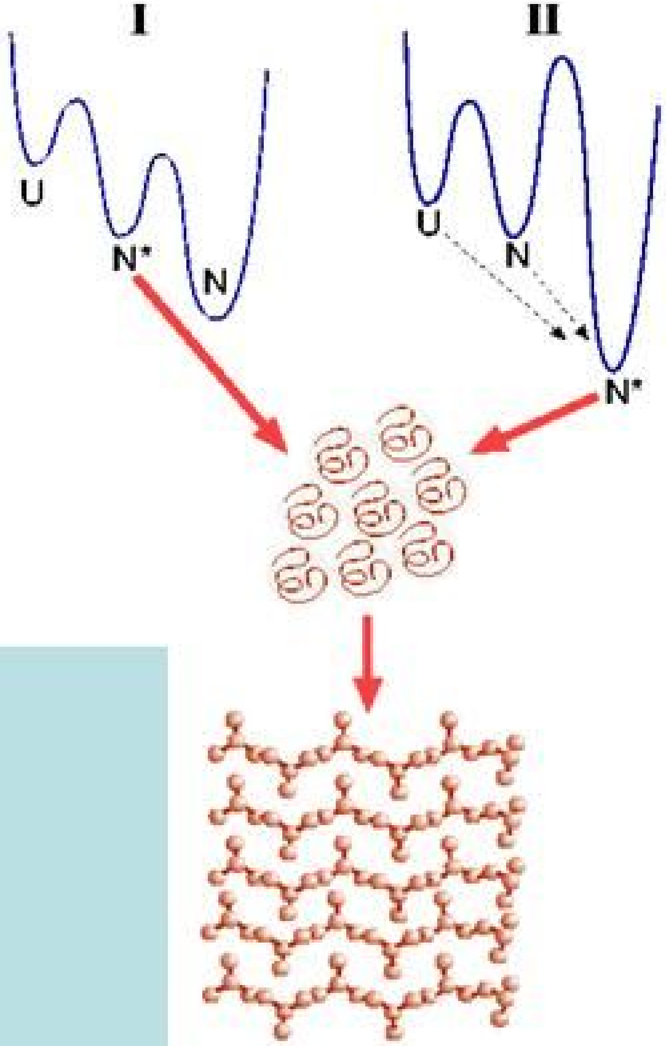
Scenarios for AGgregation

(dt, D. Klimov and R.Dima, Curr. Opin. Struct. Biol., 2003)

Example TTR

N* = metastable (conformational variations)

N* formation = partial unfolding



Mammalian Prions

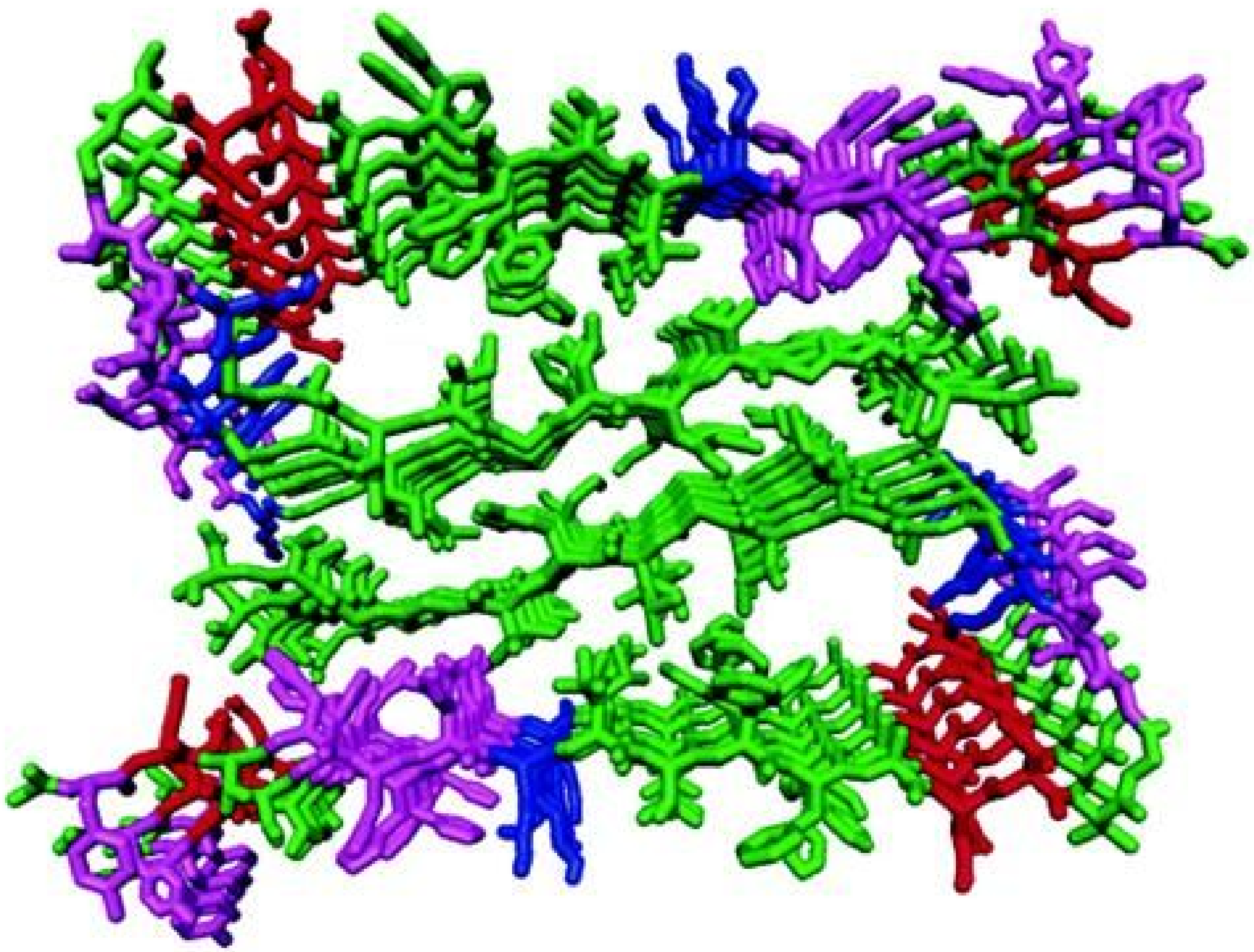
N* = stable

N* formation in prions = unfolding of N

K_G depends on rate of formation of N^* from N or U (Evidence from J. Kelly for TTR)

PrP^c is metastable with respect to PrP^* aggregation prone Particle; Prediction Dima and dt PNAS (04) Surewicz PNAS (08)

Towards a Aβ Inspired Toy Model

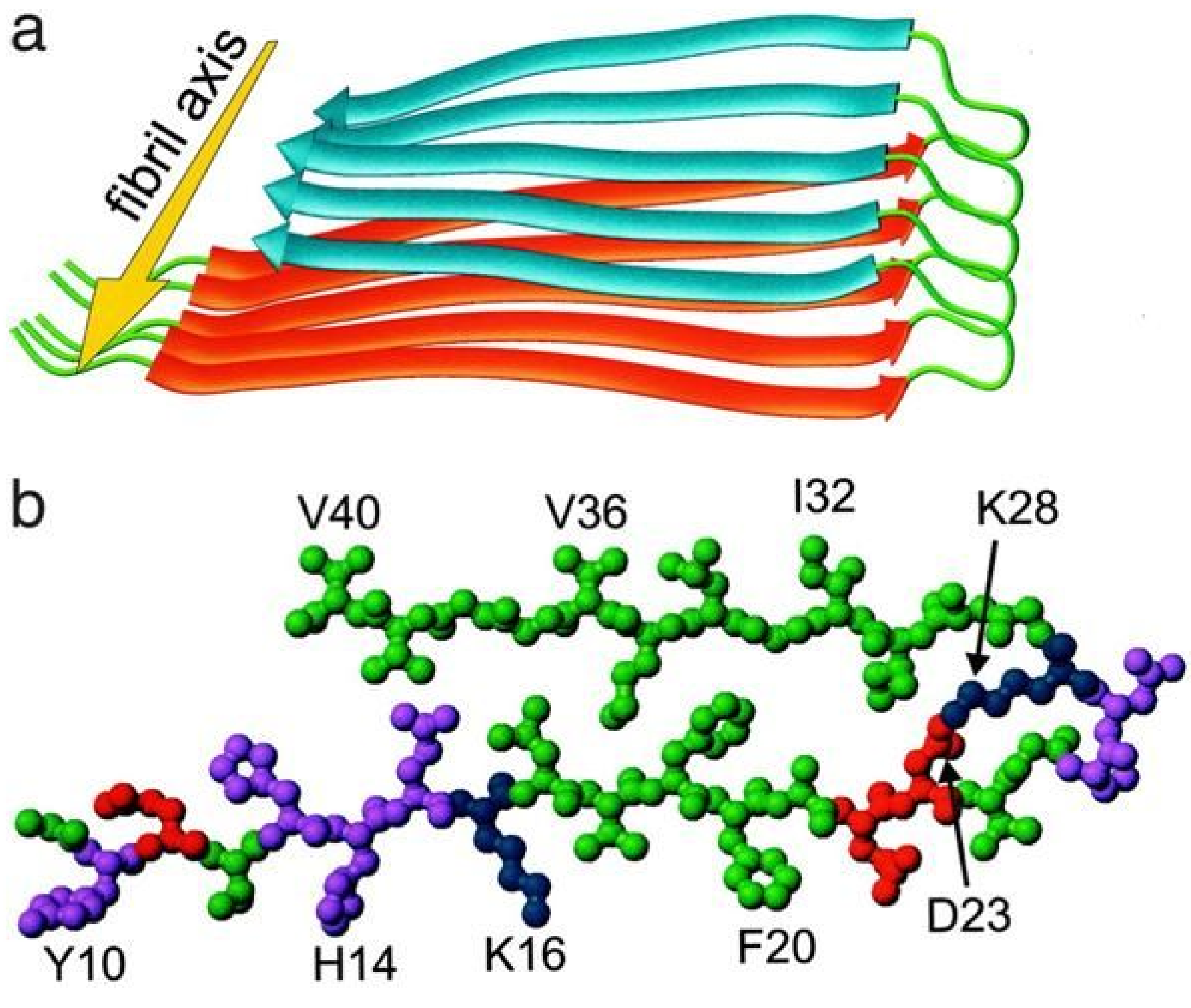


Double-layer (two) protofilaments ($A\beta_{1\text{-}40}$)

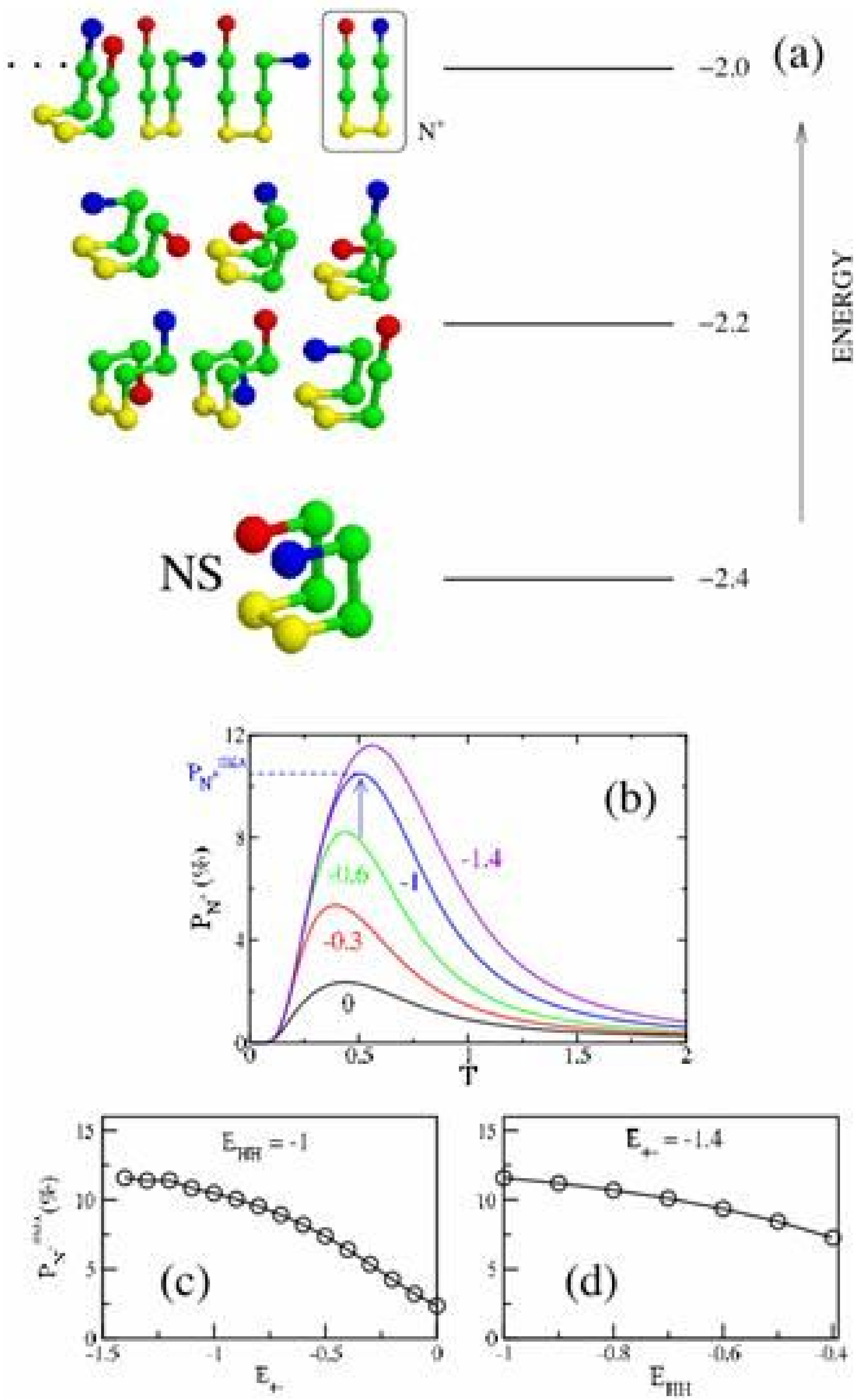
Strands (β-turn-β) Perpendicular to fibril axis

Tycko and company (NIH)

A$\beta_{1\text{-}40}$ Structural Model (Tycko)



Toy Model (Is the fibril structure encoded in monomer spectrum) Prot Sci 2002; JCP 2008



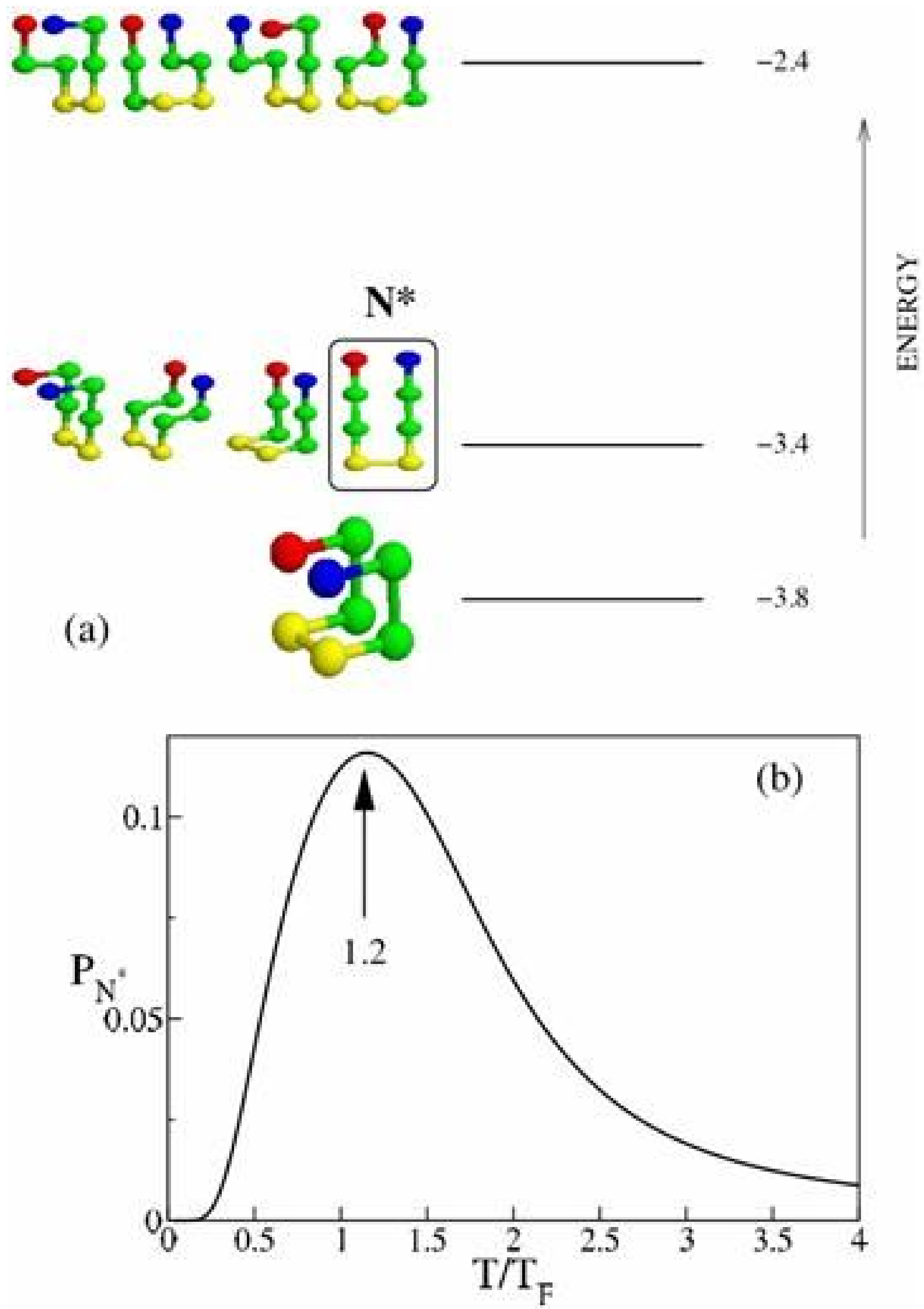
4 types of monomers (H, P, +, -)

