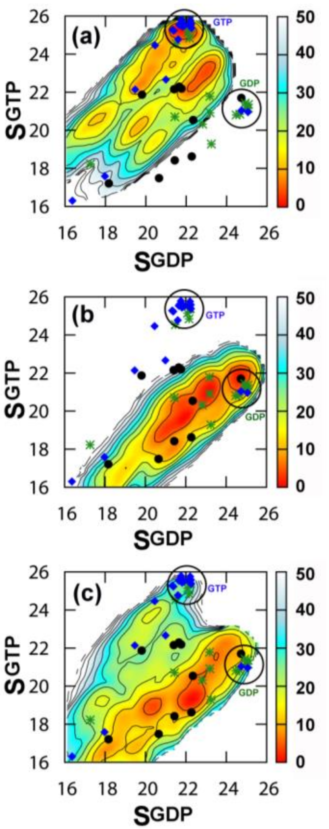
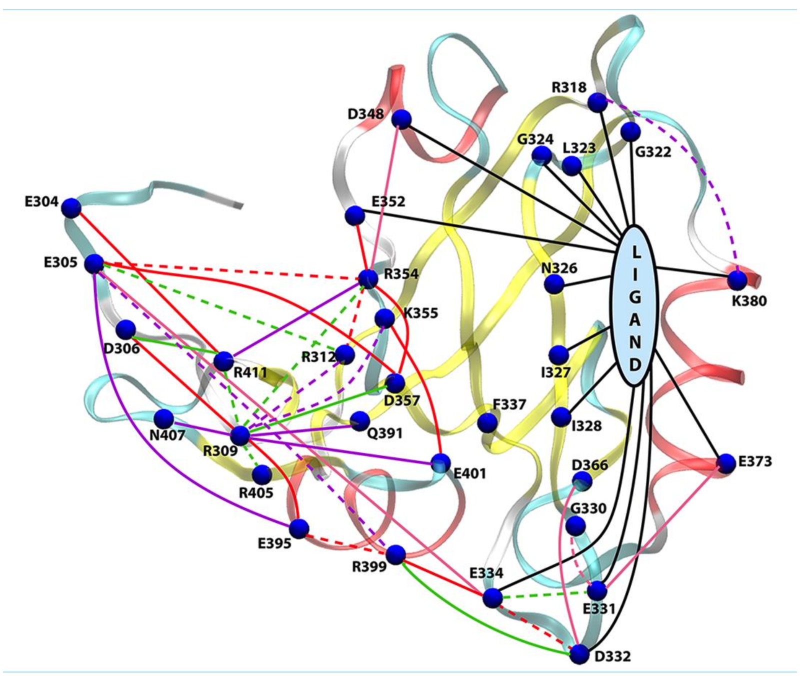
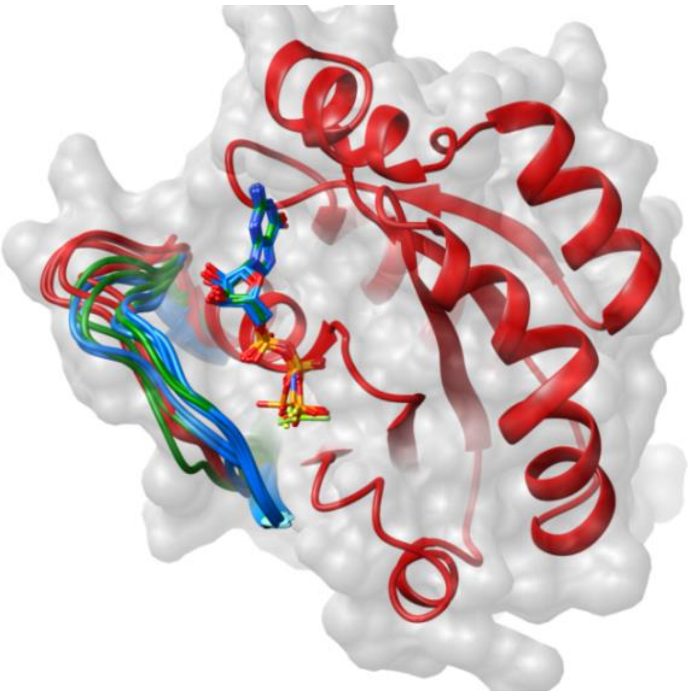
Conformational free energy landscape of misfolding and aggregation in Prion proteins: A challenge for enhanced sampling techniques







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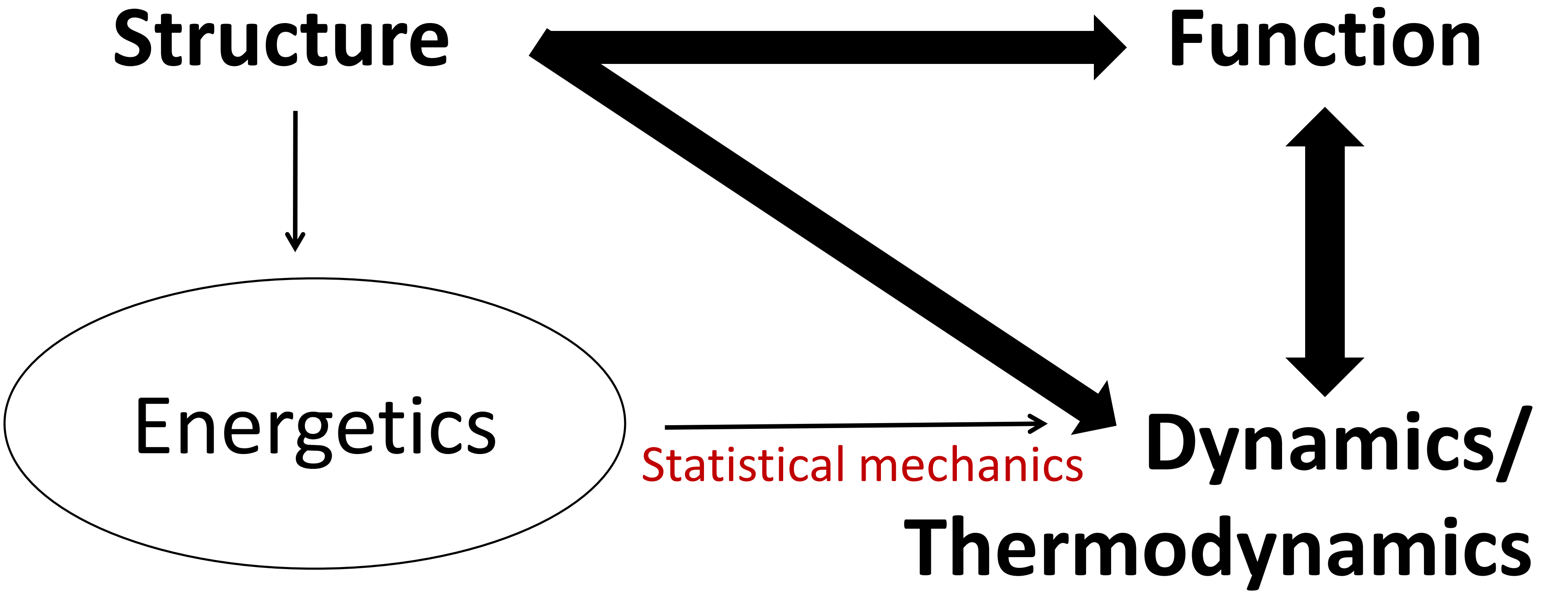
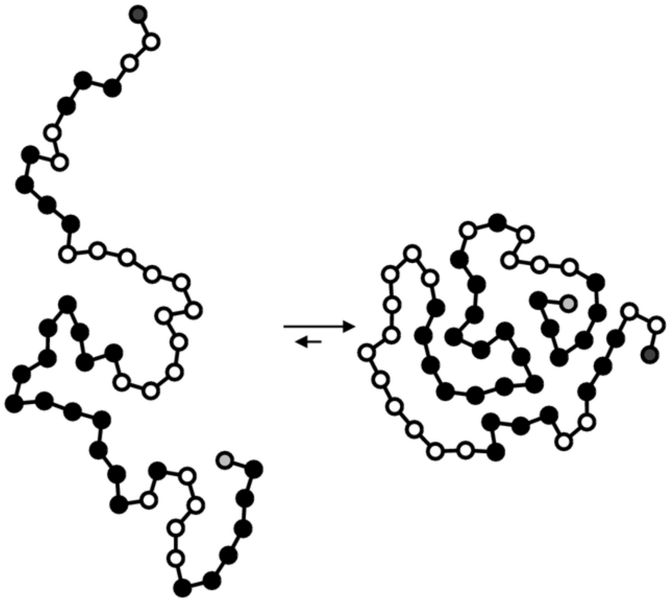
❑ The goal: Structure – Interaction - Dynamics - Function



❑ Molecular thermodynamics (an oxymoron?!)