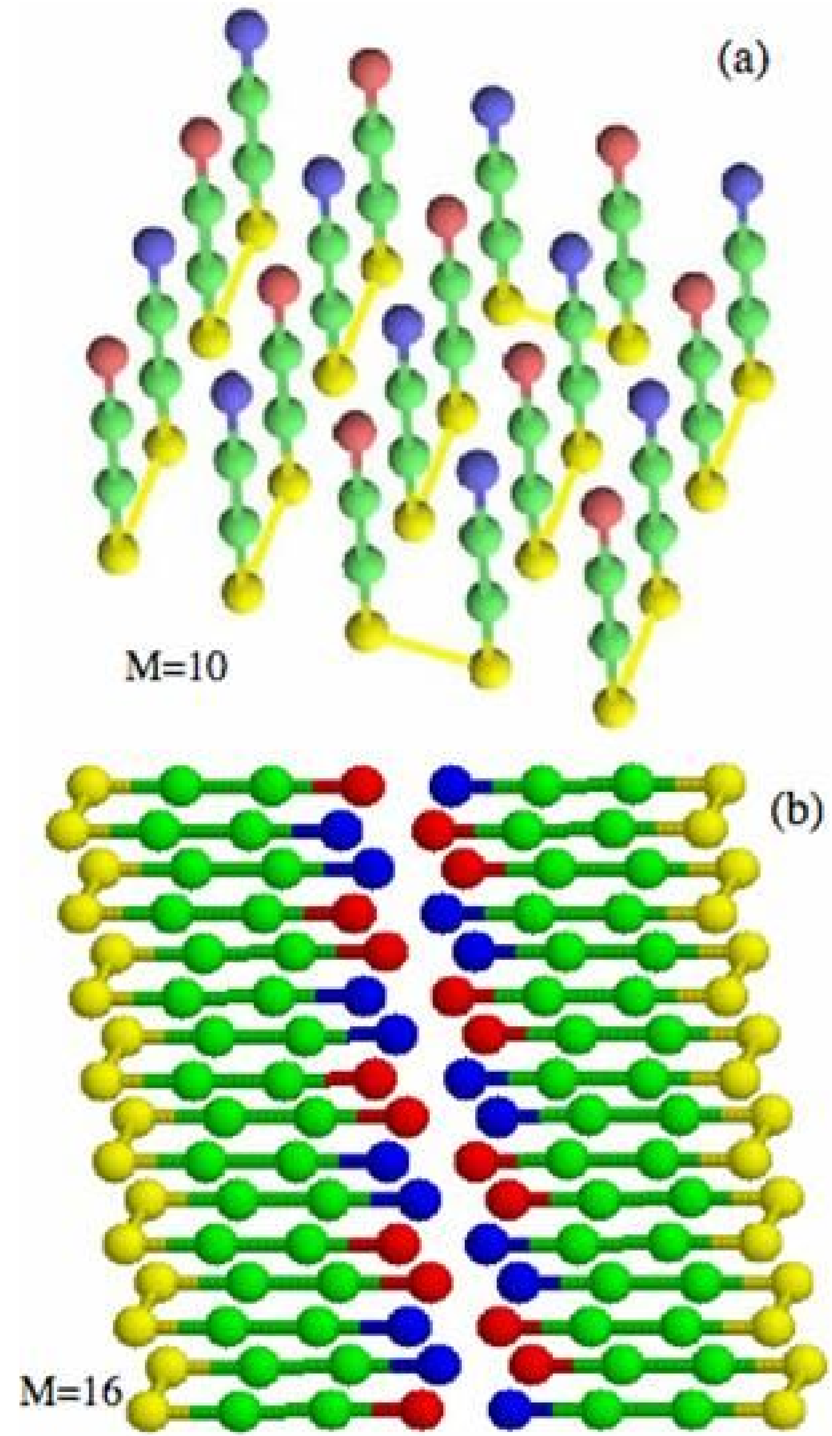
Monomer has 8 beads

# of sequences = 4^8 (amylome)

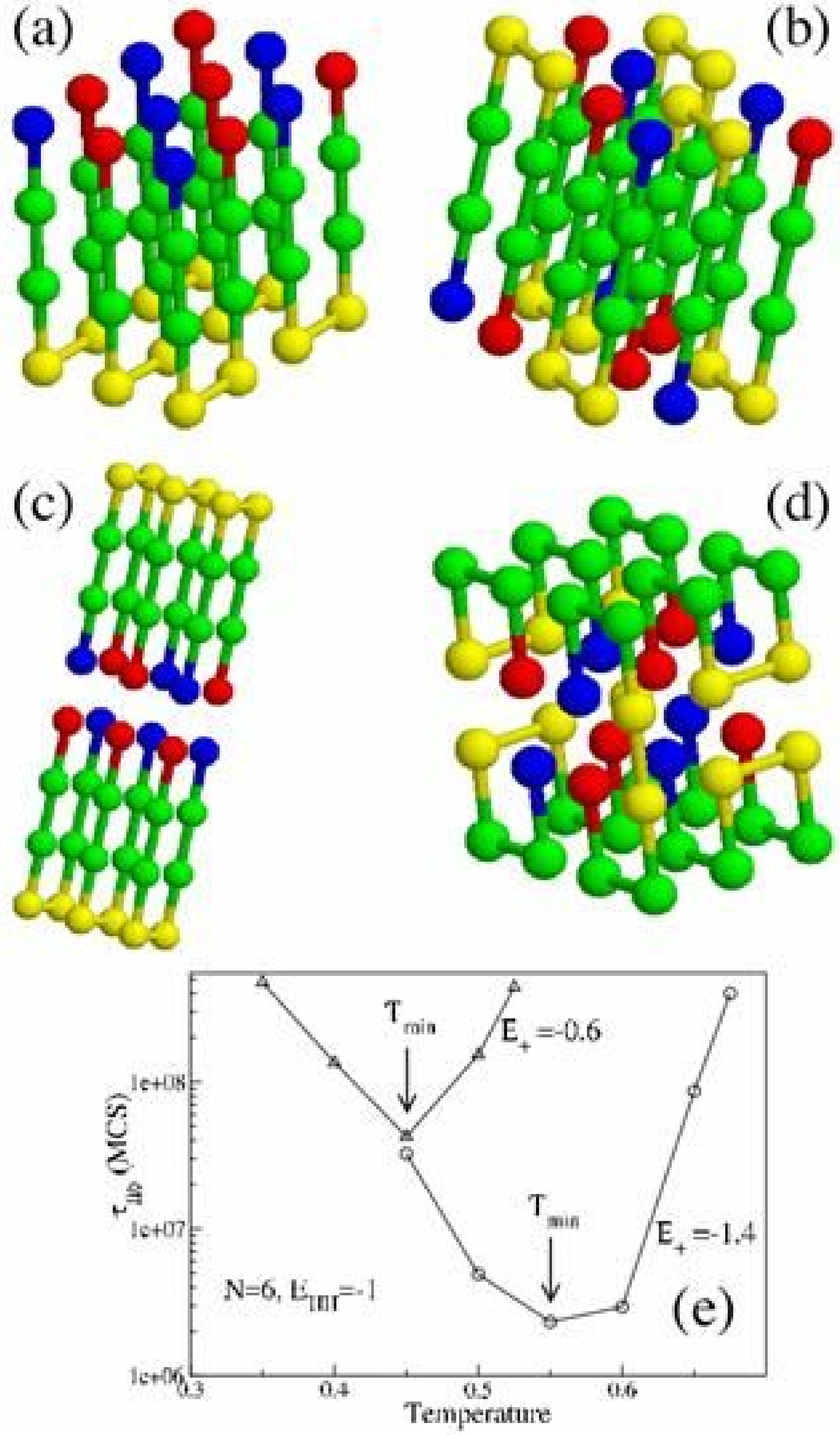
# of conformations on cubic lattice = 1,841

Structure of “protofilament” + “fibril”
Single and double layer





Interplay of E_{+-} and E_{HH}



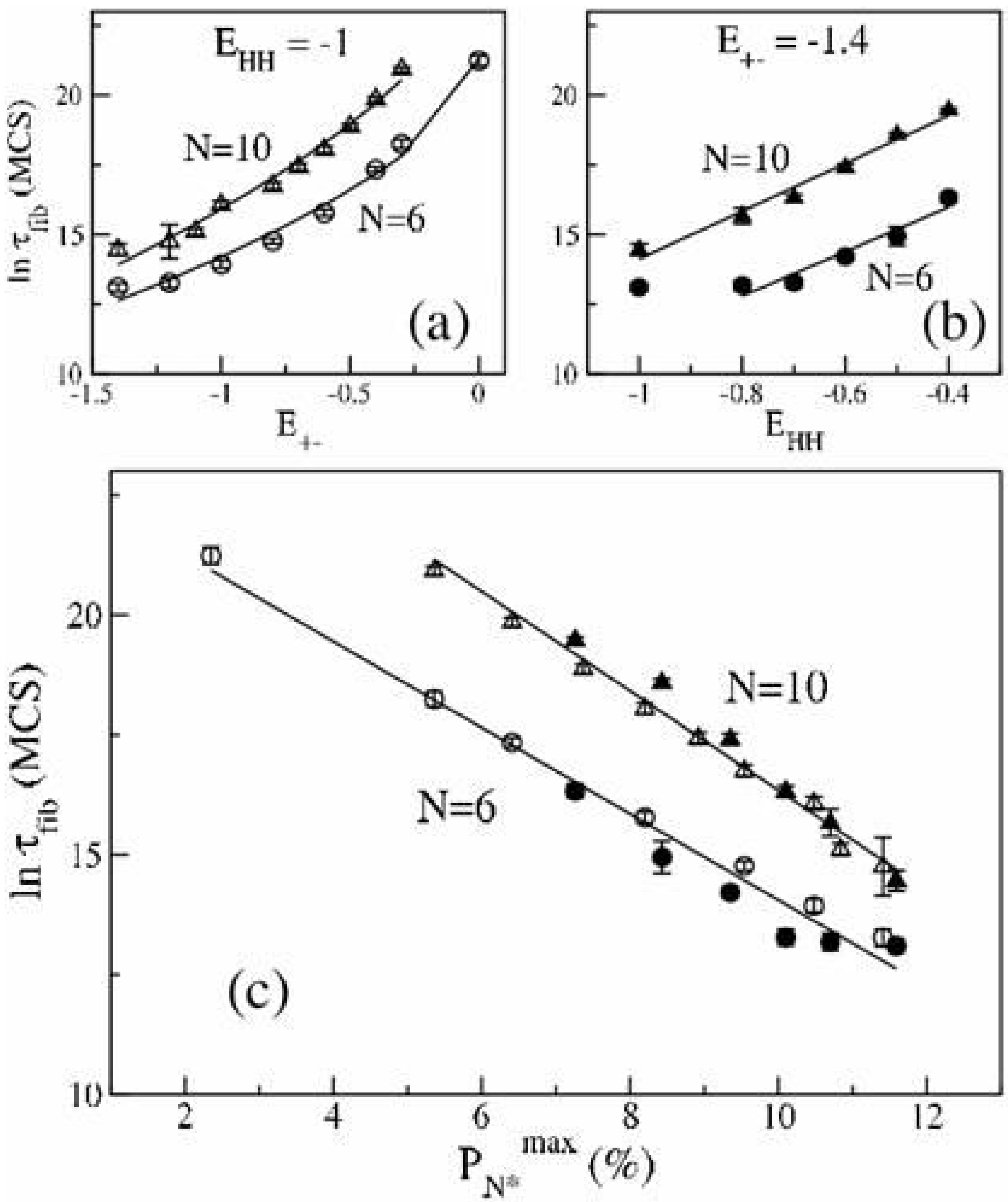
a: Monomers parallel
b: Monomer alternate
c: Double layer
d: No fibril compact

Optimal growth temp
$\tau_{fib} = (10^4 - 10^n)\tau_F$

Largest n about 9

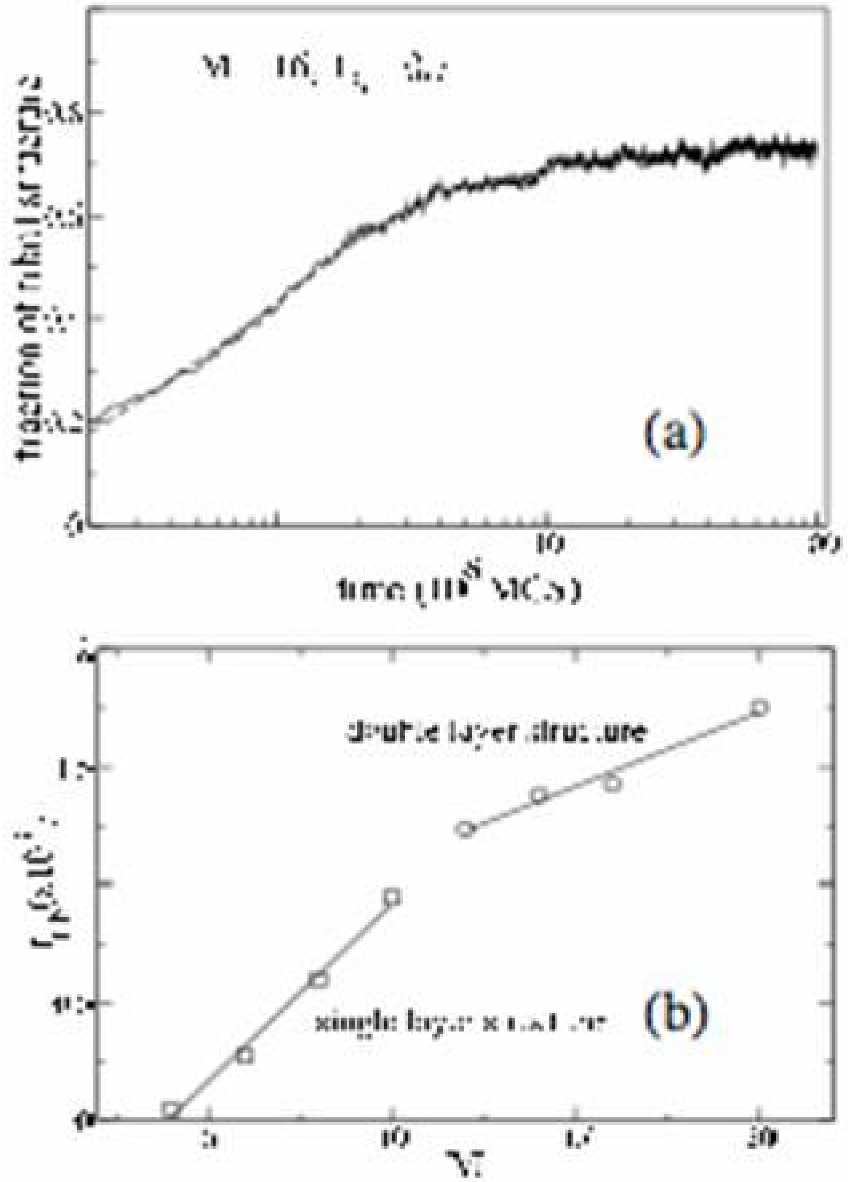
Seeding speeds up fibril rate formation

Growth rate depends on N^* population $P_N{}^*$
Depends on sequence



Sequence + N^* ensemble fibril kinetics ⟹ monomer landscape encodes structure + growth rate

Lifshitz-Slyazov Growth Law
Supersaturated solution
J. Phys. Chem. Solids (1961)

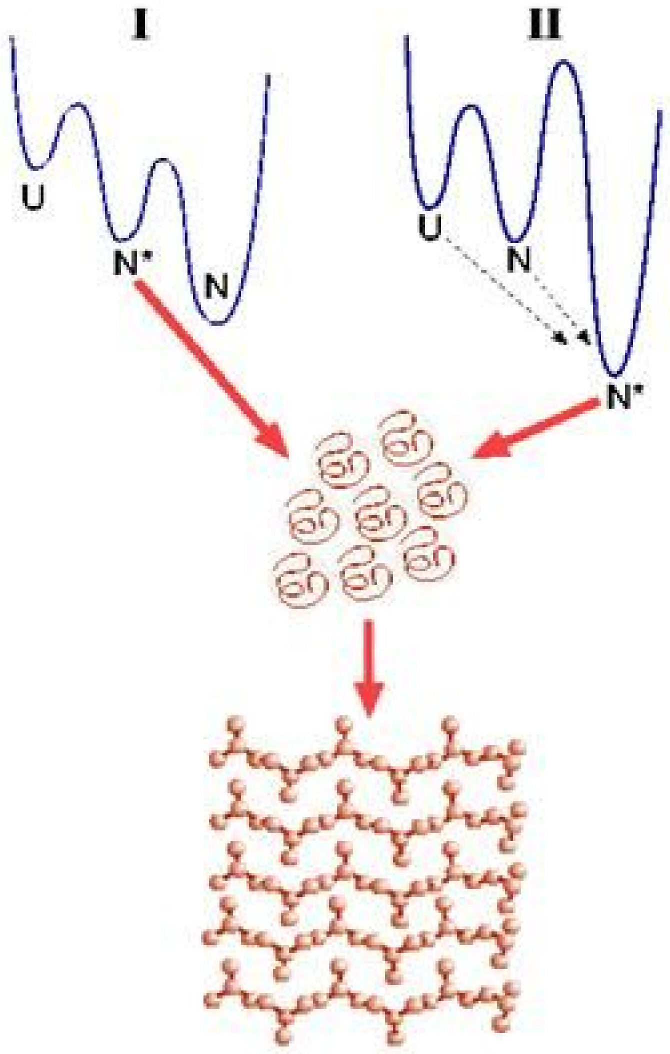


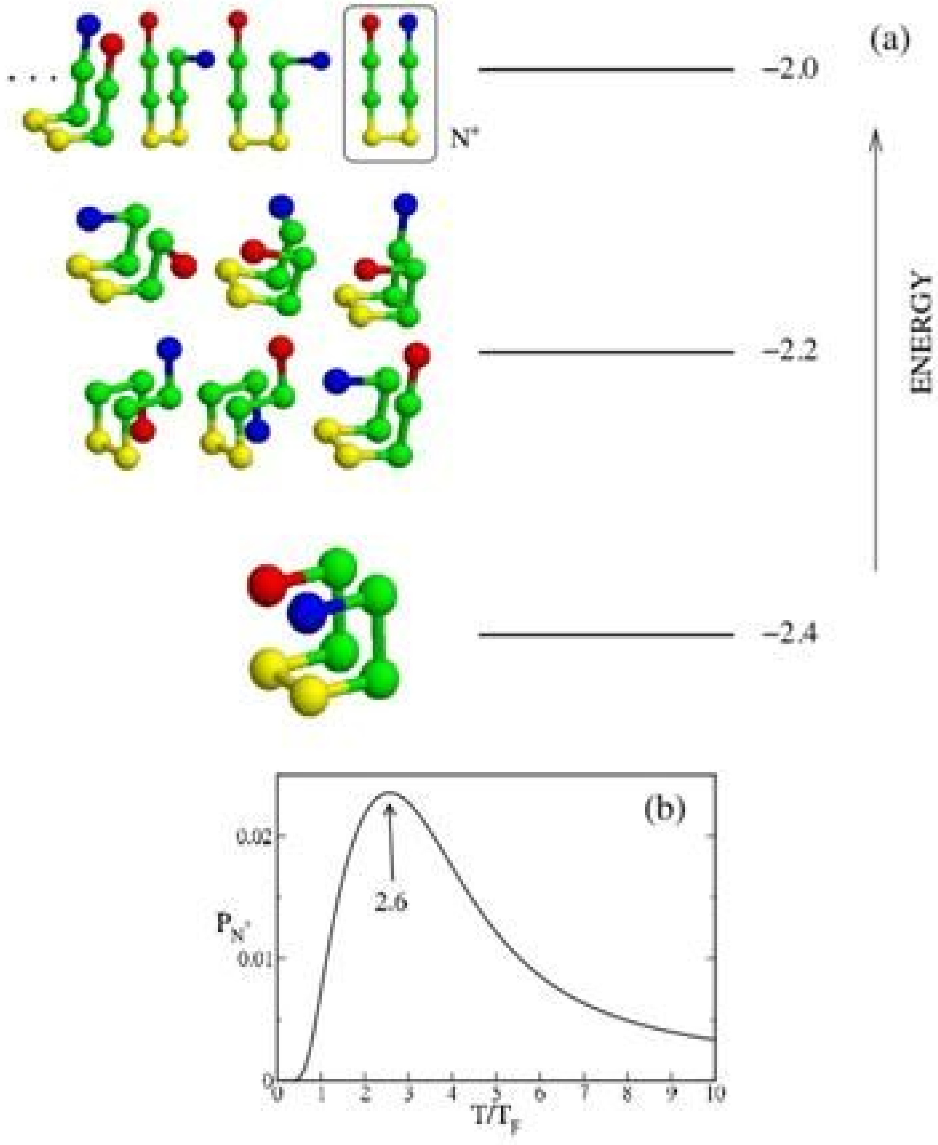
$\tau_G \approx \tau_0 M^{1/3}$

Large clusters incorporate small oligomers

$M \rightarrow M_n^* \, [\rightarrow PF \rightarrow Fibrils]$

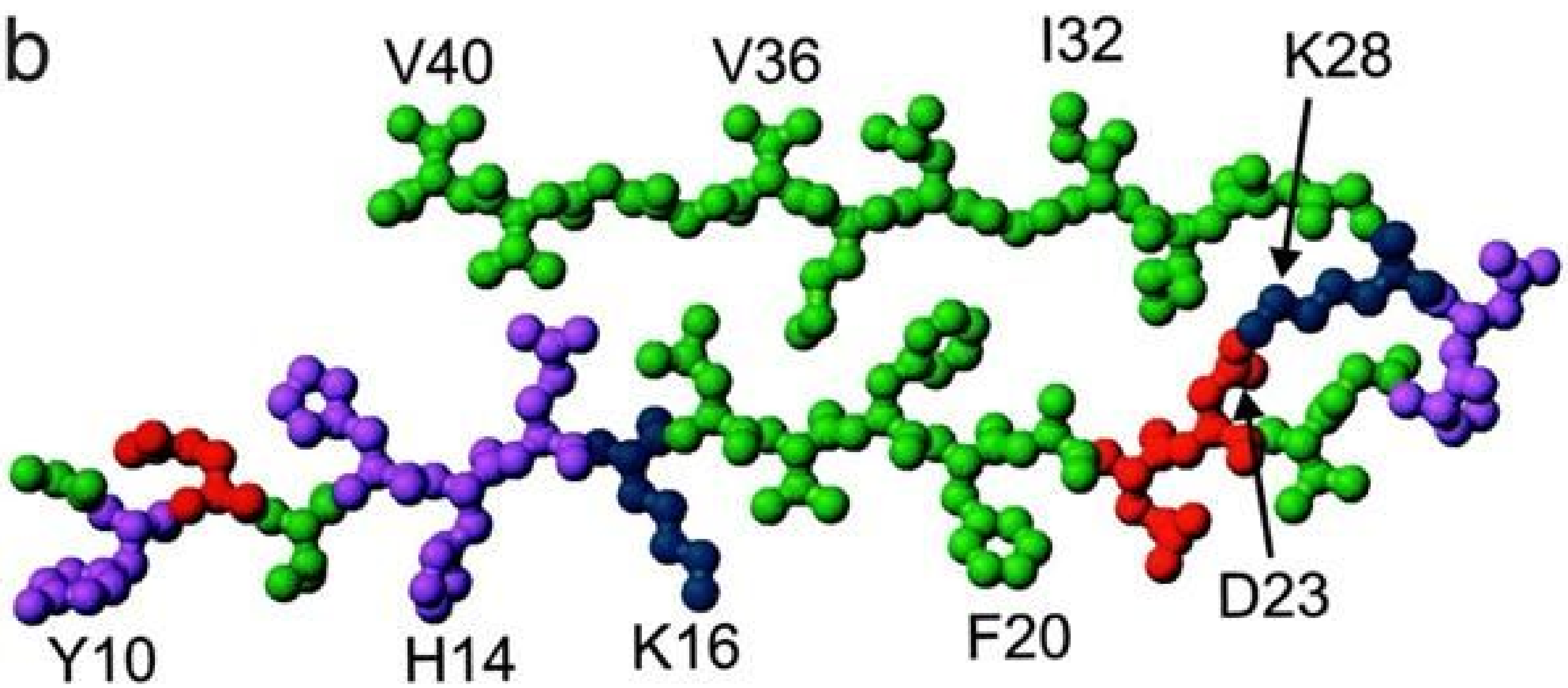
Heterogeneity of N* determines polymorphism?





Nature of N* depends on sequence

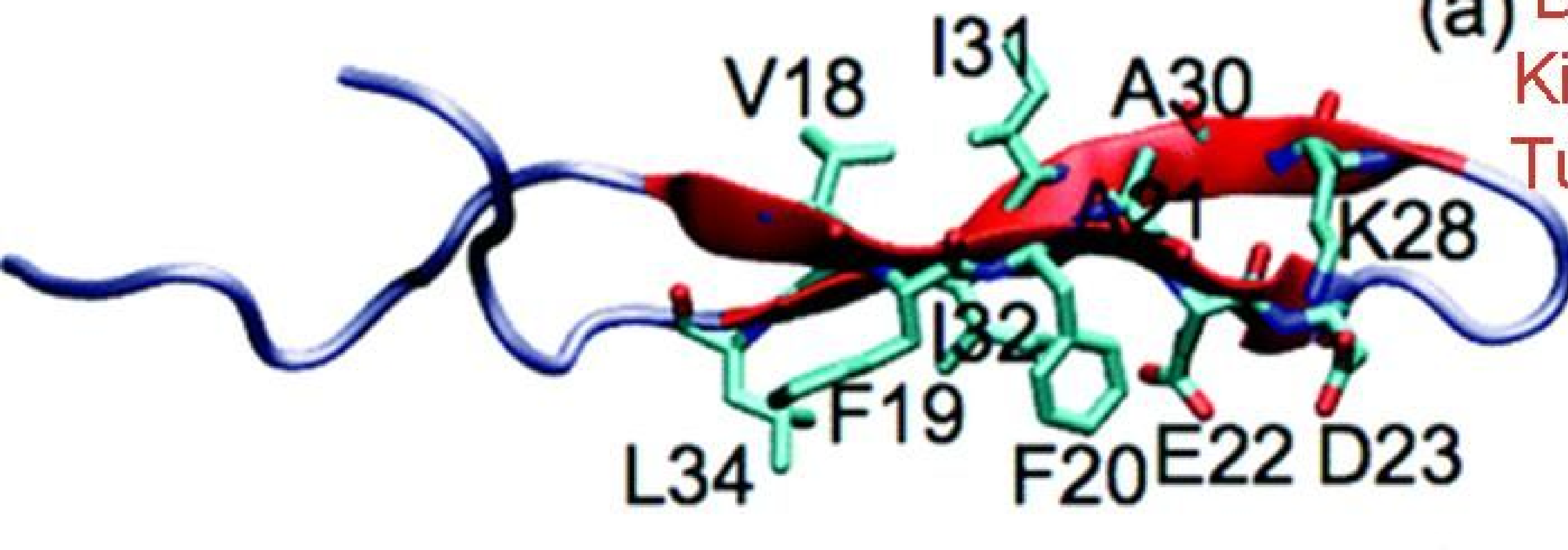
N* and Fibril monomer



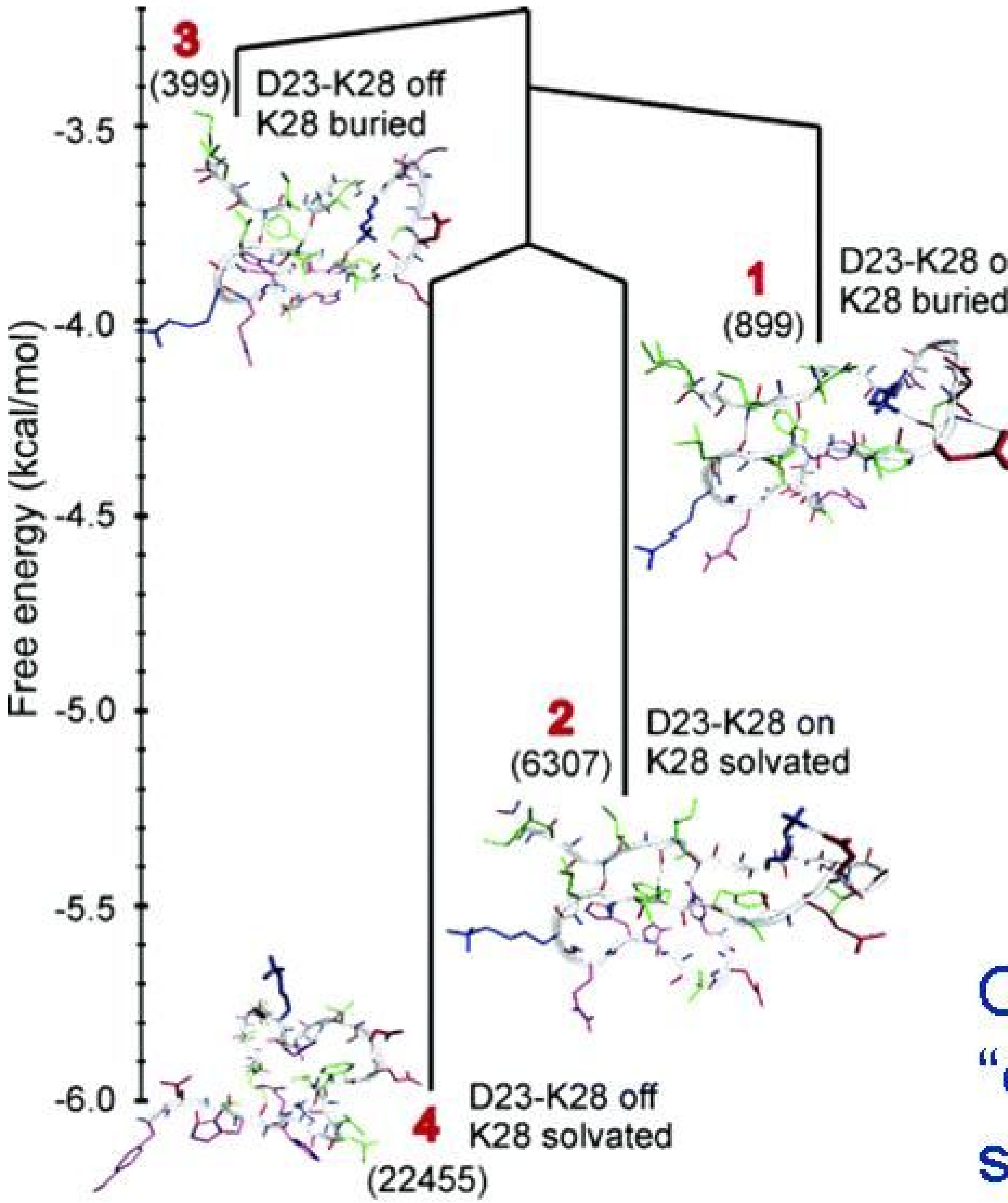
Tycko PNAS (02)

Reddy JPCB (09)

(a) D23-K28 + Kirschner-Straub Turn(VGSN)



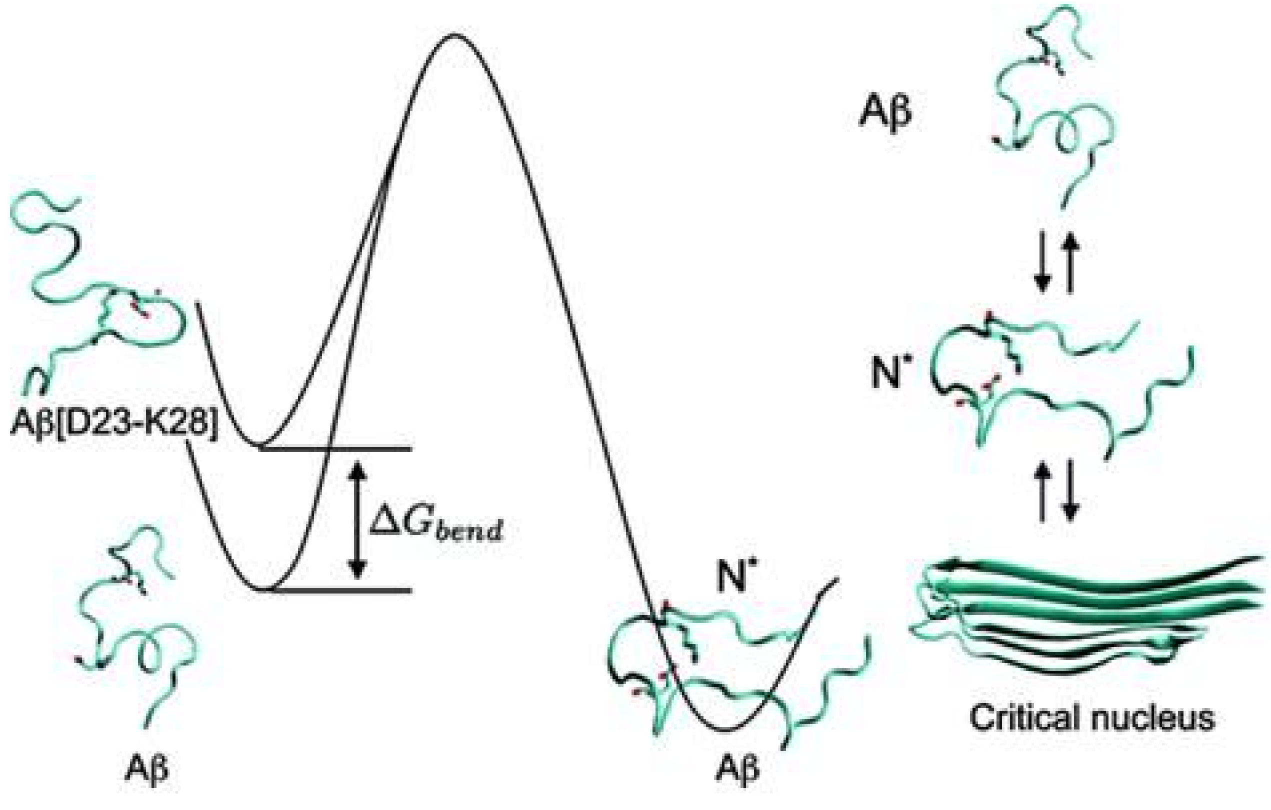
Free energy "spectra" for Aβ(10-35)
Tarus, Straub & dt JACS (2006)



1 & 2 are N* states!

Conjecture: Polymorphism "encoded" in spectra of N* states

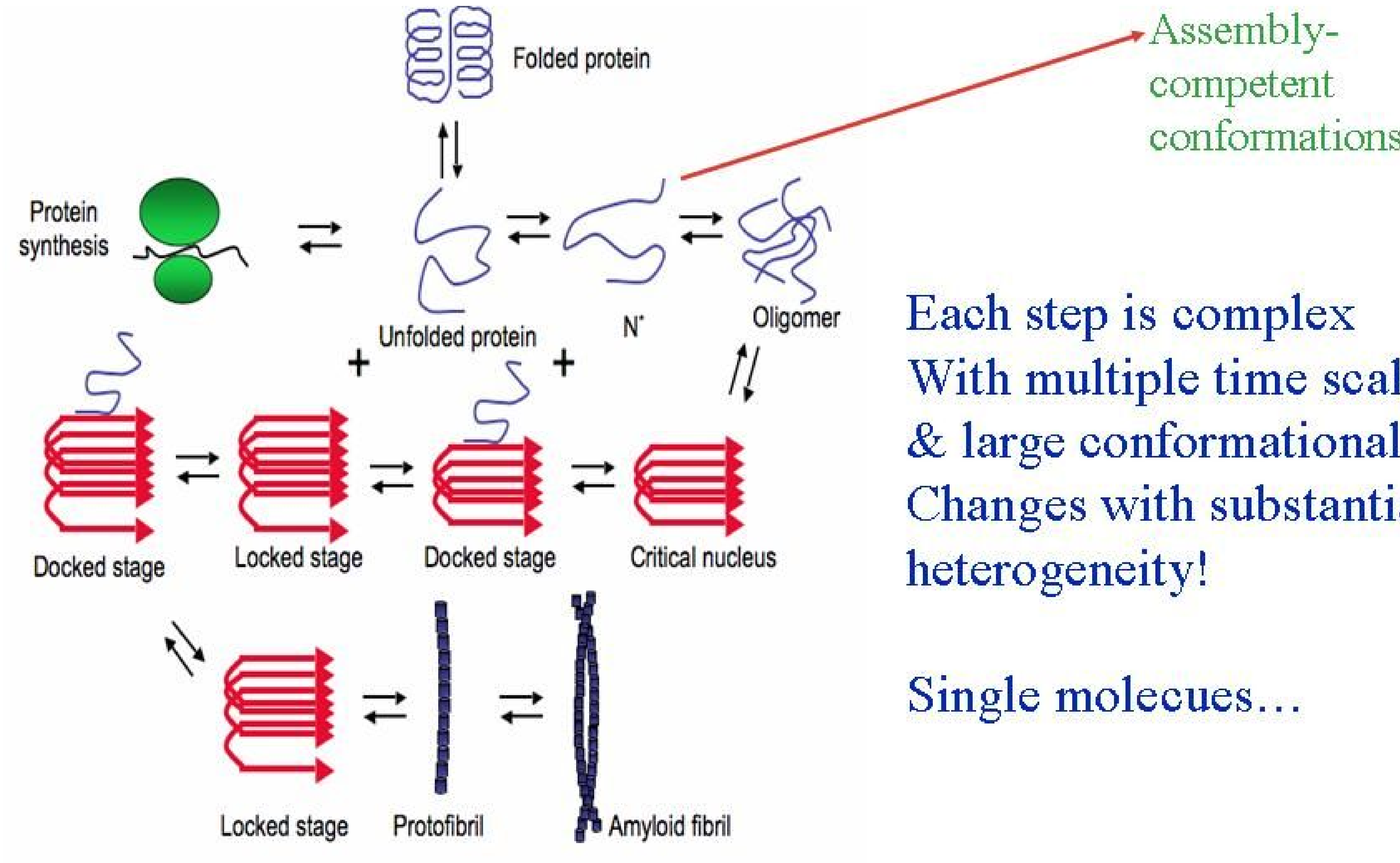
D23-K28 salt bridge enhances fibril rate by 1000 (Meredith + Tycko)



Distinct N*
States could
Grow into
Different
Fibrils..Monomer
Spectra "encode"
The distinct strains

Desolvation (water dehydration)
a free energy barrier to form N^* in WT

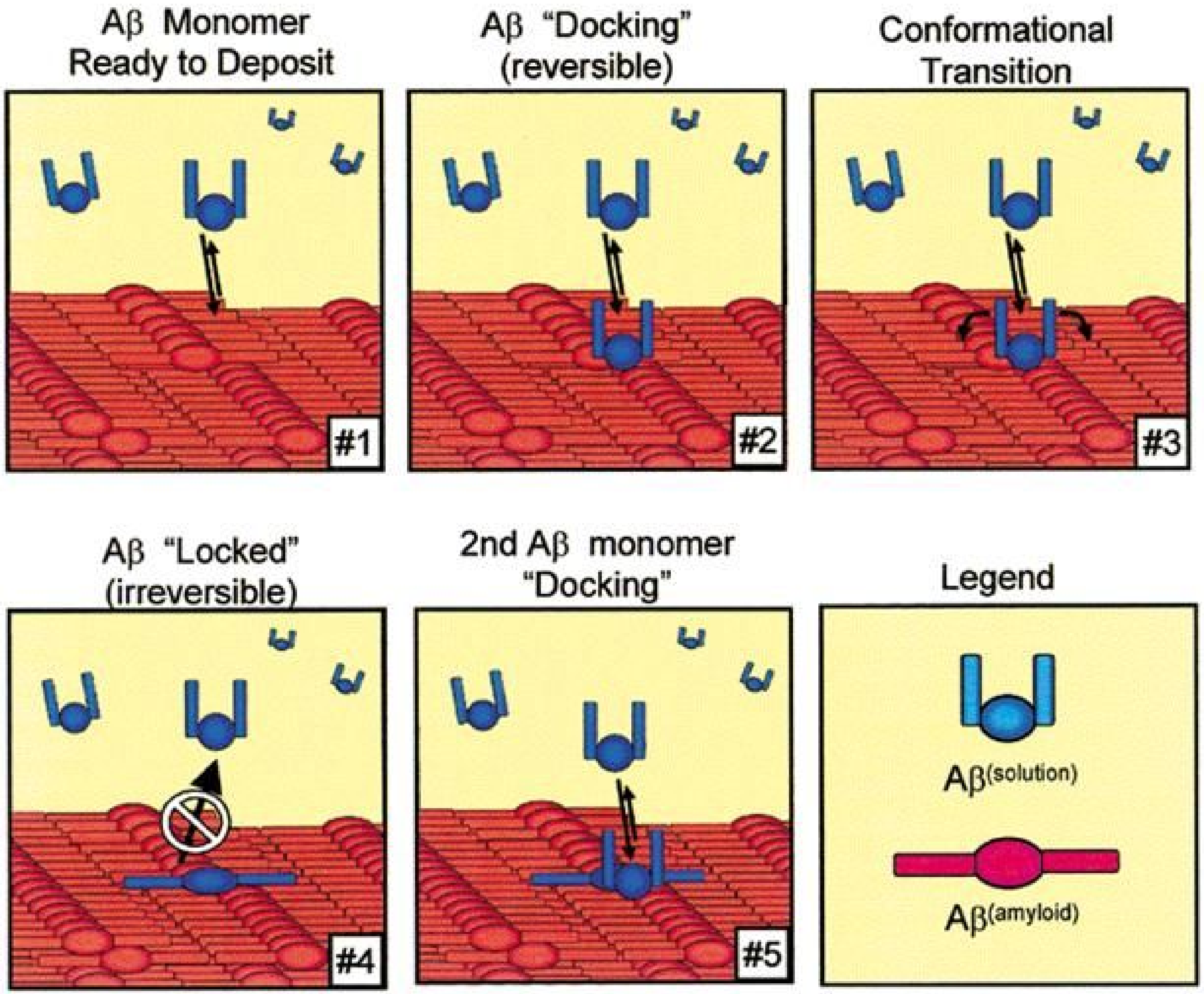
Cascade of events in protein aggregation
(no chaperones, crowding..)