Unfolded ⇌ Folded

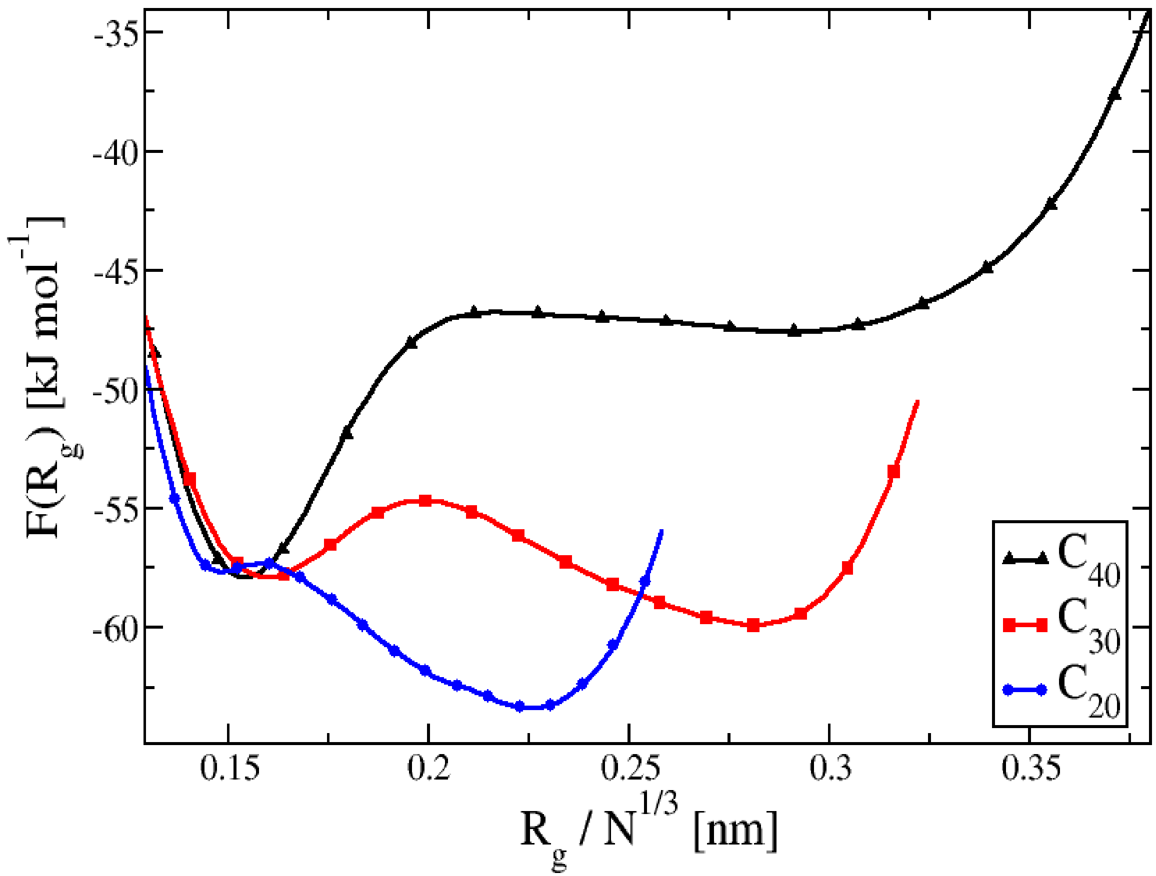
$$\Delta A = \Delta E - T\Delta S$$

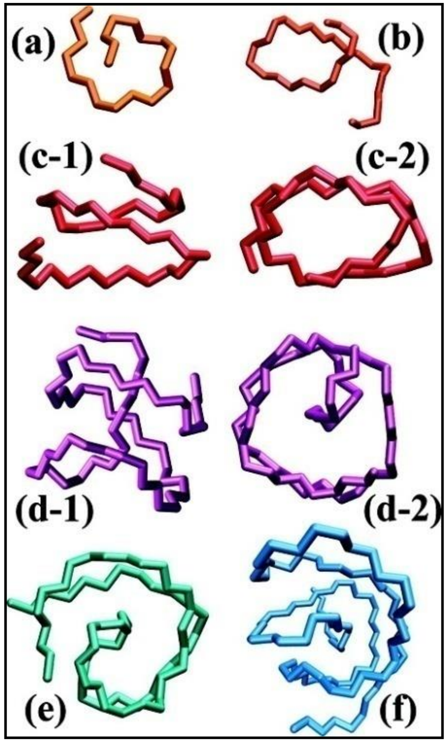
$$= [\Delta E_{torsion:pol} + \Delta E_{nb:pol\text{-}pol} + \Delta E_{nb:pol\text{-}wat} + \Delta E_{nb:wat\text{-}wat}]$$

$$- T\,[\Delta S_{polymer} + \Delta S_{water}]$$

Which will win?

Free Energy Surface: Know it all!





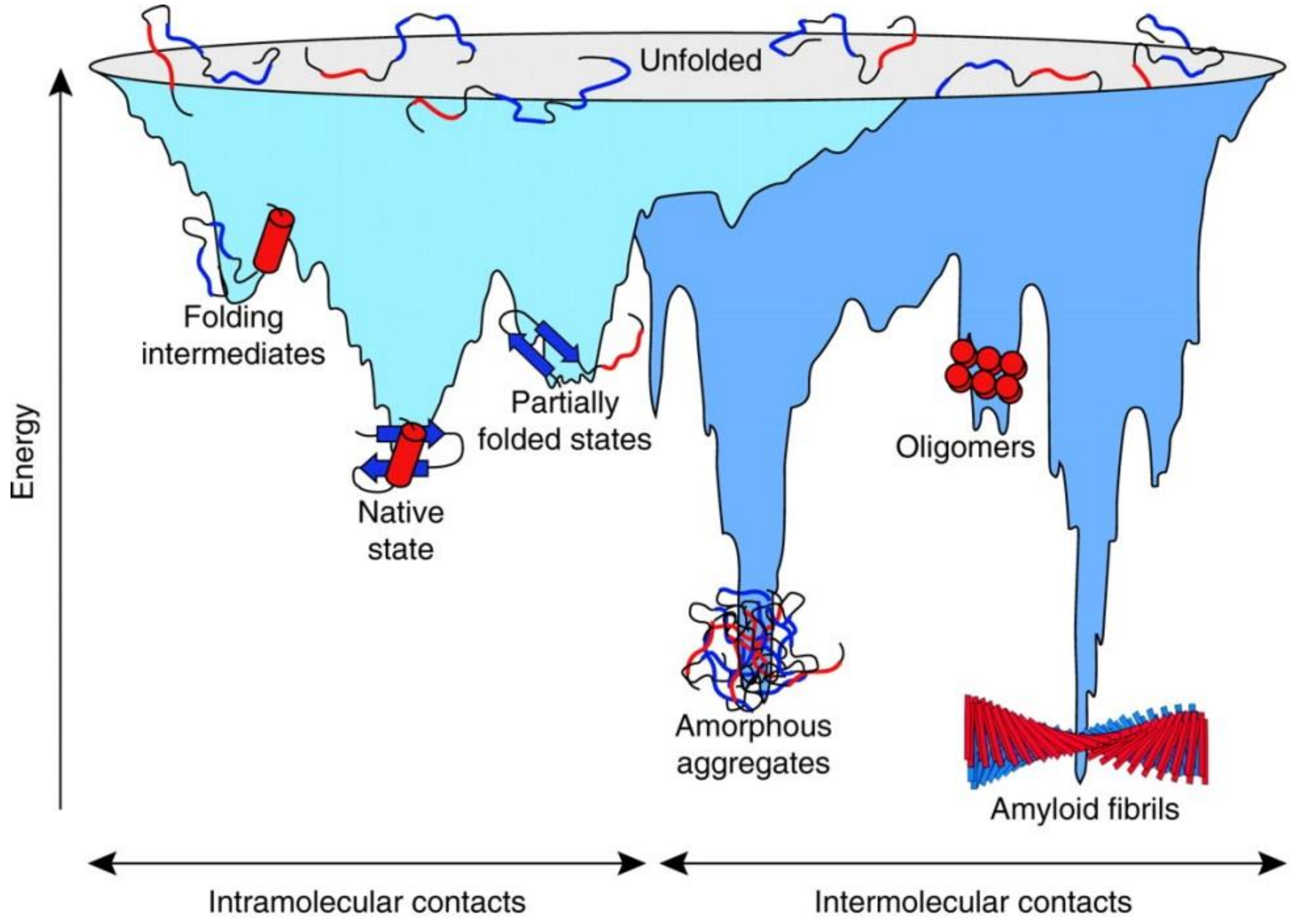
Population ratio:

$$\frac{[A]}{[B]} = \exp\left(-\Delta G_{A \to B} / RT\right)$$

Rate constant:

$$k = \frac{k_B T}{h} \exp\left(-\Delta G^{\neq} / RT\right)$$

❑ Energy Landscape View of Protein Folding, Misfolding and Aggregation:



Source: *Nature Structural & Molecular Biology* **16**, 574 - 581 (2009)

Molecular Dynamics == Newton's Equation of Motion

(i) Potential energy -> Force -> Acceleration

$$\mathbf{F}_i = -\frac{\partial E_i(\underset{\sim}{\mathbf{r}})}{\partial \mathbf{r}_i}$$

$$m_i \frac{\P^2 r_i}{\P t^2} = \mathbf{F}_i, \qquad i = 1, 2, \ldots.., N$$

But do we know $E(\underset{\sim}{r})$ accurately?

(ii) Acceleration -> Velocity -> Position

$$v(t + dt) = v(t) + a(t)dt + \ldots$$

$$r(t + dt) = r(t) + v(t)dt + \ldots$$

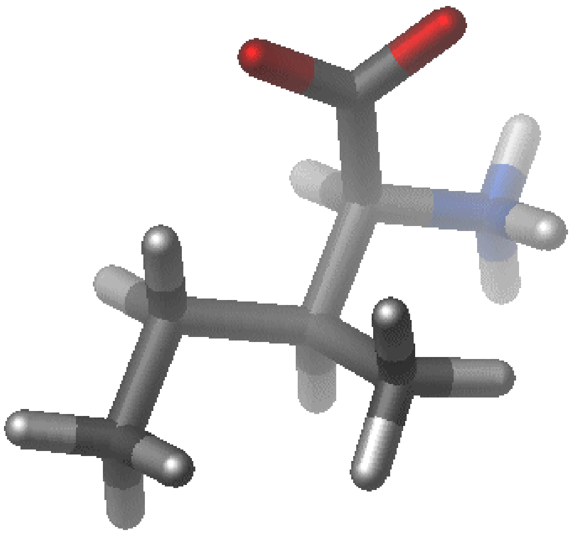
In an ideal world,
we would just solve …

$$H\Psi = E\Psi$$

… but the world is not ideal, quantum methods are prohibitively slow.

Molecular Mechanics (MM): We assume that classical mechanics works even at the molecular level!

Assumption: PES ($E(\underset{\sim}{r})$) can be broken into individual pair-wise additive terms



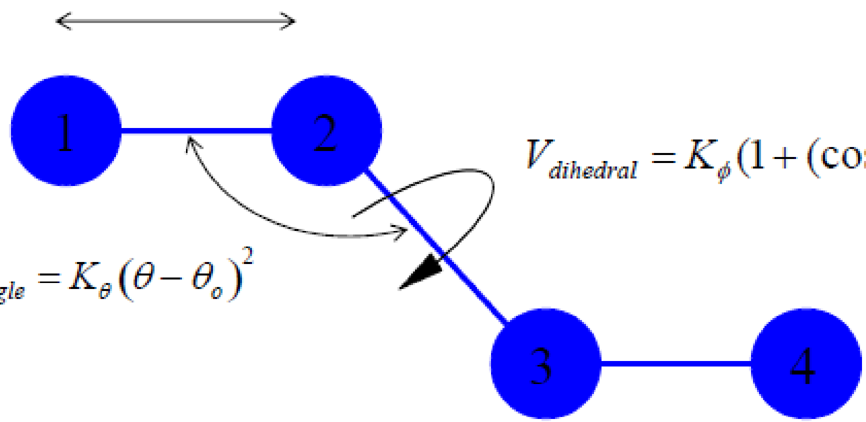
$$E_{total} = E_{bonded} + E_{non\text{-}bonded}$$

$$E_{bonded} = E_{bond} + E_{angle} + E_{torsion}$$

$$E_{non\text{-}bonded} = E_{VDW} + E_{electrostatic}$$

Molecular Mechanics: “Force field”

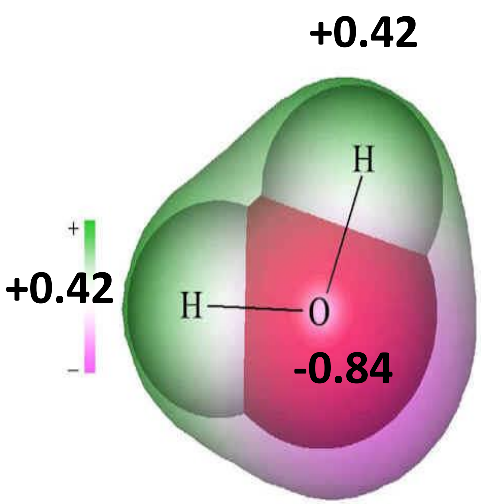
$$V_{bond} = K_b (b - b_o)^2$$

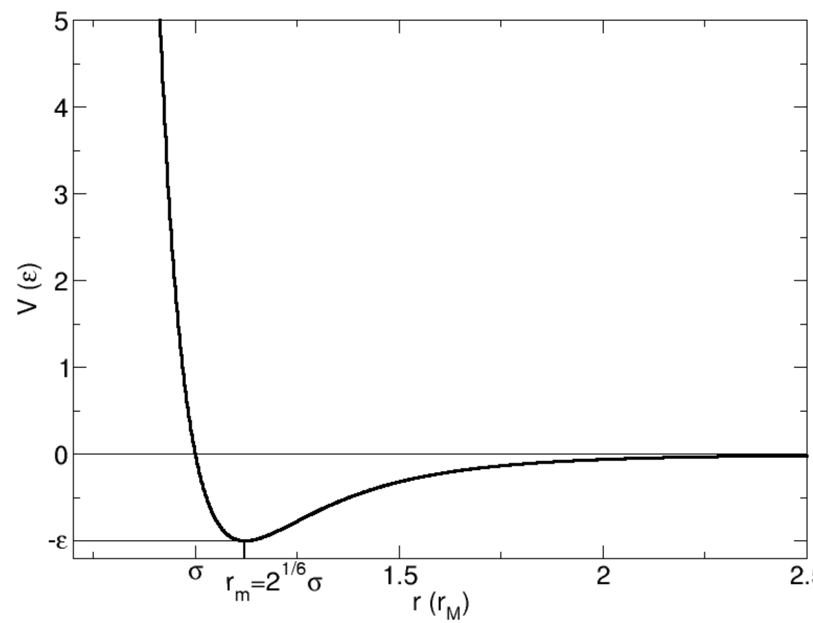


$$V_{dihedral} = K_\phi (1 + (\cos n\phi - \delta))$$

$$V_{angle} = K_\theta (\theta - \theta_o)^2$$

$$E_{VDW} = 4\varepsilon \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} \right]$$





❑ Bridging the gap between microscopic & macroscopic worlds:

✓ **Boltzmann distribution:** $P(\underset{\sim}{r}) \propto \exp\left[-E(\underset{\sim}{r}) / k_B T\right]$

✓ **Average observable:** $\langle O \rangle = \int d\underset{\sim}{r} O(\underset{\sim}{r}) P(\underset{\sim}{r})$

✓ **Free energy:**

$$Q = \int d\underset{\sim}{r} \exp\left[-E(\underset{\sim}{r}) / k_B T\right]$$

$$A = - k_B T \ln Q$$

Nature is driven by free energy !