Each step is complex
With multiple time scales
& large conformational
Changes with substantial
heterogeneity!

Single molecues…

Schematic View of Dock-Lock Process
Template = existing fibril



Lee-Maggio (2000)
Wetzel (2004)

What are the
Molecular
Events?
Role of water?

MD simulations
$A\beta_{16\text{-}22}$ oligomers
Addition of
Sup35 (7-13) pD
$A\beta_{35\text{-}40}$ & $A\beta_{37\text{-}42}$ to
fibrils

Growth of oligomers & Fibrils

MD simulations
$A\square_{16-22}$ oligomers

Addition of
Sup35 (7-13) pD
$A\square_{35-40}$ & $A\square_{37-42}$ to
Fibrils

Starting structures
From atomic coordinates
(Eisenberg & company)

All atom representation

Thermodynamics: Replica
Exchange (implicit solvent)

Dynamics: MD
Simulations explicit water..

Monomer addition to $A\square_{16-22}$ oligomers
Nguyen & Li PNAS (2007)

$$A\beta_{16-22} + [A\beta_{16-22}]_{n-1} \Leftrightarrow [A\beta_{16-22}]_n \ (n = 2 \ldots 5)$$

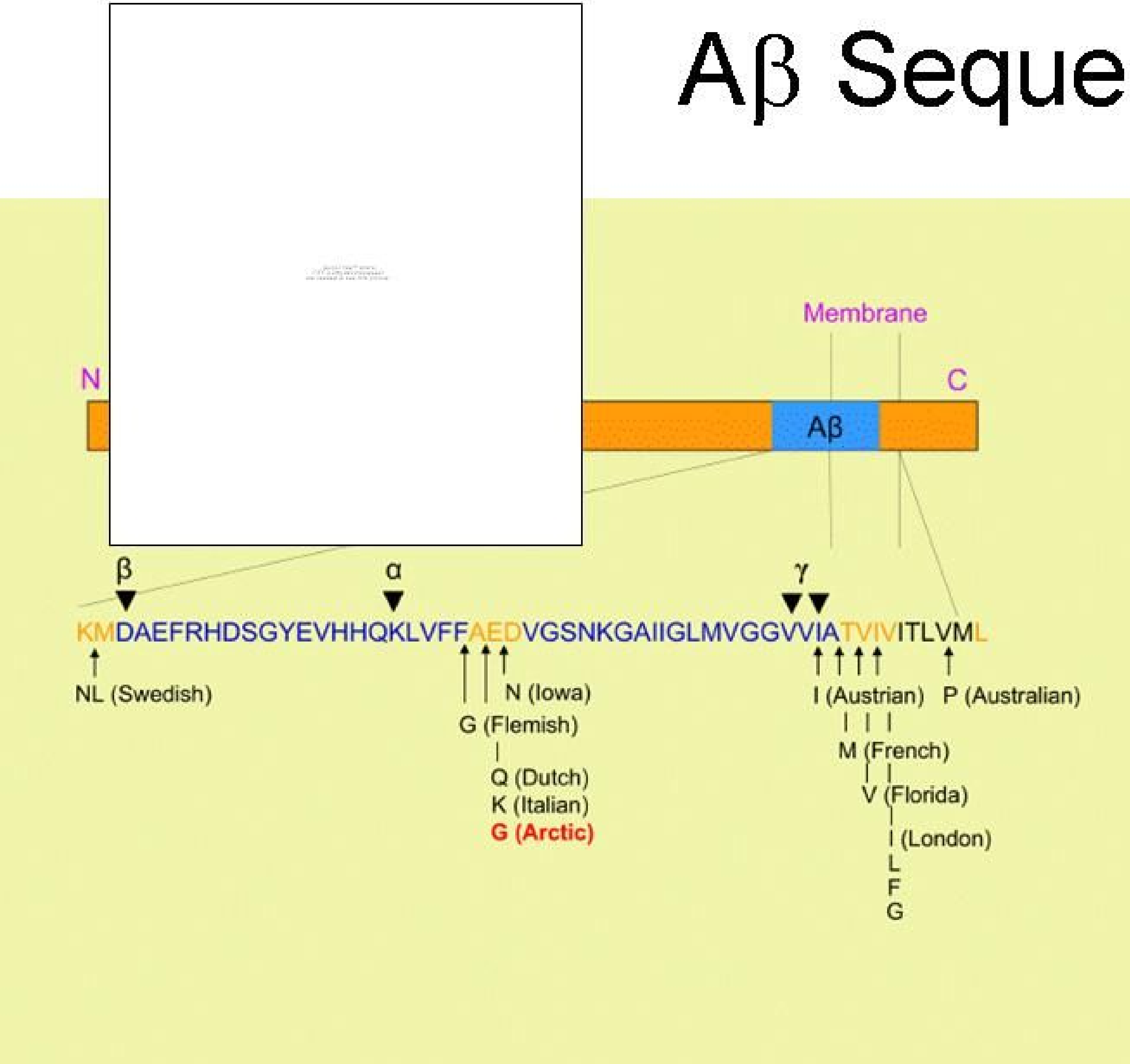
Random Coil

Preformed

Simulations get difficult to carry out for n bigger than 5

AD (Deposition diseases) oligomers are neurotoxic with plaques being end product discovered in autopsy

Aβ Sequence

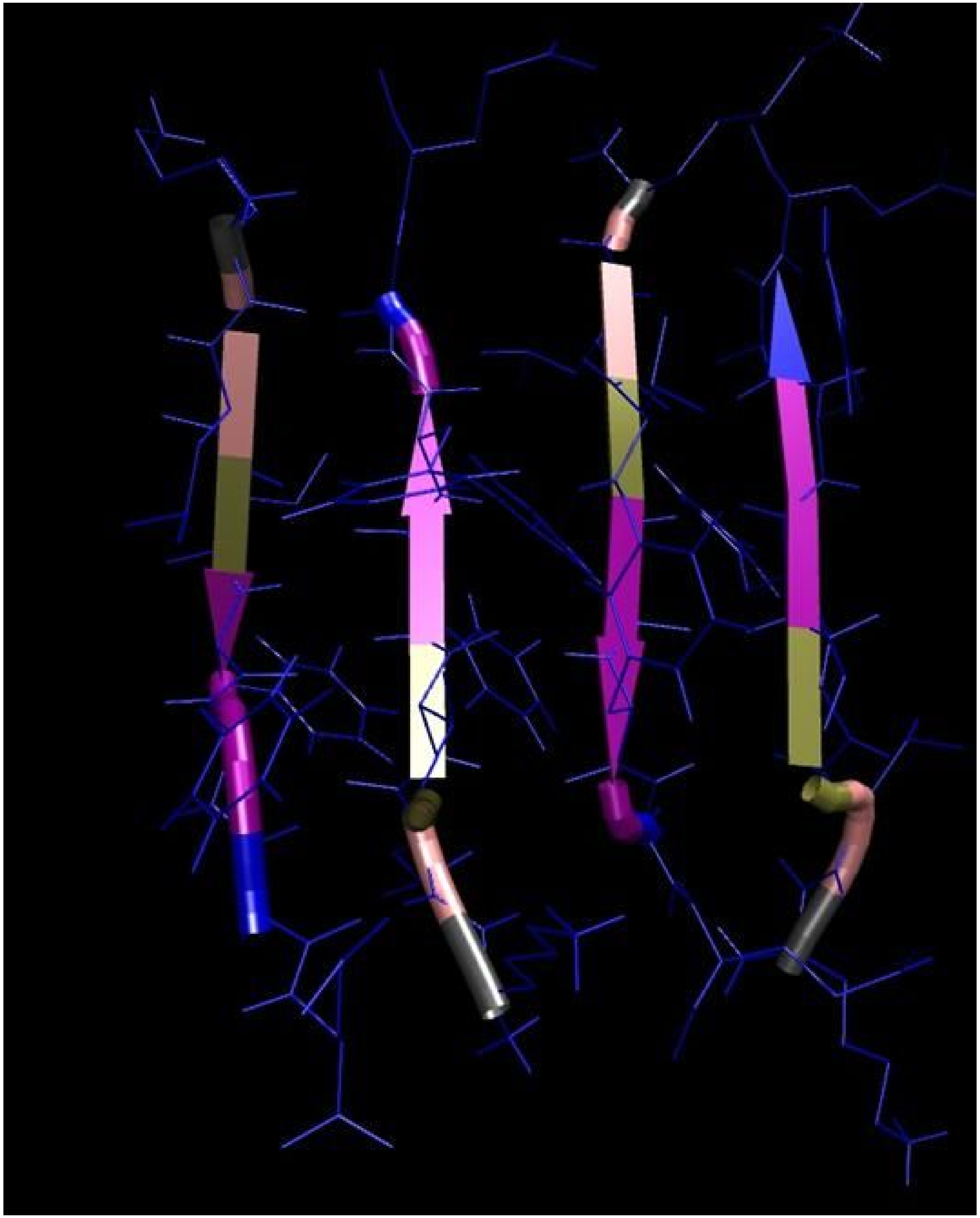


^{16}KLVFFAE^{22}
Central hydrophobic cluster

Hexapeptides

^{35}MVGGVV^{40}
Antiparallel

^{37}GGVVIA^{42}
Parallel



Oligomer assembly

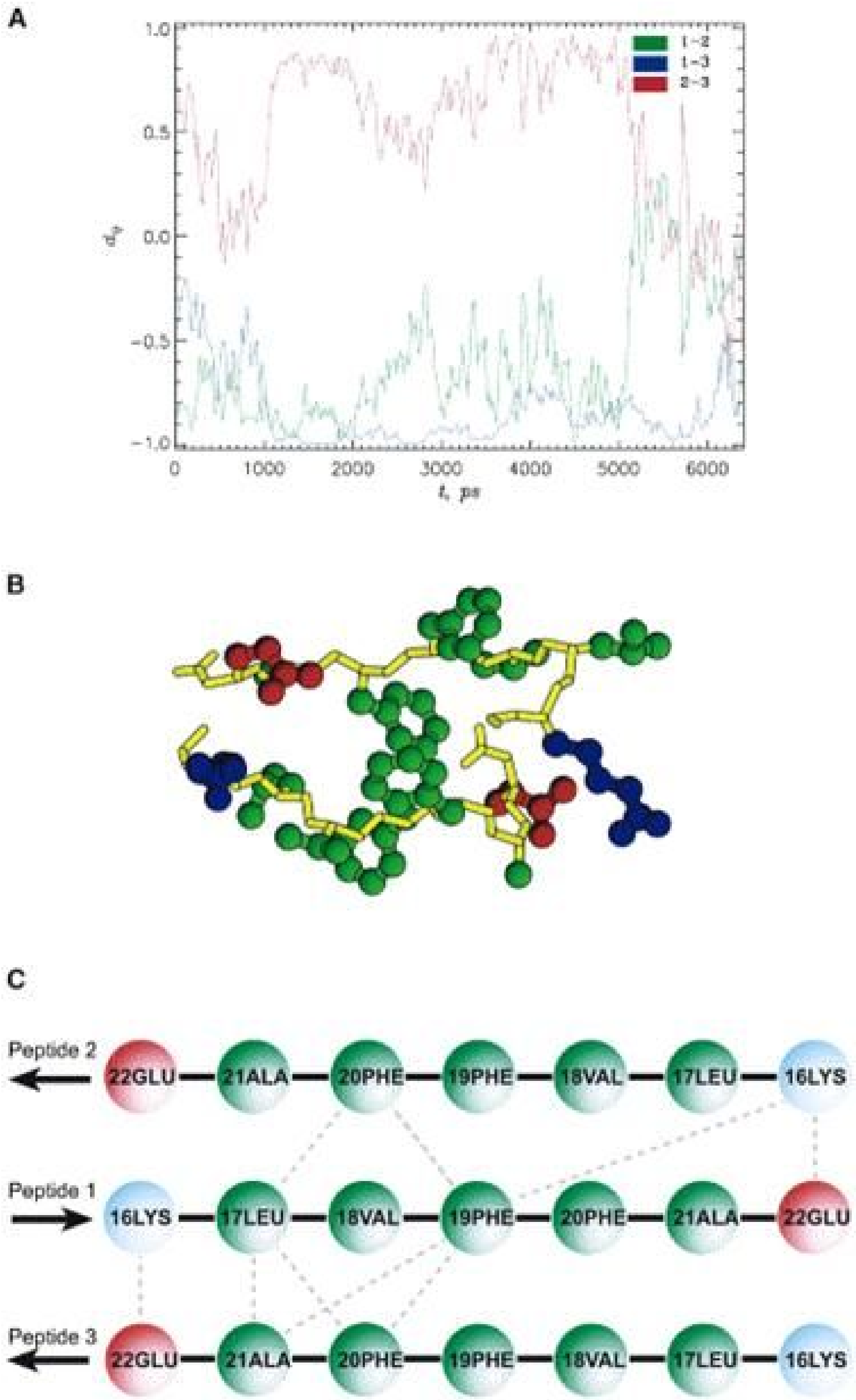
Insert $A\beta_{16\text{-}22}$ monomer

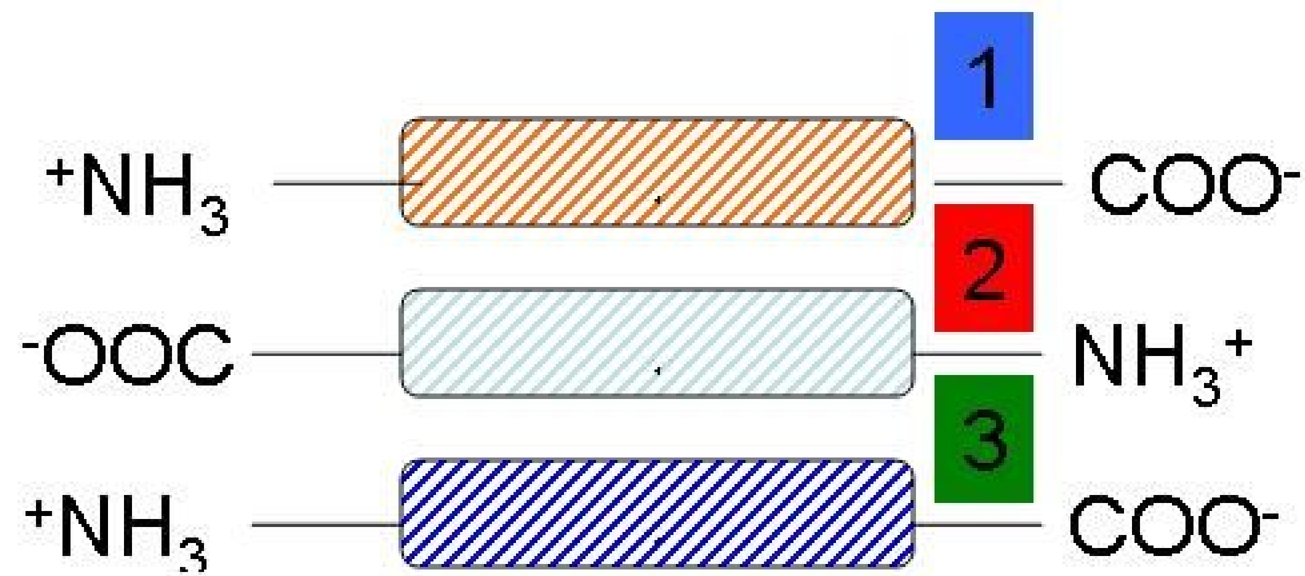
$(A\beta)_{n-1} + A\beta \Leftrightarrow (A\beta)_n$

n = 4, 5, and 6

MD simulations in water

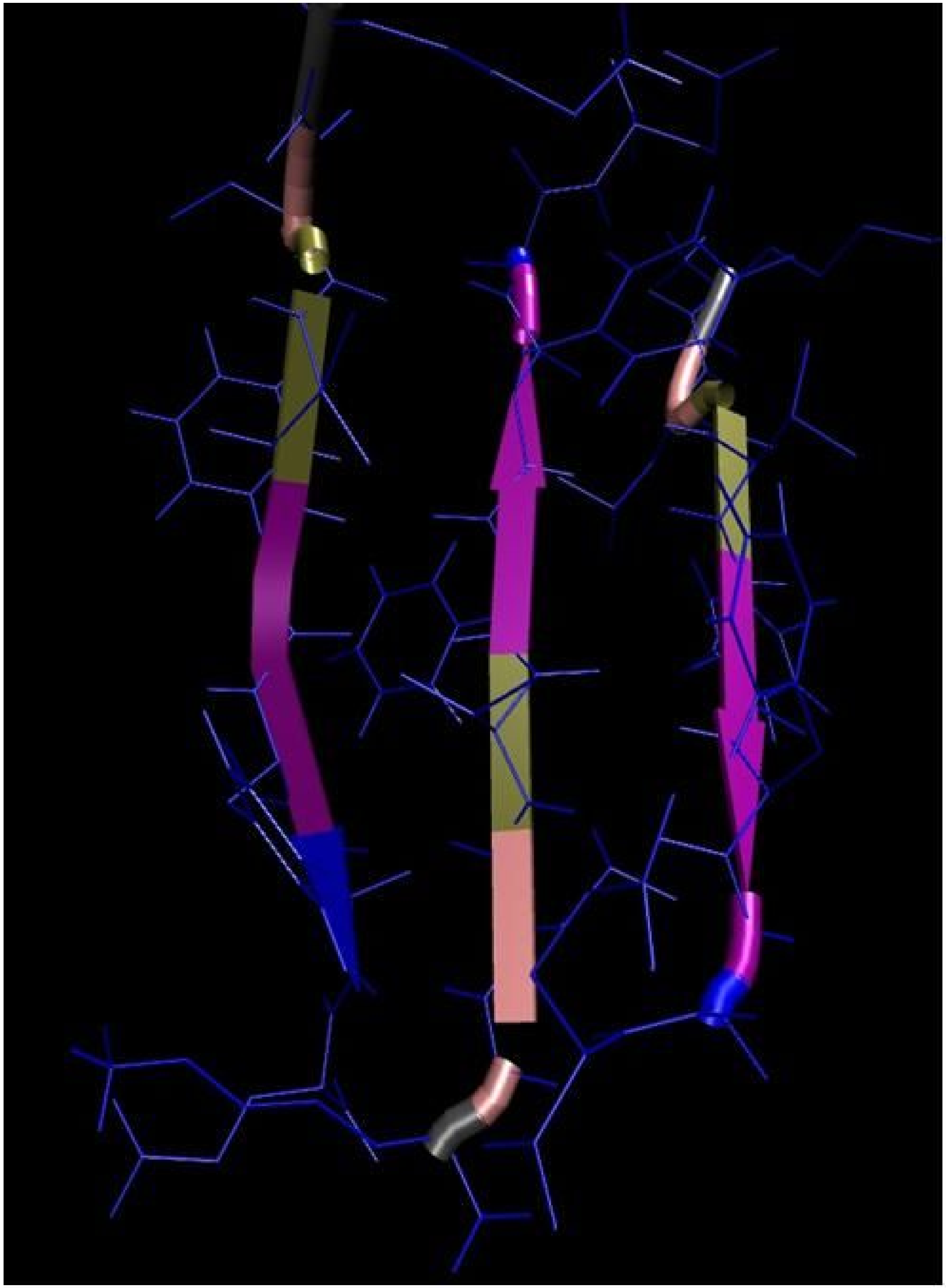
Hydrophobic and charged residues stabilize oligomers





Principle of Amyloid Self-assembly (PASA)

Anti-parallel registry satisfies Hydrophobic and charged interactions



Trimer Structure from MD

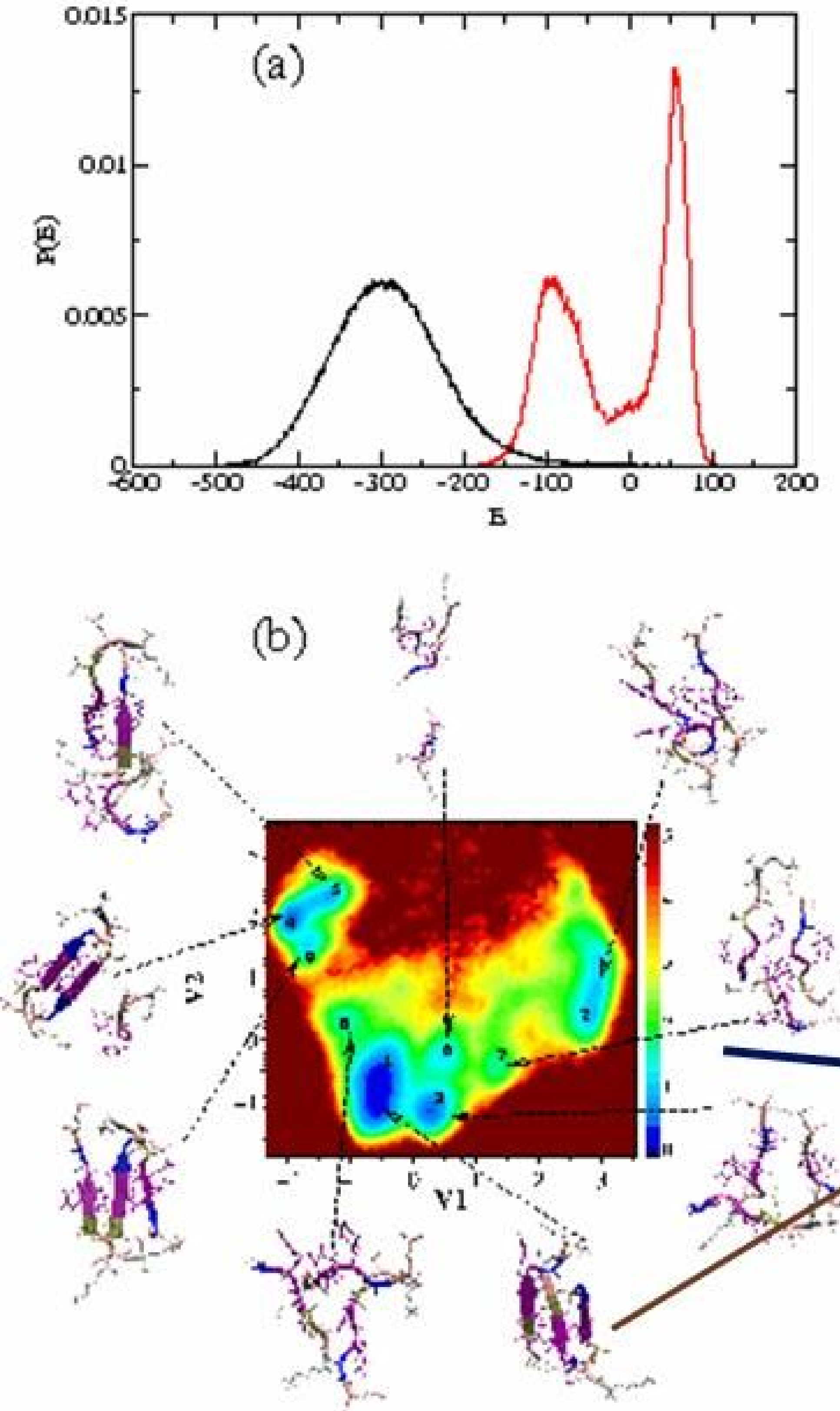
Antiparallel β sheets

Monomer is Random Coil
Negligible α or β content

Structure: Interaction Driven

DKlimov & dt
Structure *11*, 295 (2003)

A□$_{16-22}$ trimer forms antiparallel structure



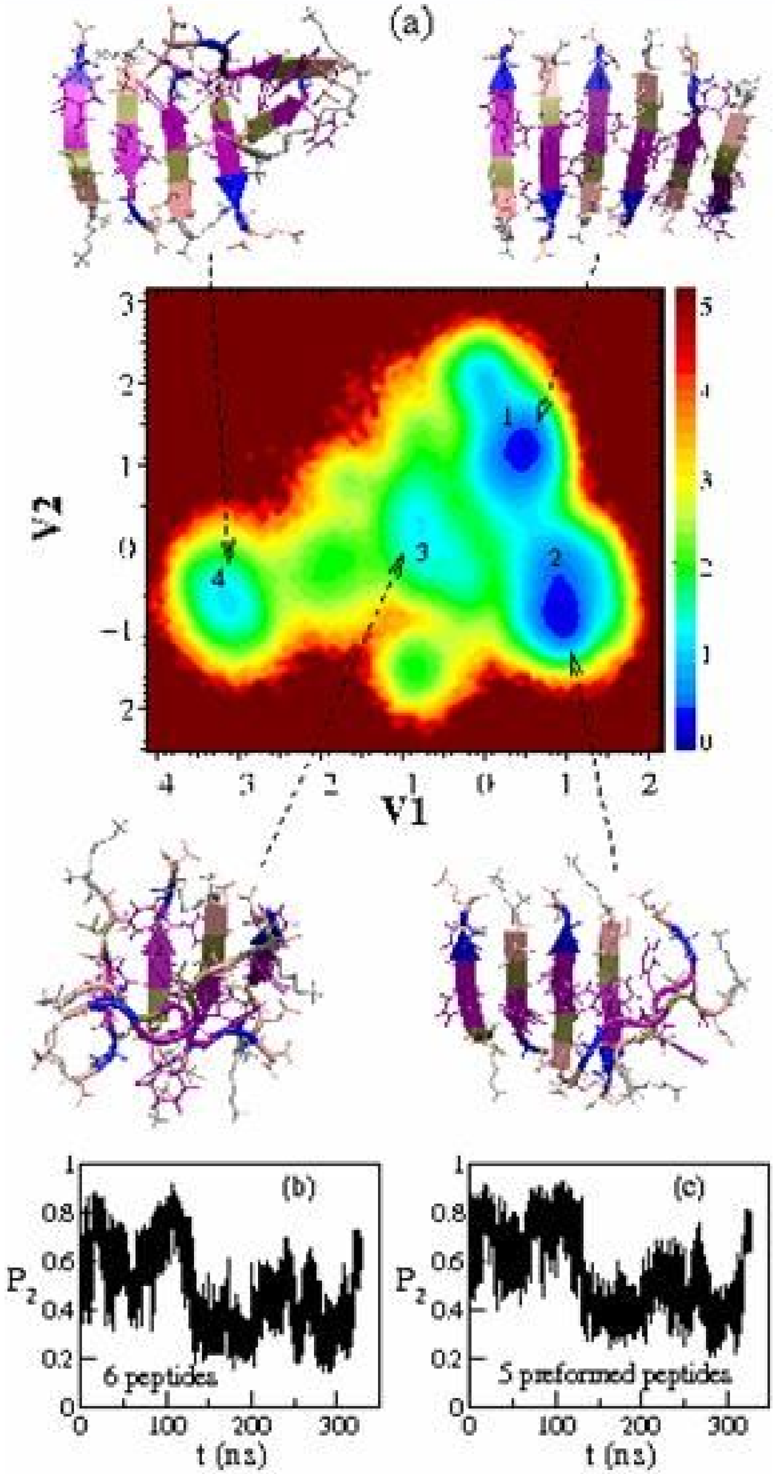
Proc. Natl. Acad. Sci. ***104***, *111 (2007)*

Trimer undergoes substantial conformational fluctuations (Fluid-like)

Most stable

Energy Landscape
PCA

Ordered oligomer fluctuates greatly to accommodate added monomer



P_2 = nematic order parameter

$= 1.5(\cos^2\theta - 1)$

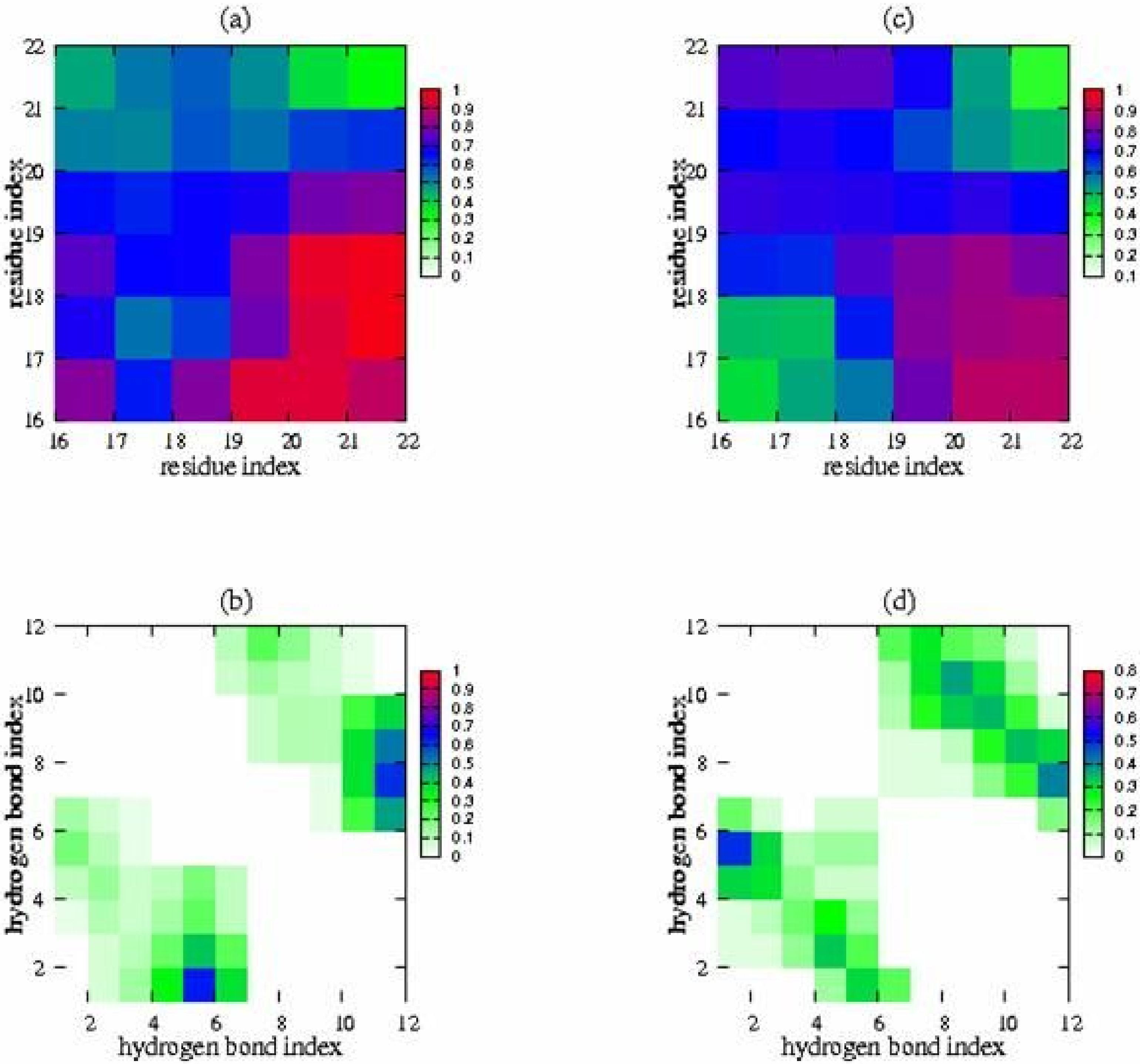
$\Delta P_2(n)$ (fluctuations in nematic parameter) decreases as n increases

$(A\beta)_5 + A\beta \rightarrow (A\beta)_6$

Oligomers are fluctuating Nematic droplets!

Interior is dry, few hydrogen bonds, stabilized by side chain contacts

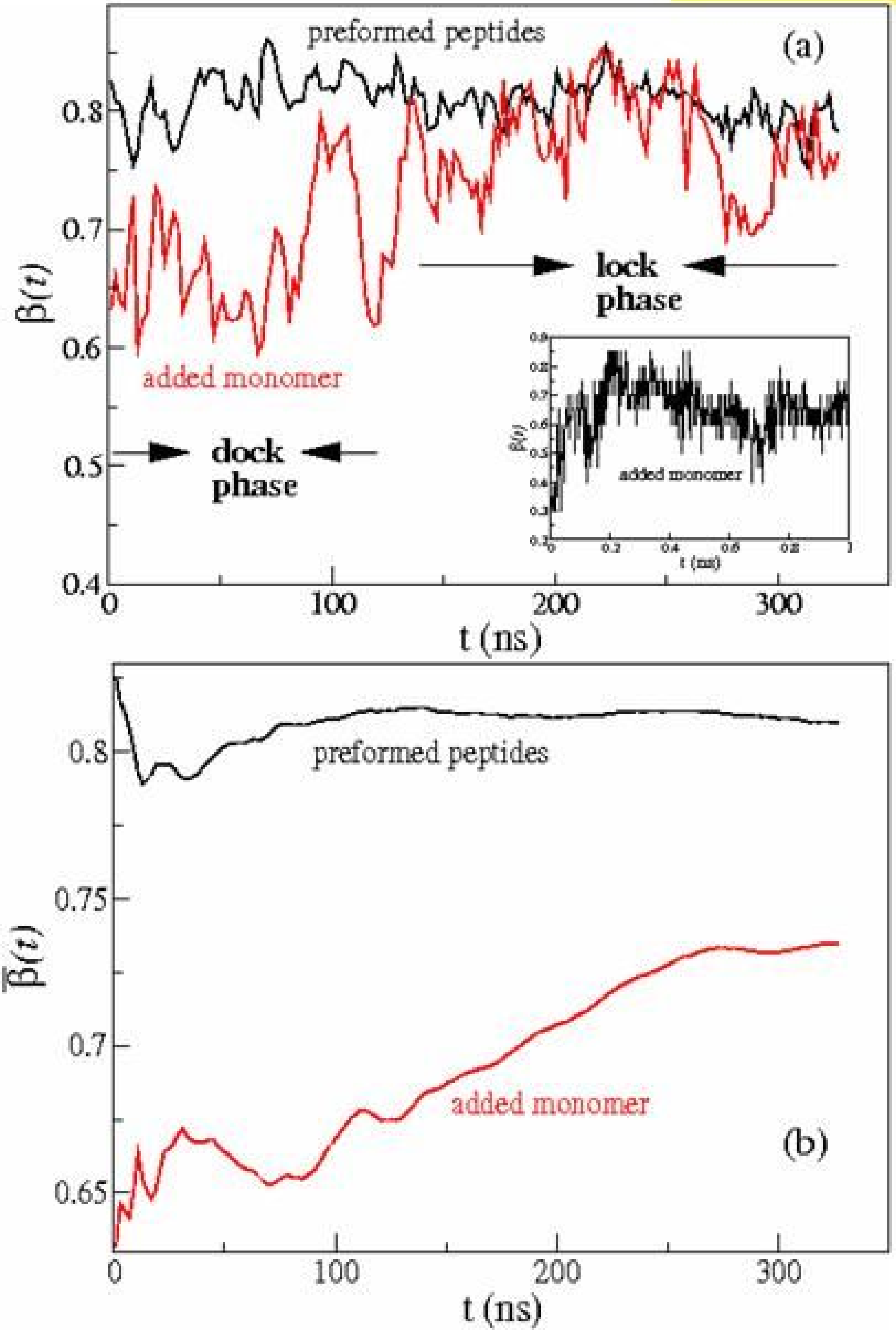
Tetramer



Pentamer

Drying an early event

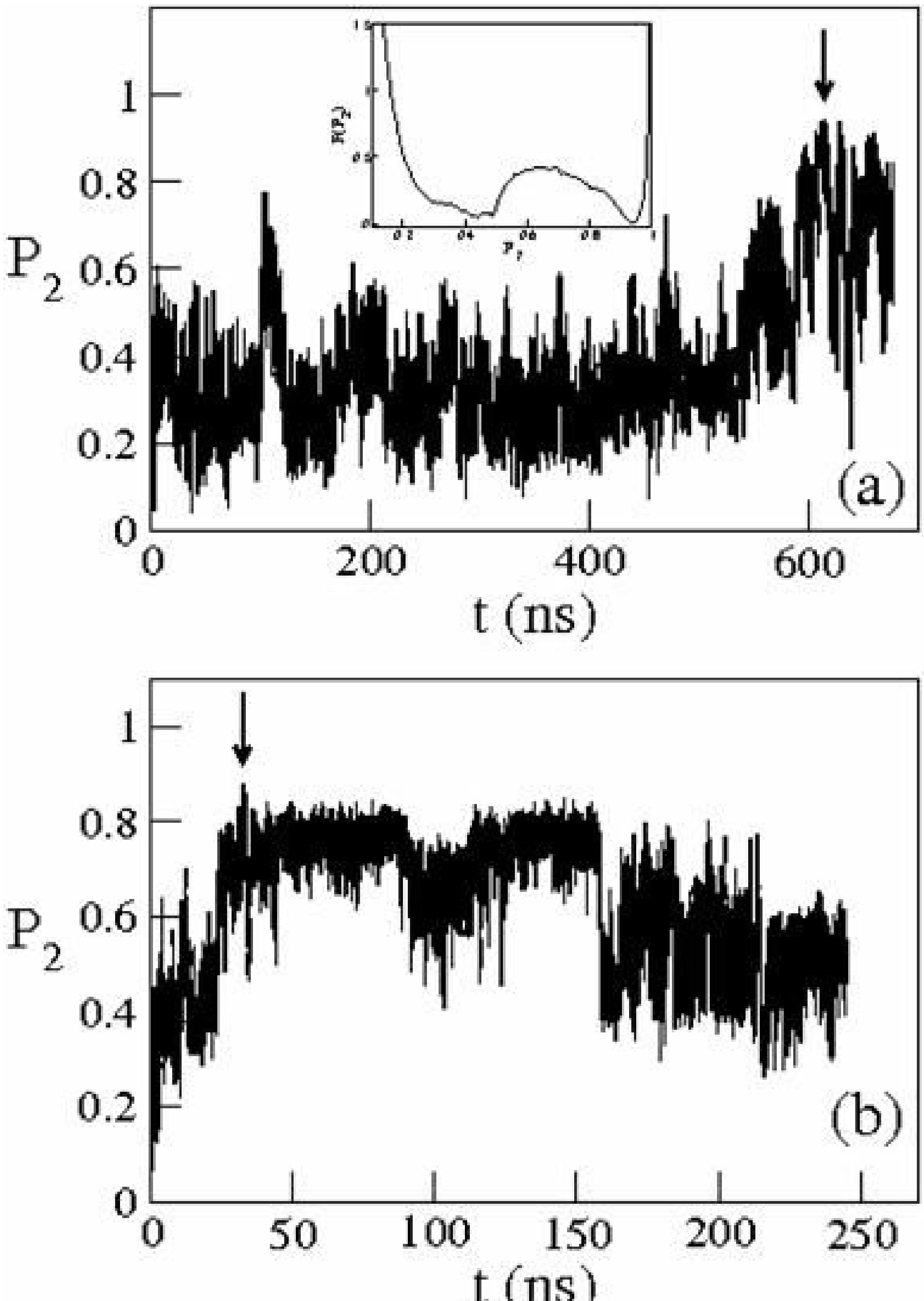
Oligomers grow by a multistage Simplified as (Dock-Lock) mechanism



- Docking (rapid) □ content small
- $\square_{LOCK} >> \square_{Dock}$
- Ordered oligomers orientationally melt but retain □-strand content
- Dock-Lock mechanism is be sequence independent.