- **Statistical Mechanics:** Connecting molecular interactions to thermodynamics

  - ✓ Sample configurational space: **Molecular Dynamics / Monte Carlo**

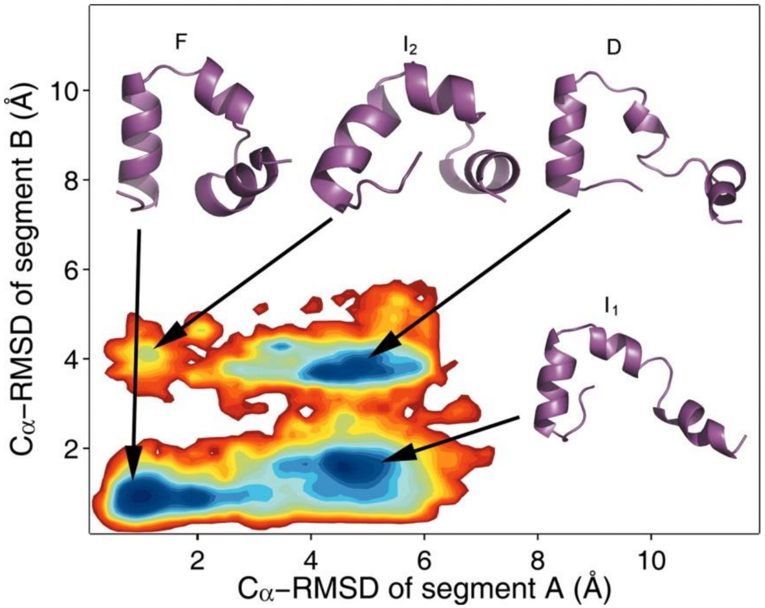
$$Q = \int d\underset{\sim}{r} \exp\left[-E(\underset{\sim}{r}) / k_B T\right]$$

- **Absolute free energy:** $A = -k_B T \ln Q$

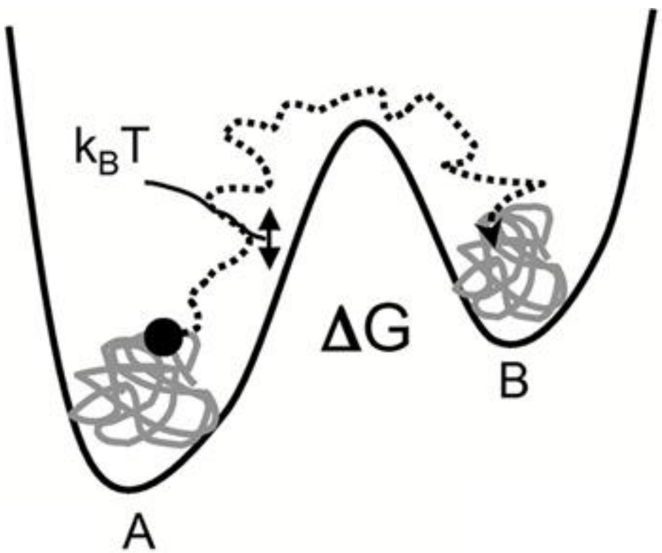
- **Free energy surface / potential of mean force:**

$$\exp\left(-\beta A(X)\right) = \left\langle \exp\left(-\beta U(\underset{\sim}{r})\right)\right\rangle_{f(\underset{\sim}{r})=X}$$

$$A(X_1, X_2, \ldots) = -\, k_B T \ln P(X_1, X_2, \ldots)$$



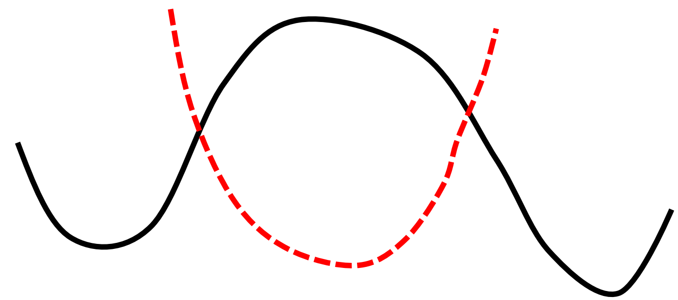
How "rare" is a rare event?



$$k \simeq \frac{k_B T}{h} \exp\left[-\beta \Delta G^{\neq}\right]$$

Barrier (kcal/mol)	Time scale (300K)
5	< 1 ns
10	> 1 µs
15	> 10 ms
20	1 min
25	~ 3 days
30	~40 years!

Umbrella sampling: The background



With original hamiltonian: $U(\underset{\sim}{r})$

$$< O >_U = \int d\underset{\sim}{r} O(\underset{\sim}{r}) P(\underset{\sim}{r})$$

With perturbed hamiltonian: $U'(\underset{\sim}{r}) = U(\underset{\sim}{r}) + w(\underset{\sim}{r})$

For example:

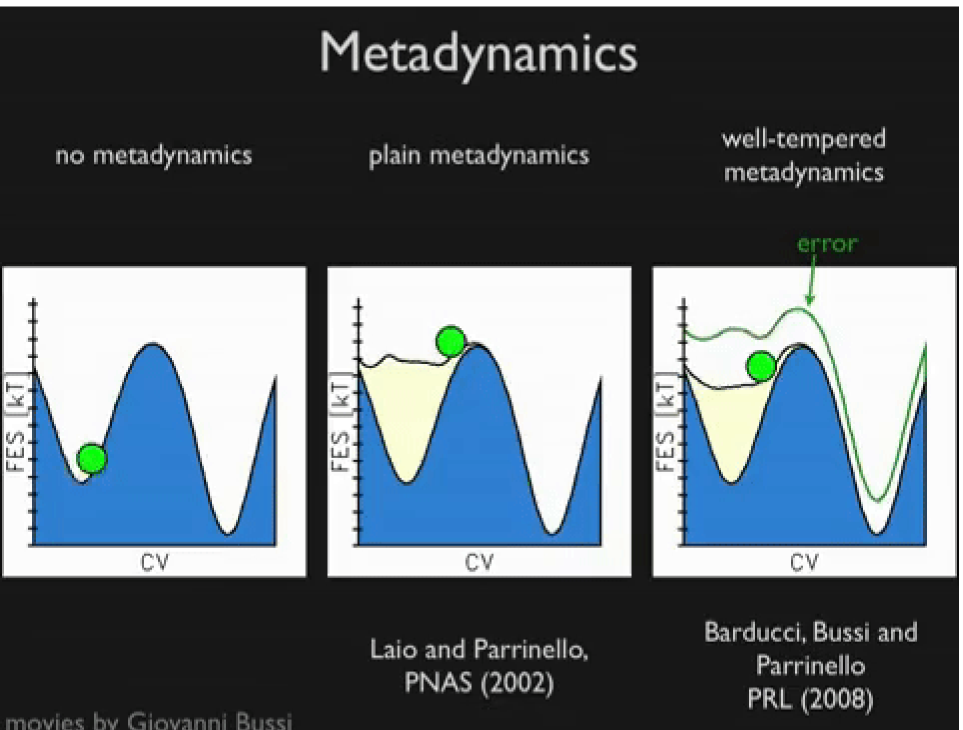
$$w(\underset{\sim}{r}) = -k\left[X(\underset{\sim}{r}) - X_0\right]^2$$

$$< O >_U = \frac{\left\langle O.\exp\left[bw\right]\right\rangle_{U+w}}{\left\langle \exp\left[bw\right]\right\rangle_{U+w}}$$

Metadynamics: Escaping minima

- **History dependent bias potential: Sum of Gaussians**

$$V_G(s(x),t) = w\sum_{t'\leq t}\exp\left(-\frac{(s(x)-s(x_G(t')))^2}{2(\delta s)^2}\right)$$