→ Time averaged

Estimating Oligomer Formation Time Scale (C-dependent)



Multiple Assembly Pathways
Random coil monomers add fast (rate ∝ contact area)

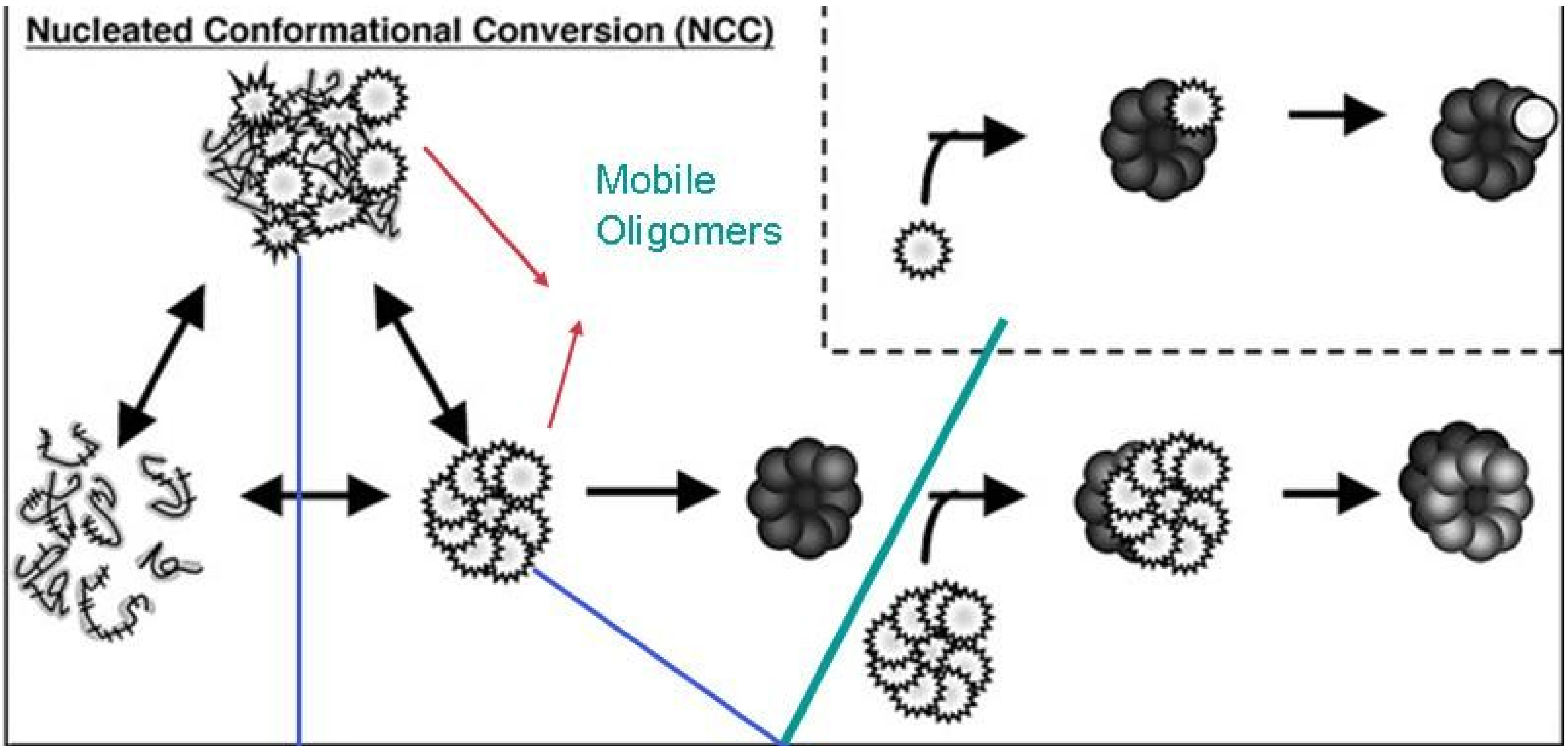
τ(n) (MFPT) from F(P_2)
Using Kramers theory

$\tau(n) \approx \exp(\beta\, G(n))$

$n > n^{*}$ τ(n) diffusion limited

$n^{*} \sim$ (6-8) Using Kramers + naïve nucleation theory

Picture of Oligomer growth



Serio…..Lindquist *Science* (2000)

$(N^{*})_m + (RC)_{n-m}$

$(N^{*})_n$

Diffusion-limited growth: concentration dependence

(no prayer of computing $\beta\Delta G‡$)

Smoluchowski Theory of association
(preformed fibril stationary)

k_G . $4\pi\alpha\sigma \exp(-\beta\Delta G‡)/(\eta_0 + \eta_c C + k_H \eta_c C^2)$

k_G . C (small C)

k_G . $C/(1 + \delta C)$ (moderate C)

k_G . $1/C$ (large C)

Fit Experiments to C dependent k_G

Dynamics of locking of a monomer to a growing fibril

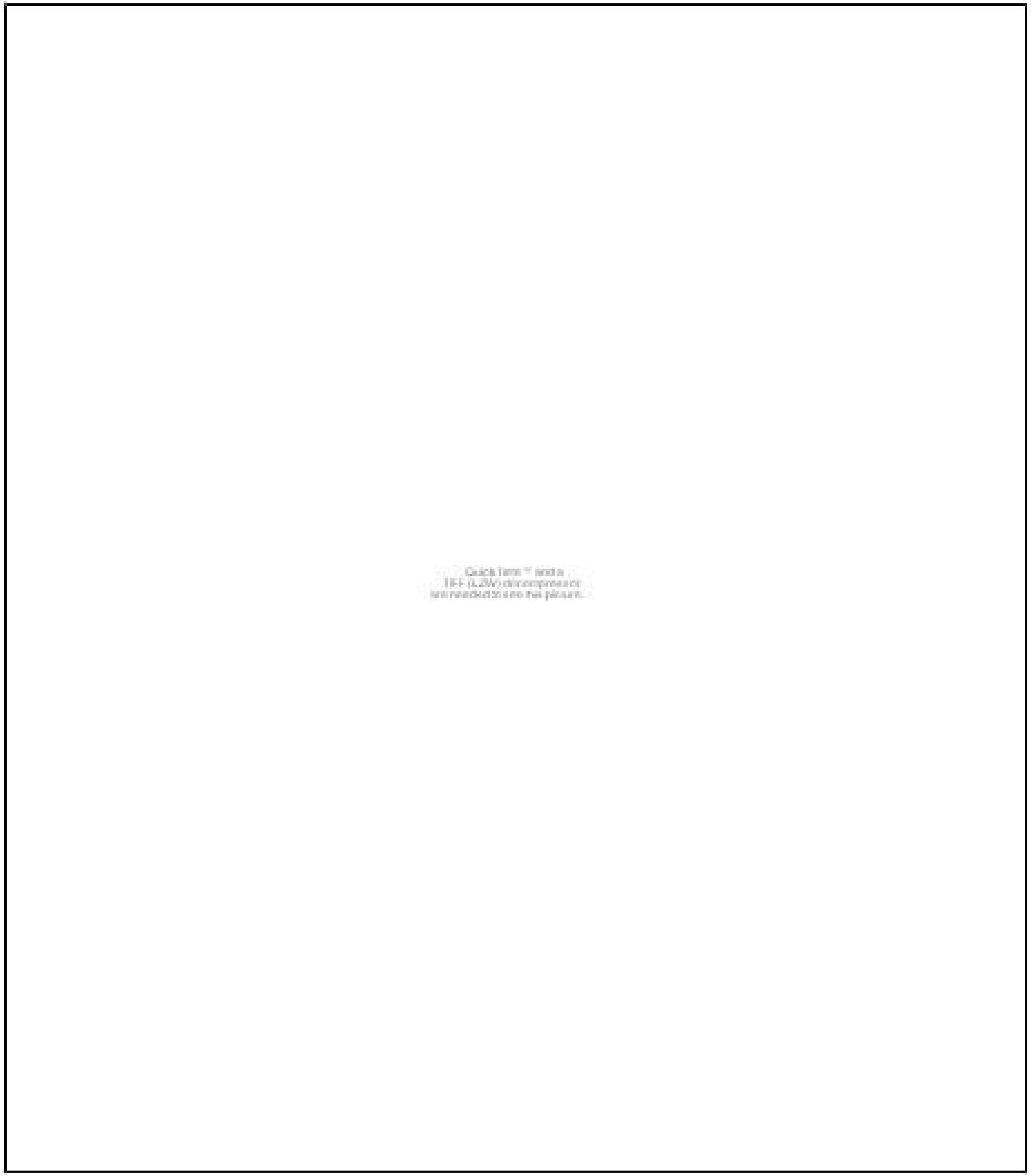
Molecular events in the growth of fibrils Peptides from Sup35 and Aβ peptides

Preformed fibrils (Eisenberg and company)
add an "unstructured" peptide

Role of water in creating a dry interface

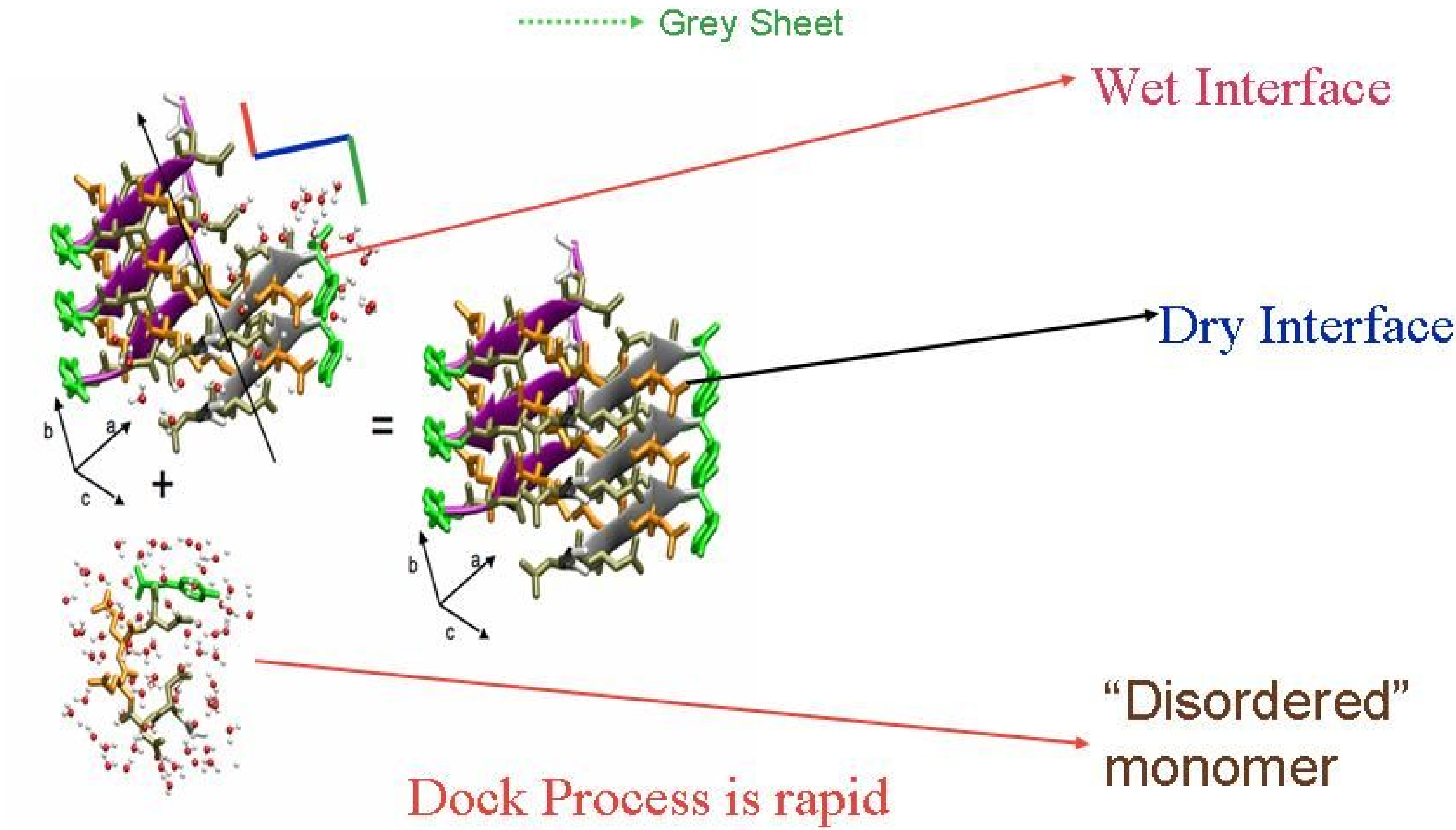
X-ray Structure of a Fibrillar Peptide

Crystal structure of a 7-residue peptide from Sup35 prion (7-13) (David Eisenberg) shows that the peptides stack parallel

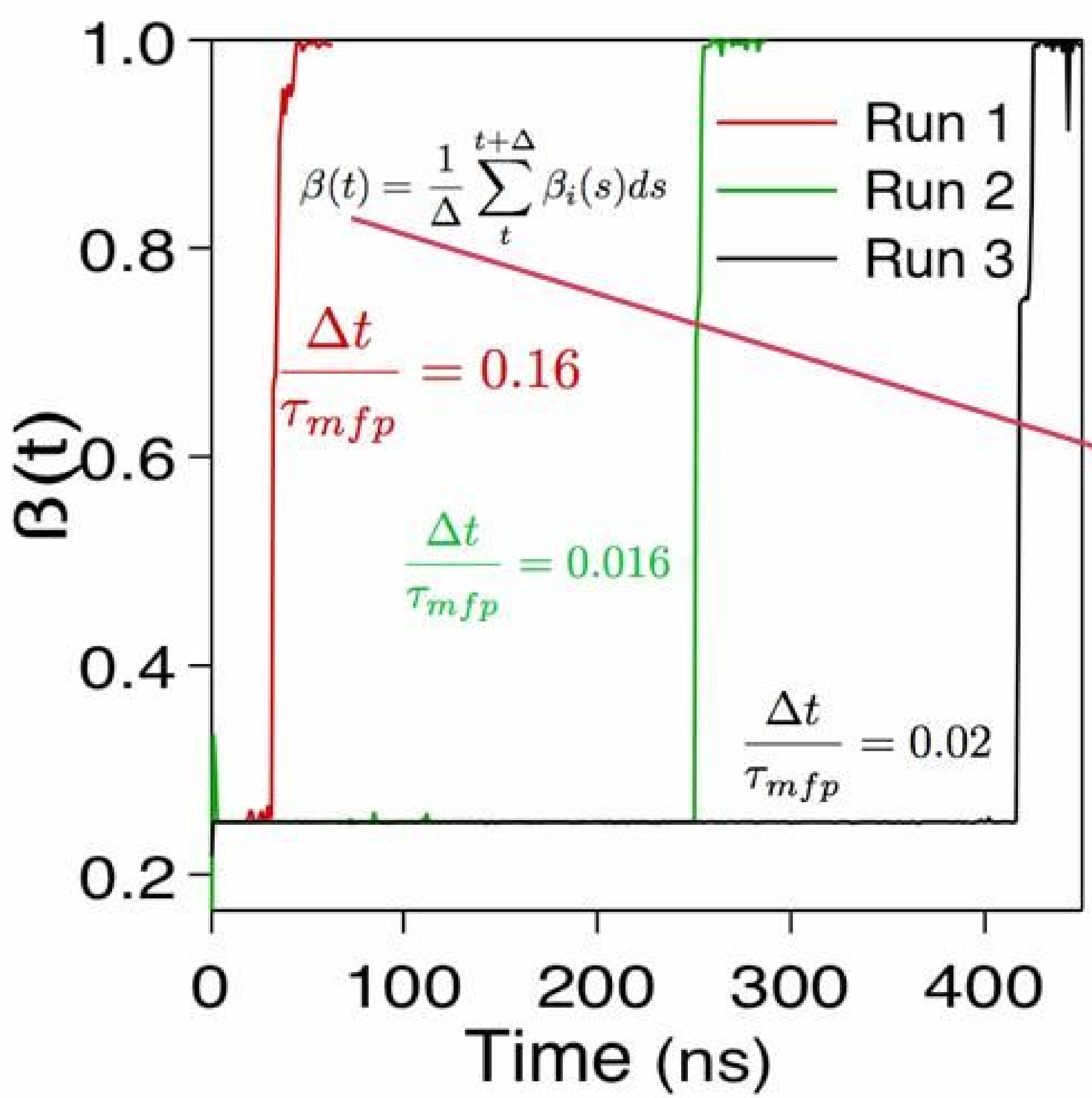


GNNQQNY
From Prion Domain;
Sheets antiparallel
Dry interface
"Steric Zipper"
Full inter digitation
H-bonds

Locking of GNNQQNY & Role of Water
All Atom MD Simulations



Growth by docking is dynamically highly cooperative!



$\beta(s) = (1/4)\, \Sigma \delta_{i,\beta}$

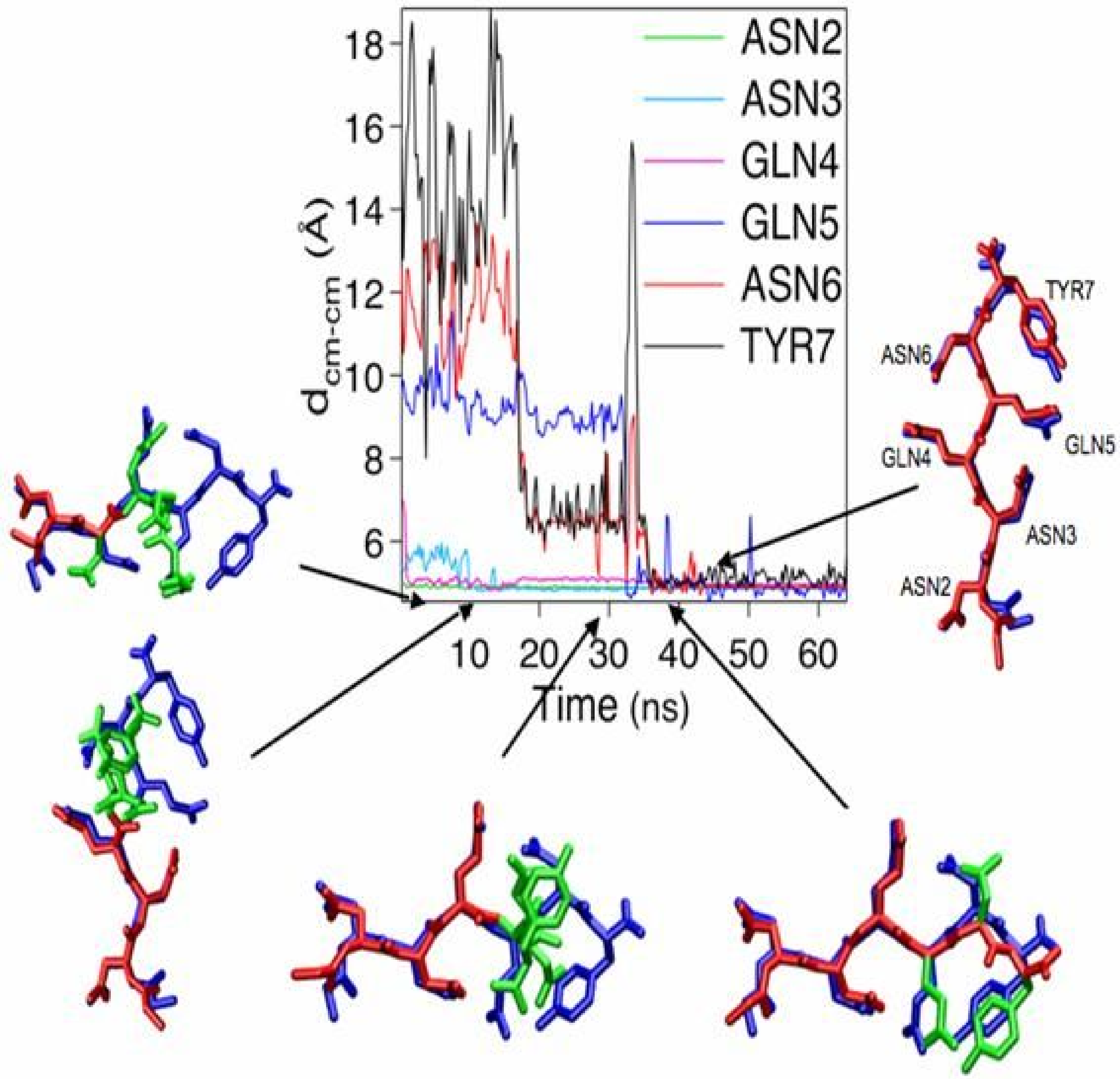
$\delta_{i,\beta} = 1$ if ith residue is in a strand "βstate"

β(t "long") = 1 implies monomer is locked onto the crystal

Δt = transit time

τ_{mfp} = first passage time

Dynamics of Ordering at residue level

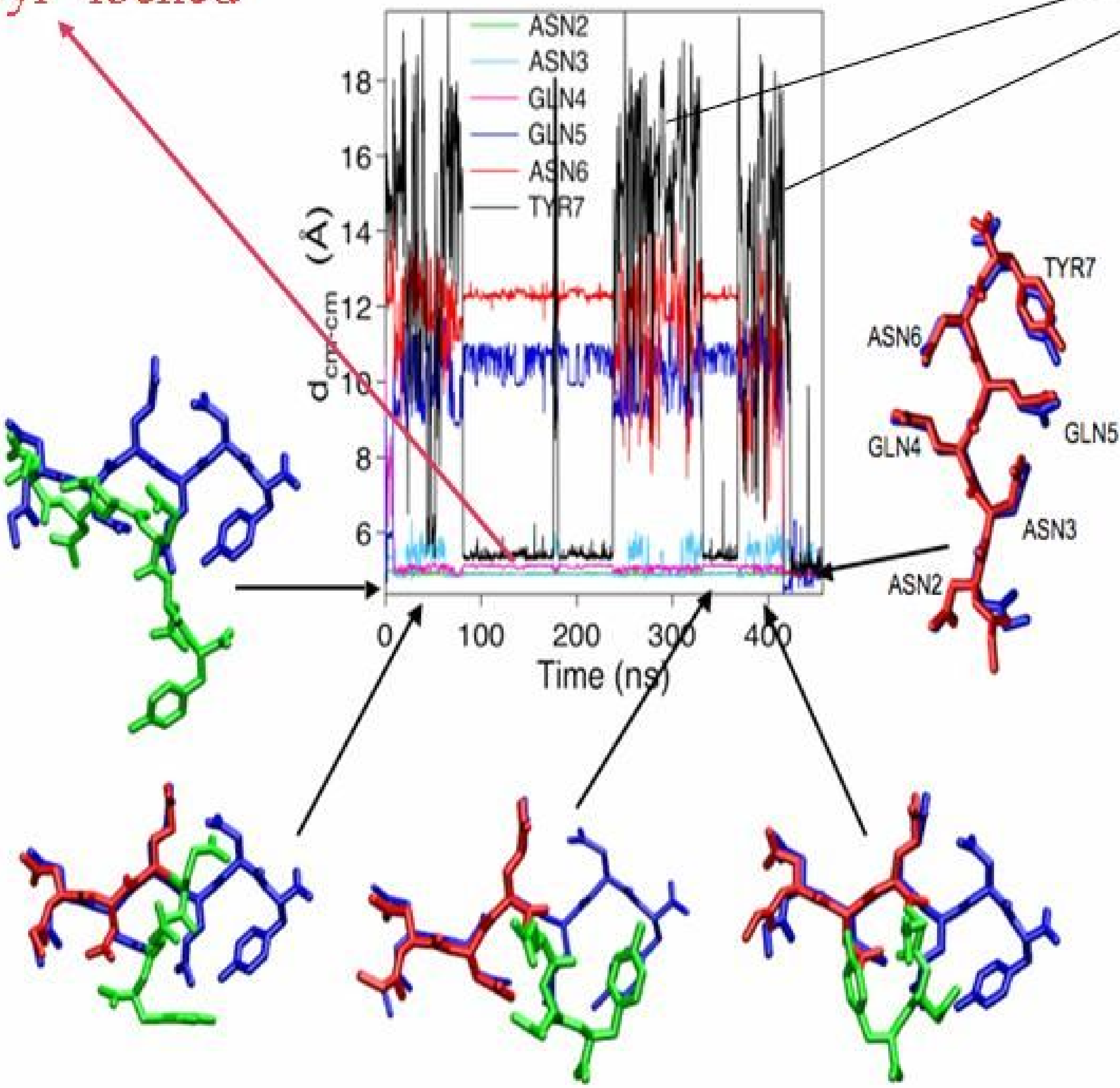


Red = ordered
Blue = fibril
Green=disordered

Assembly Heterogeneity: Every event is special!

Tyr[7] backtracks

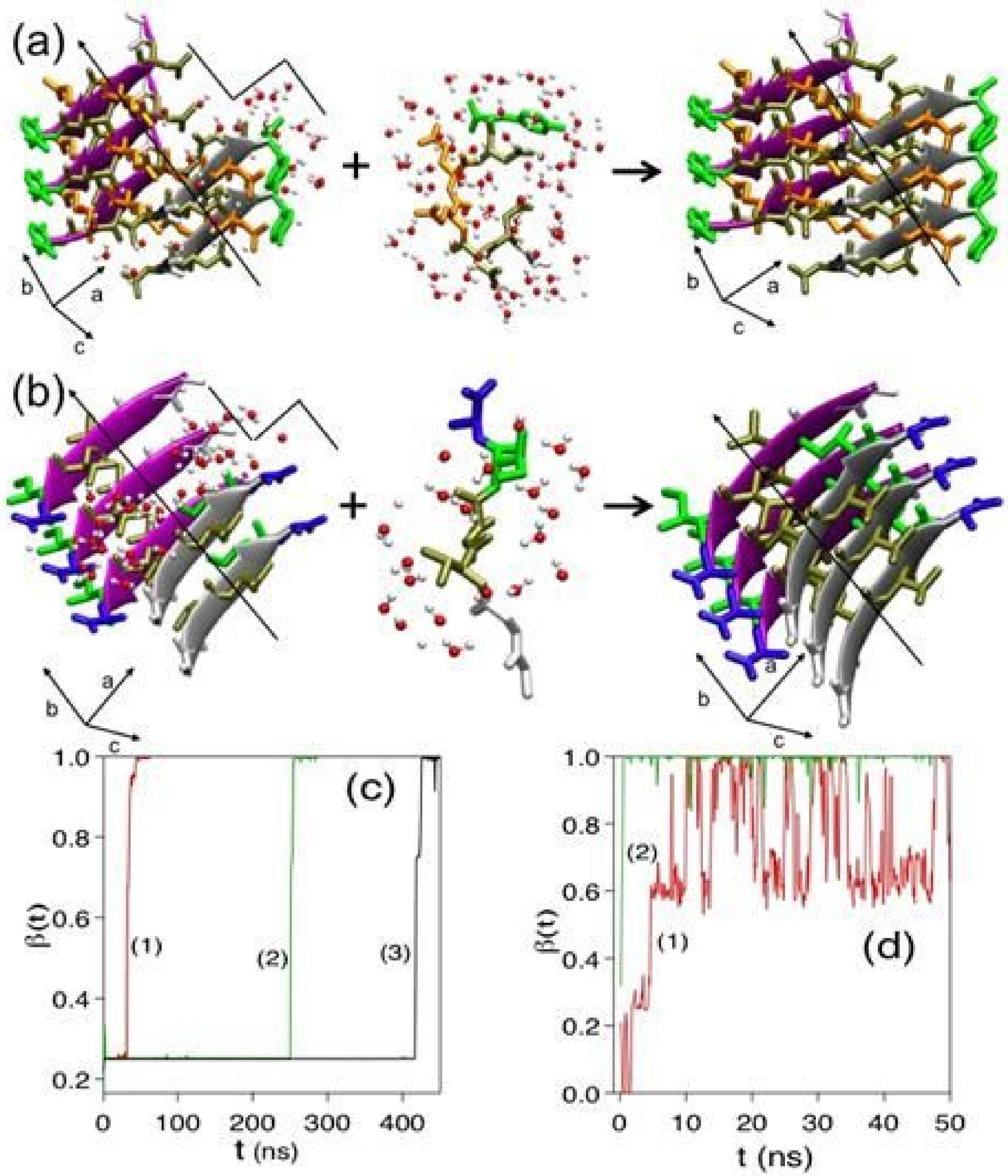
Tyr[7] locked



$N^3Q^4Y^7$ lock rest misassembled; GNNQQNY "backtracks" (C-term melts) before in registry locking