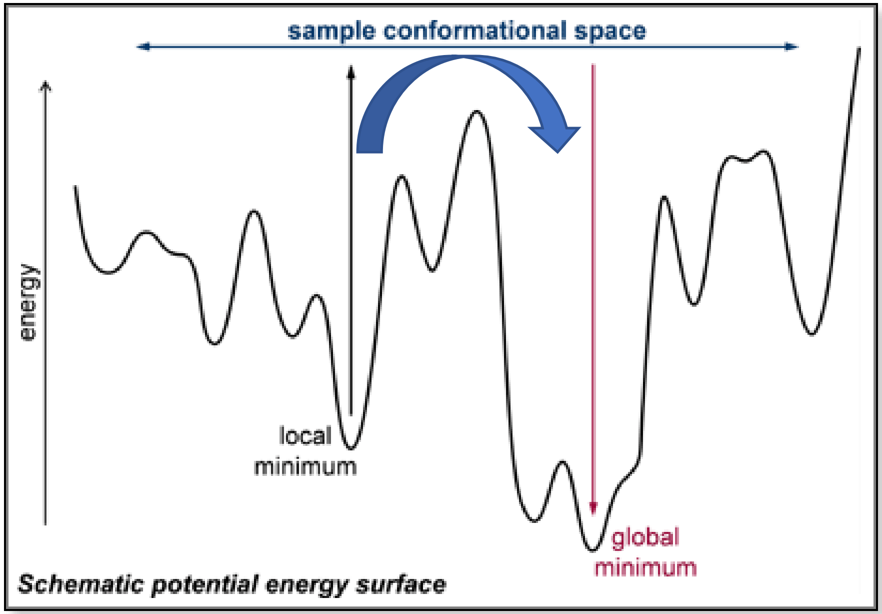


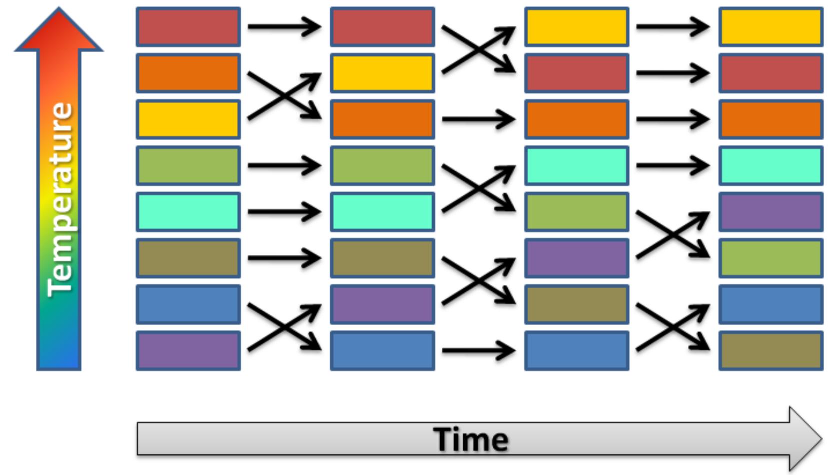
- If Gaussians are added sufficiently slowly CVs tend to diffuse to closest local minimum
- Ability to accelerate rare events

Laio and Parrinello, *PNAS* **99**, 12562 (2002)

When you don’t know **what you’re looking for**,
and **where to look for it**,
what is your “**reaction coordinate**”?

Replica Exchange Molecular Dynamics





$$(E_i,T_i)(E_j,T_j) \longrightarrow (E_i,T_j)(E_j,T_i)$$

$$p = \min\left\{1, \frac{\exp\left[-(E_j/k_BT_i)-(E_j/k_BT_i)\right]}{\exp\left[-(E_i/k_BT_i)-(E_j/k_BT_j)\right]}\right\}$$

$$= \min\left\{1, \exp\left[(E_j - E_i)(\beta_j - \beta_i)\right]\right\}$$

✓ At each temperature, the trajectory will be discontinuous, but follow a proper Boltzmann distribution for that temperature.

✓ No need to know “reaction coordinate” a priori!

Acceleration of sampling / Free energy calculation

Simplified models: Avoiding unnecessary details

- ✓ Coarse graining
- ✓ Multi-scale modeling
- ✓ Lattice models
- ✓ Implicit solvent
- ✓ Thermodynamic cycles

Enhanced sampling methods: application of "bias" / pathway

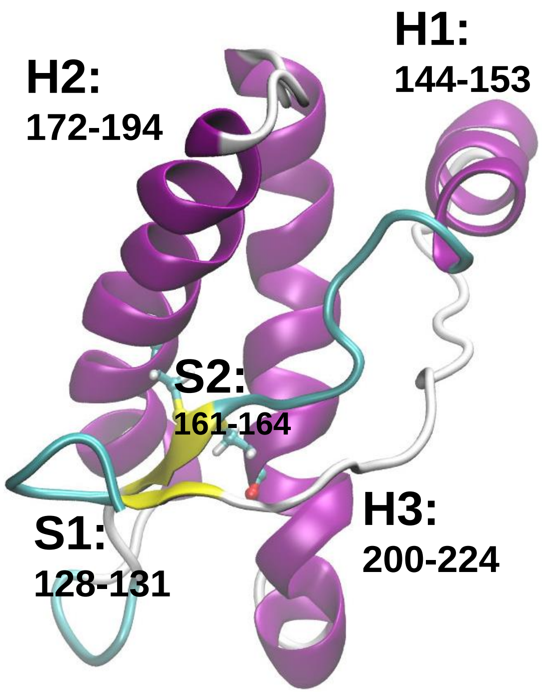
- ✓ Metadynamics
- ✓ Parallel tempering (temperature, Hamiltonian, pH etc)
- ✓ Free Energy Perturbation
- ✓ Steered MD

Hardware acceleration:

GPUs etc

Make the best use of available hardware

❑ About Prion: Structure, function, dysfunction



Mouse prion domain (124-226)
PDB ID: 1AG2

- Linked to a range of neurodegenerative diseases known as transmissible spongiform encephalopathy

- **Hypothesis:** Normal cellular prion (PrP^{C}) misfolds into an infectious oligomeric *scrapie* form (PrP^{Sc}) which tends to aggregate in amyloid plaques.

- Cellular prion (PrP^{C}) has a high α-helix (43%) and little β-sheet (3%). PrP^{Sc} should have extended β-sheet (upto 50%).

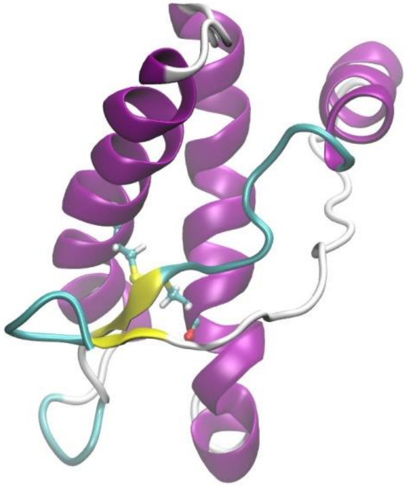
- PrP^{Sc} structure is still unknown!

- Molecular mechanism of PrP^{C} to PrP^{Sc} conversion remains unknown.

❑ Prion misfolding / aggregation pathways

➢ "Protein-only" hypothesis of prion Propagation:

PrP^{c} + PrP^{Sc} ⟶ 2 PrP^{Sc} ?



Nucleation?

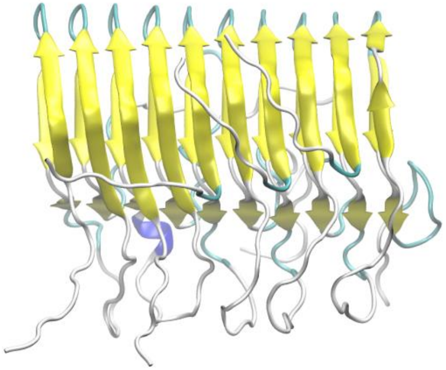
Challenges:

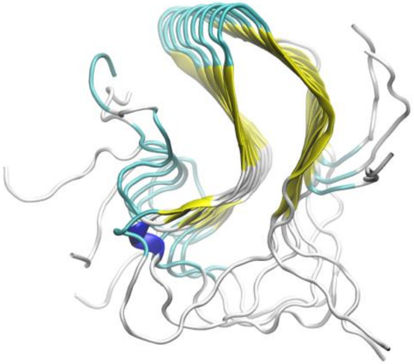
✓ **Structure of PrP^{Sc}**

✓ **Mechanism of poisoning**

Structure of the soluble toxic intermediate (PrP^{Sc}) is not known!