Similar to folding Of Interlukin Gosavi & Onuchic

Comparing Sup35 and Aβ



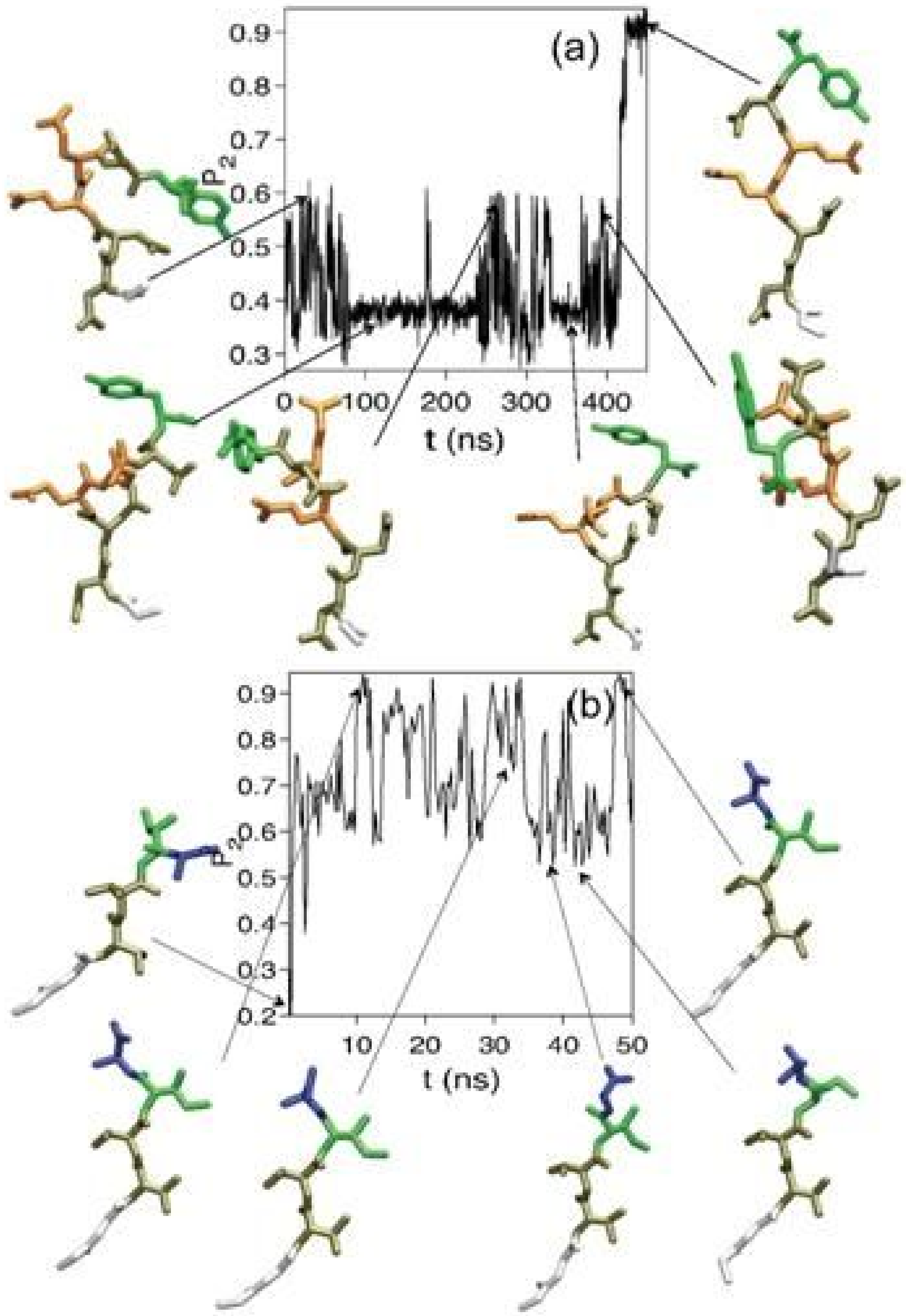
Rapid drying transition

Greater fluctuations

Stability compromised

Sequence affects energetics and growth kinetics

Contrasting ordering of Sup35 and Aβ addition

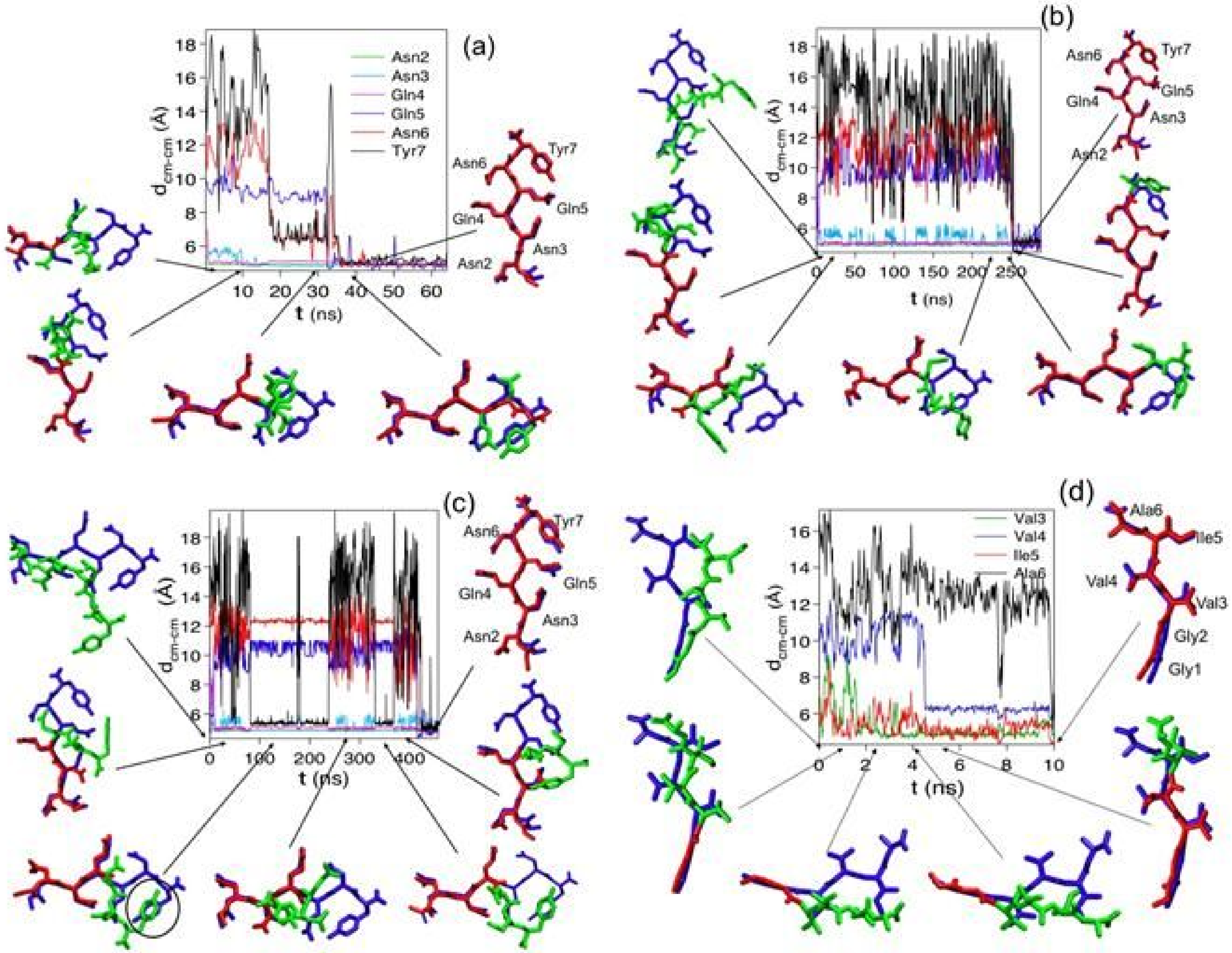


P_2 = nematic order parameter

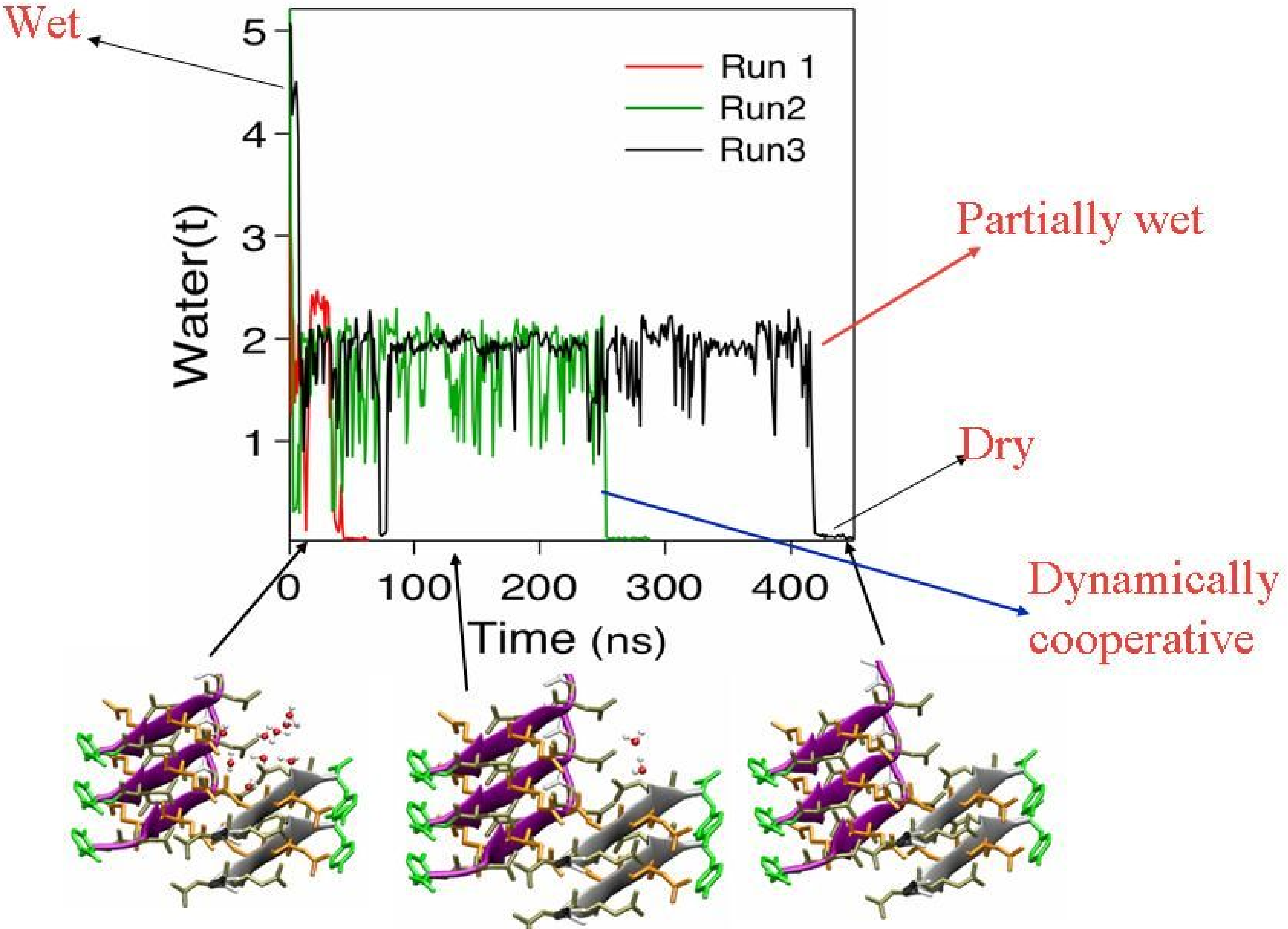
P_2 = 1 implies ordering commensurate with underlying fibril

Sup35 P_2 pinned at 1: Aβ fluctuations even after locking

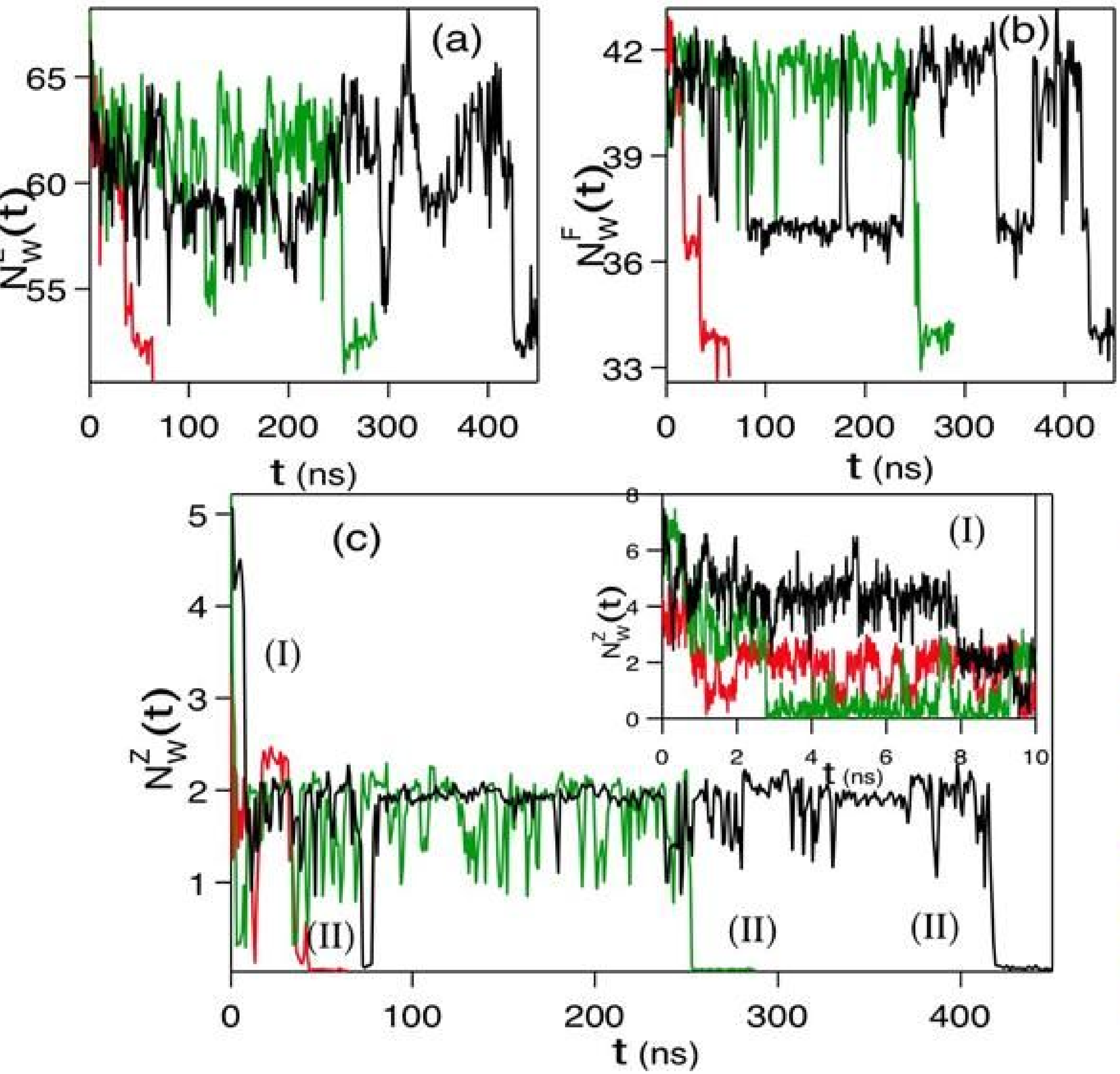
Sup35 and Aβ peptide addition



Drying of strand-strand interface coincides with amyloid growth



Dynamics of water expulsion (Sup35)



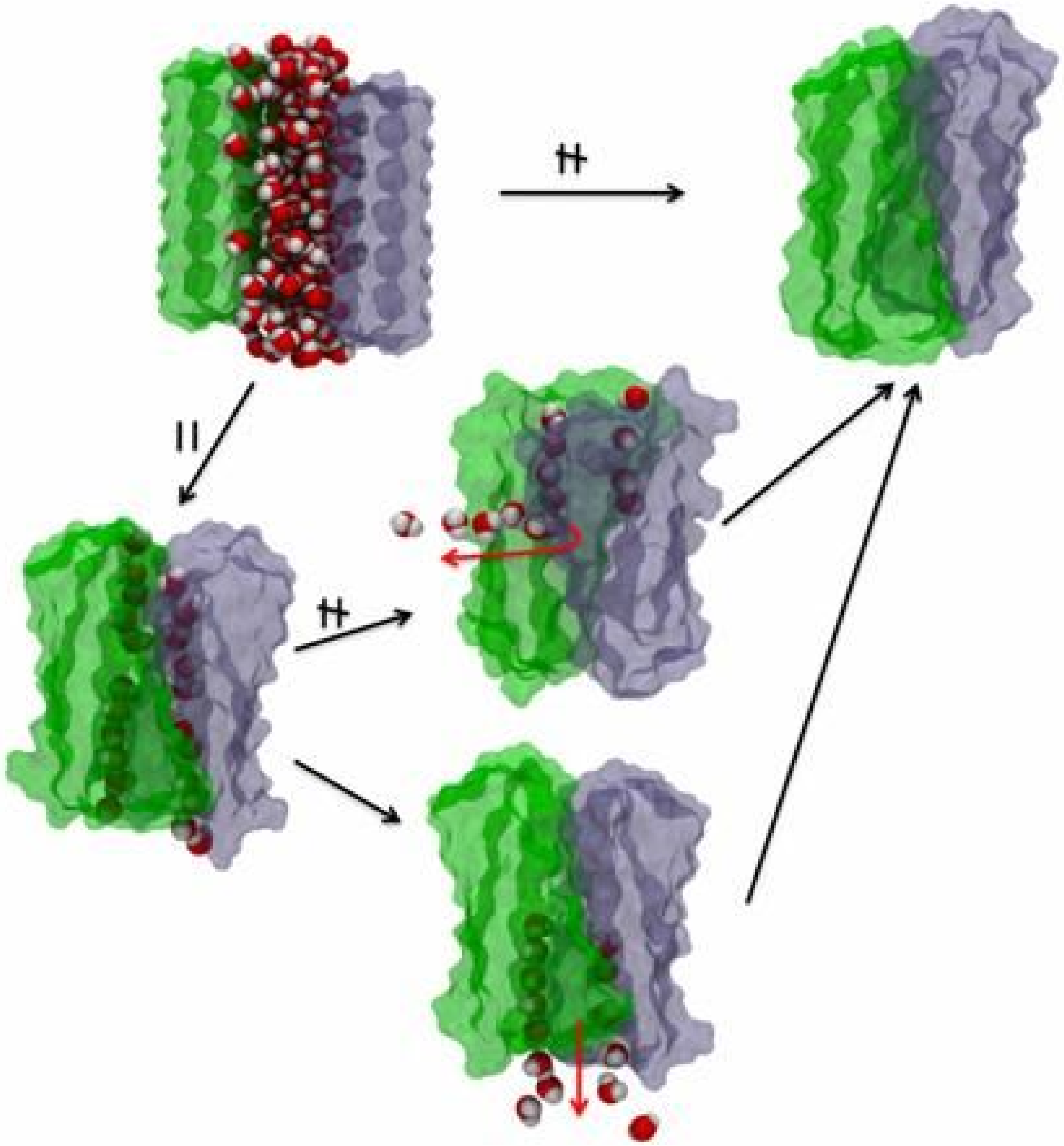
Desolvation coincident with locking

Two stage "drying"

Stage I: assocition with fibril peptide

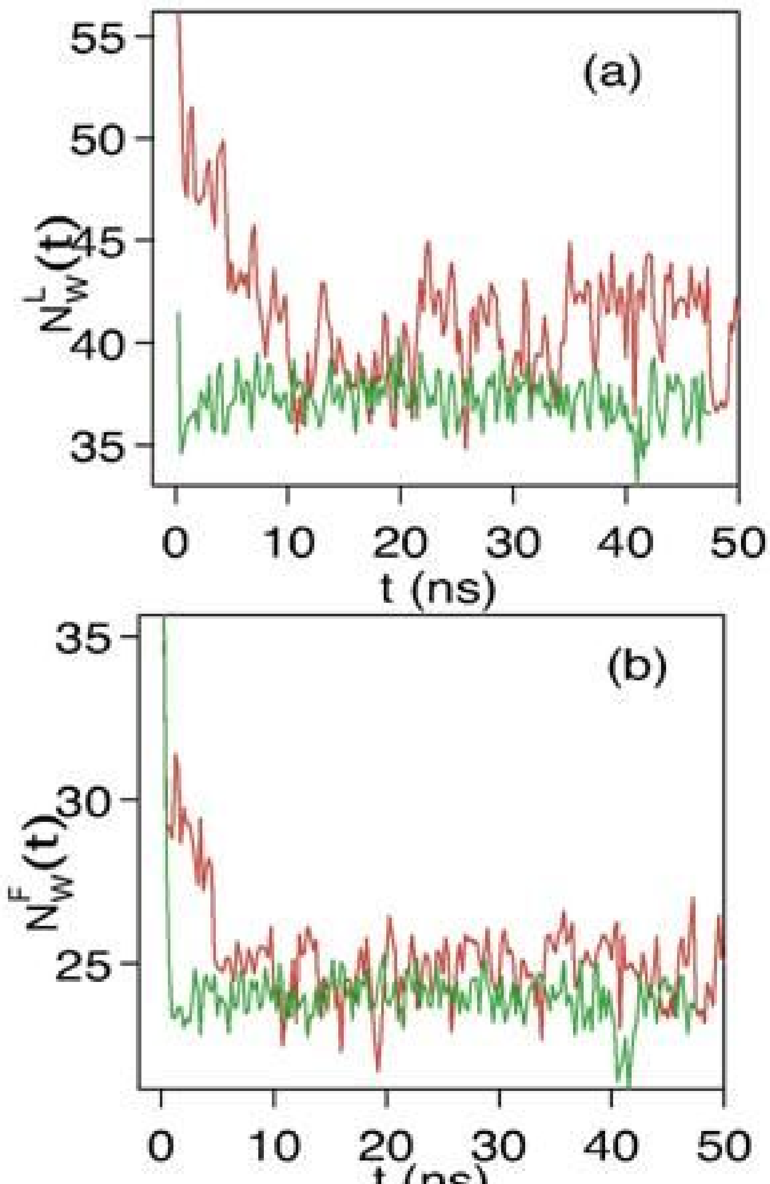
Stage II: dehydration from β-strand formation

Two 1d water wires mediate Sup35 protofilament assembly (Govardhan Reddy 2010)



QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.

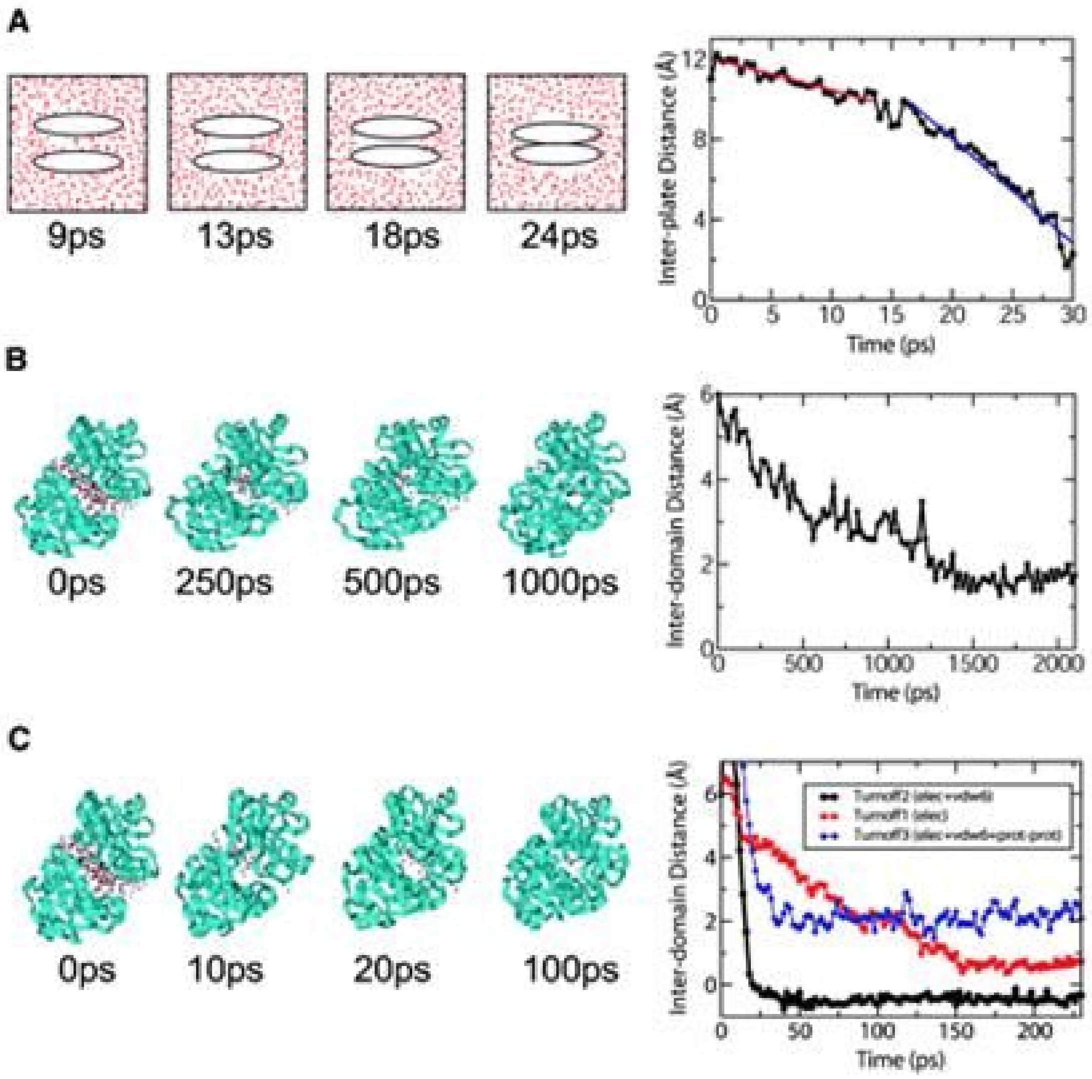
Water dynamics in Aβ fibrils



Considerable fluctuations in water after monomer locks compared to Sup35

Sheet assembly occurs by a drying step abruptly

Drying transition between hydrophobic plates similar to Aβ fibril growth



Conclusions

- Multiple routes and scenarios for fibril formation
- Electrostatic and hydrophobic interactions determine structure and kinetics
- Conformational heterogeneity in N^* controls oligomer and fibril morphology (relevant for strains)
- Phase diagram (T, C) plane for a single amyloidogenic protein is complex due to structural variations in the misfolded N^*
- Templated growth occurs by addition of one monomer at a time
- Nucleus size and growth mechanism depend on protein
- Growth of oligomers (and fibrils) by a multistage process (well separated time scales)
- Water Plays a key role (barrier to assembly + nucleation) and in a sequence-dependent manner