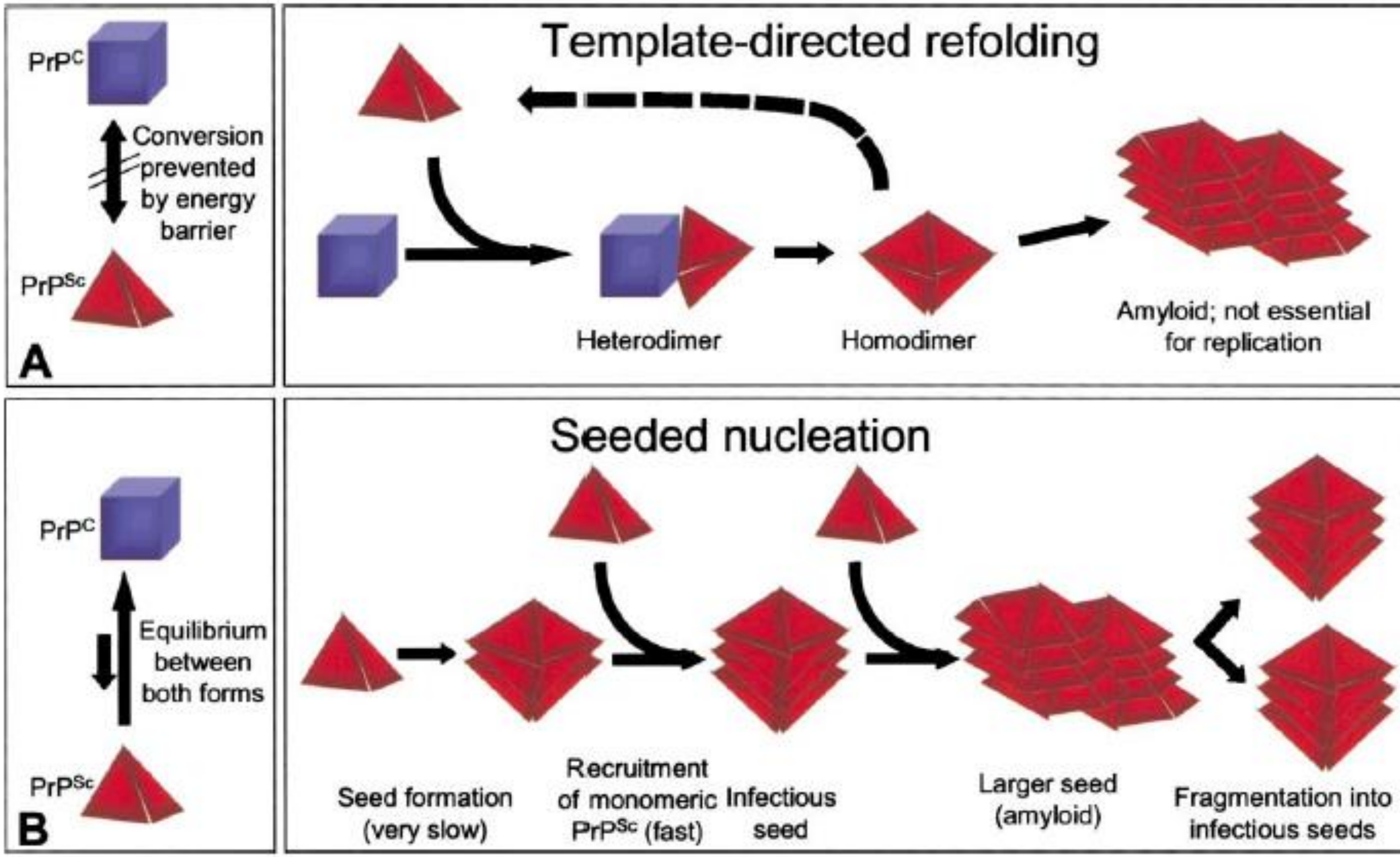
Hypothesis: Rich in β sheet





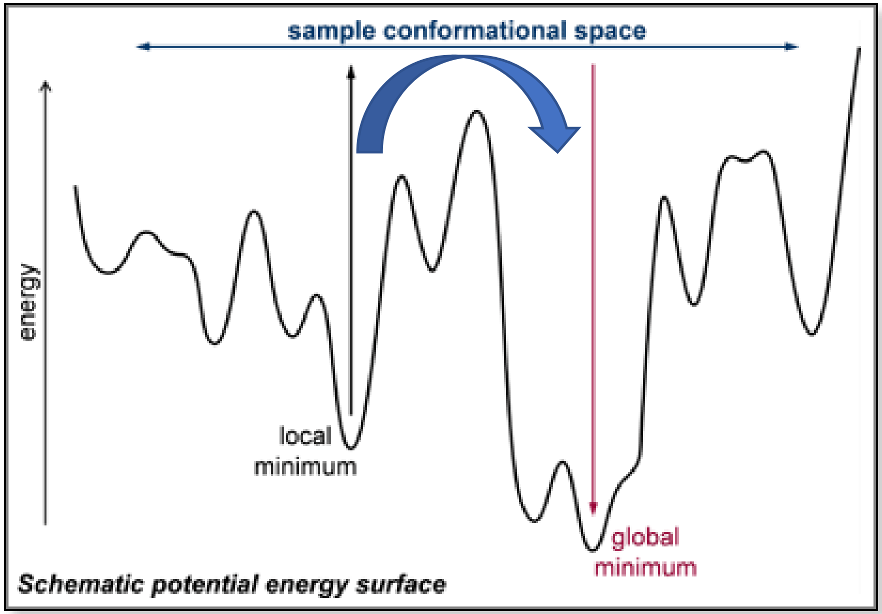
Fibril formation

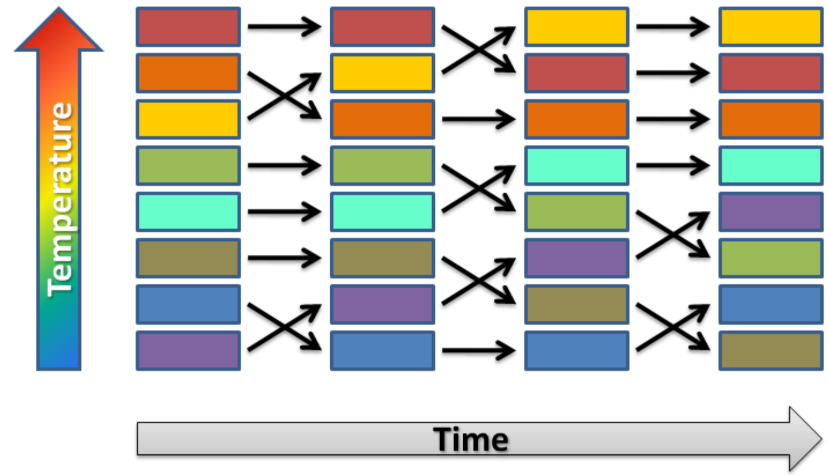
❑ Models of Prion propagation:



Source: Aguzzi *et al*, Cell **116**, 313–327 (2004)

Replica Exchange Molecular Dynamics





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$$= \min\left\{1, \exp\left[(E_j - E_i)(\beta_j - \beta_i)\right]\right\}$$

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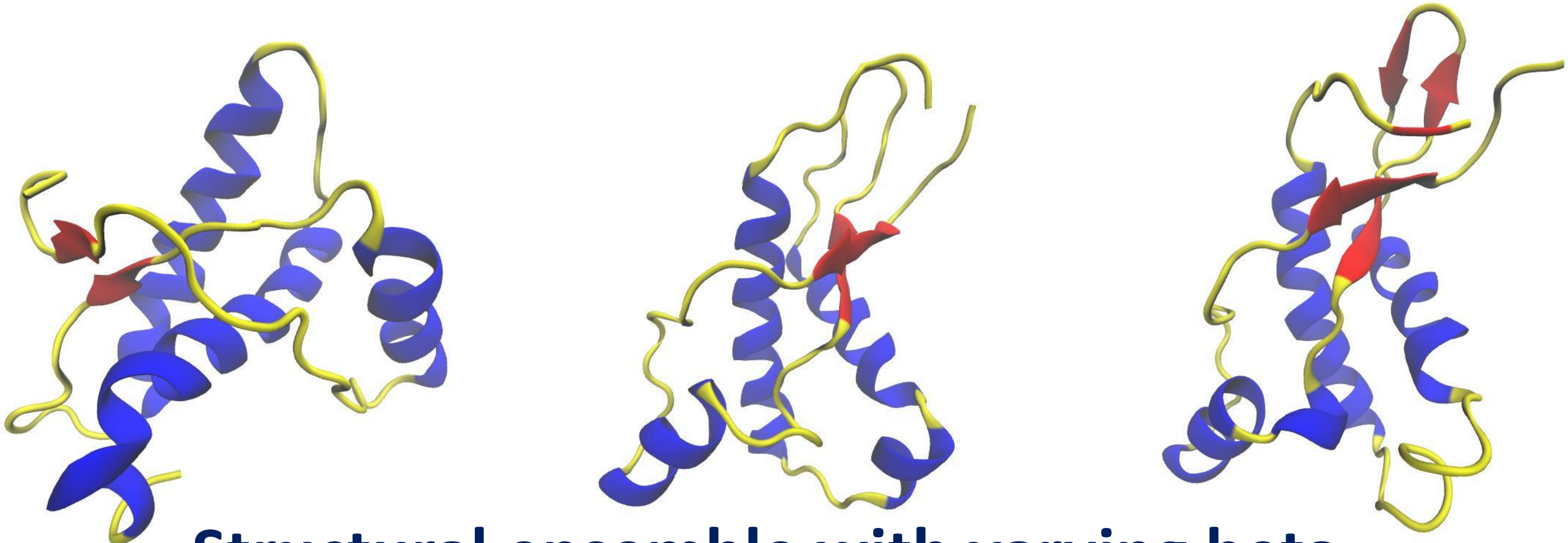
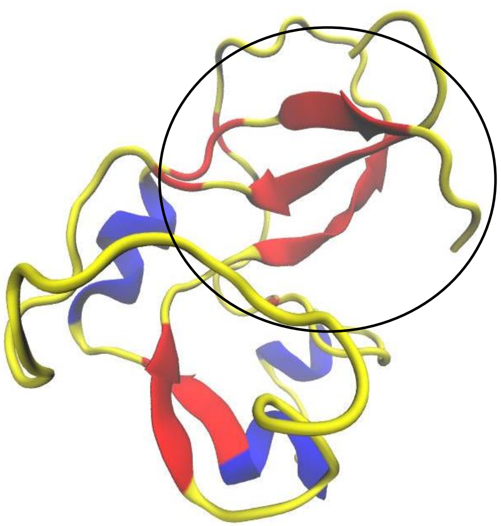
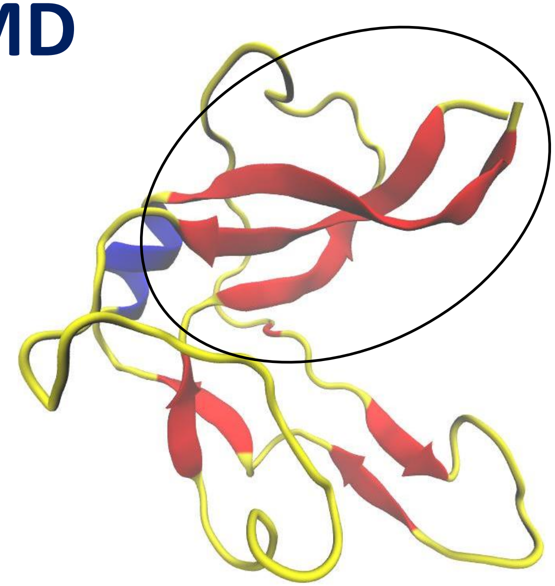


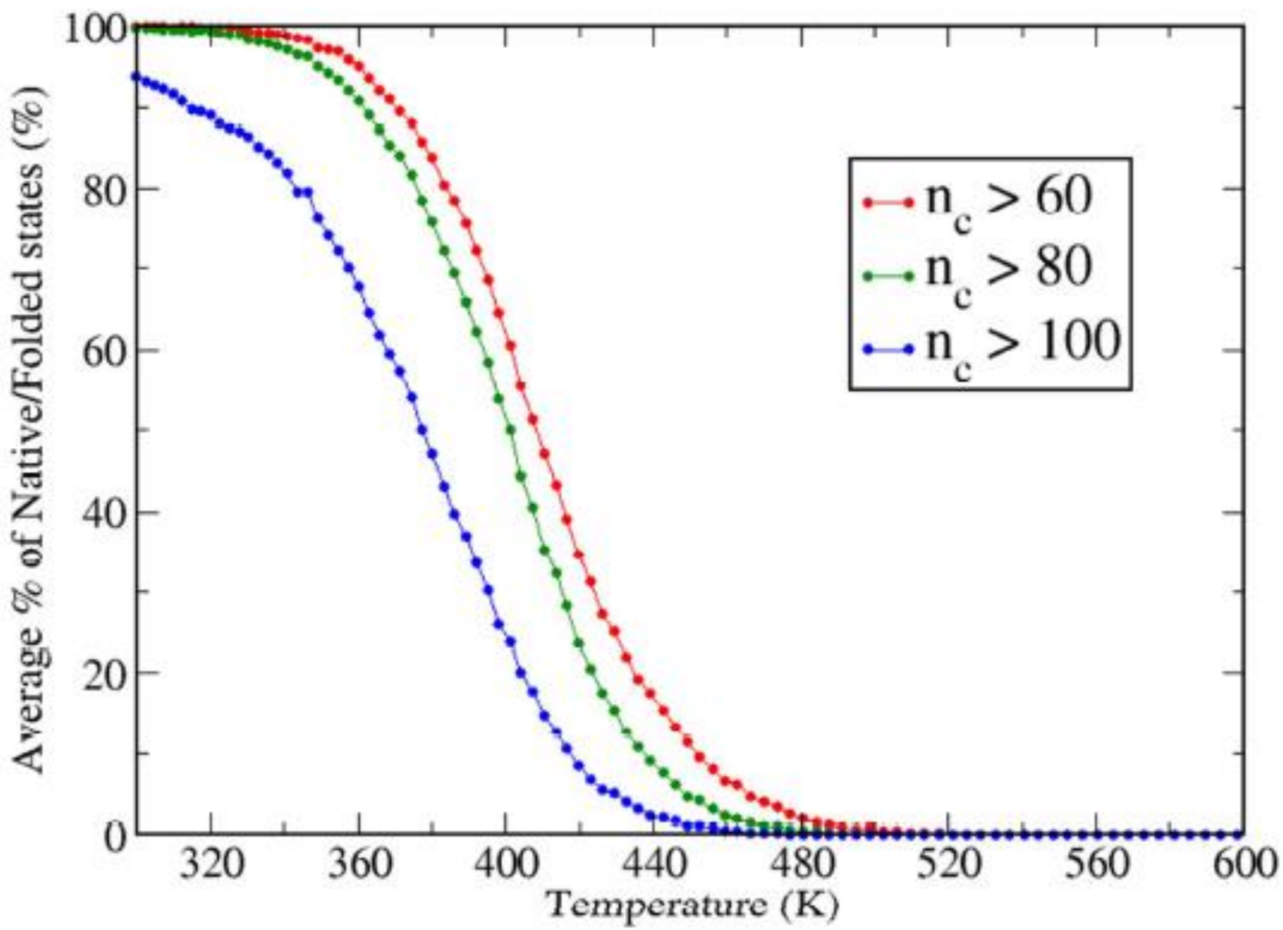
Structural ensemble with varying beta sheet content from REMD





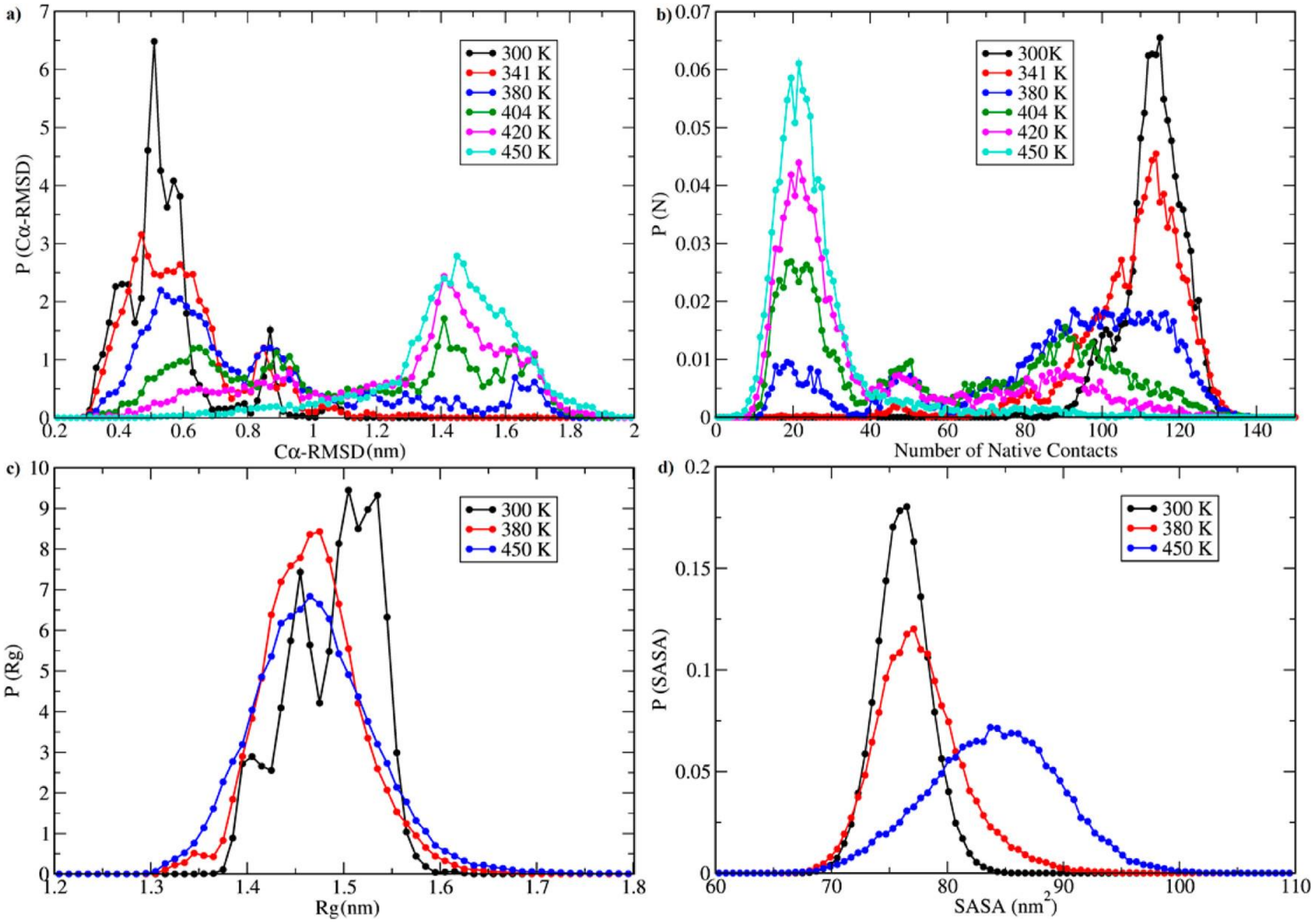
- N. Chamachi and S. Chakrabarty, *J. Phys. Chem. B* **120**, 7332 (2016)
- N. Chamachi and S. Chakrabarty, *Biochemistry* **56**, 833 (2017)

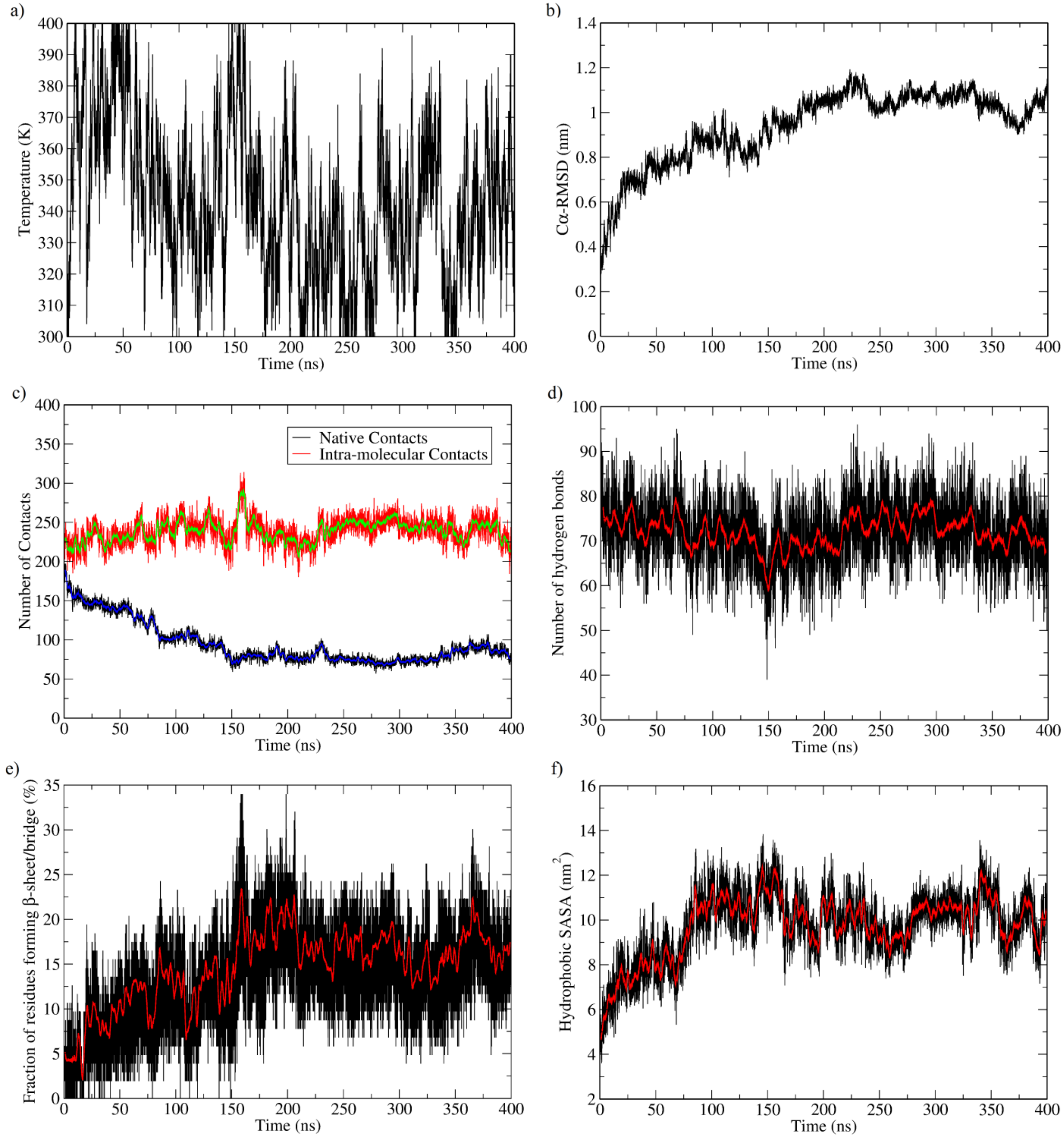
❑ **Melting curve**



We expect a first-order like transition. Two states?

❑ Temperature dependence of structural parameters





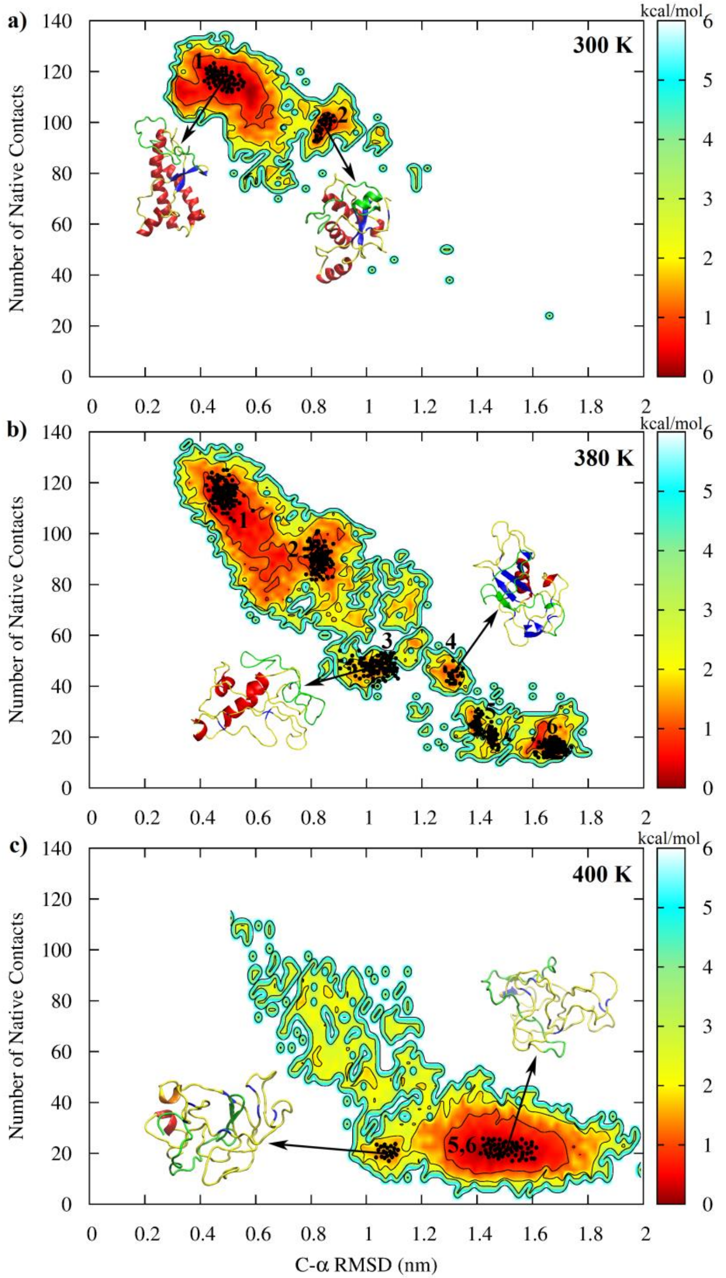
Structures from higher temperature replica have:

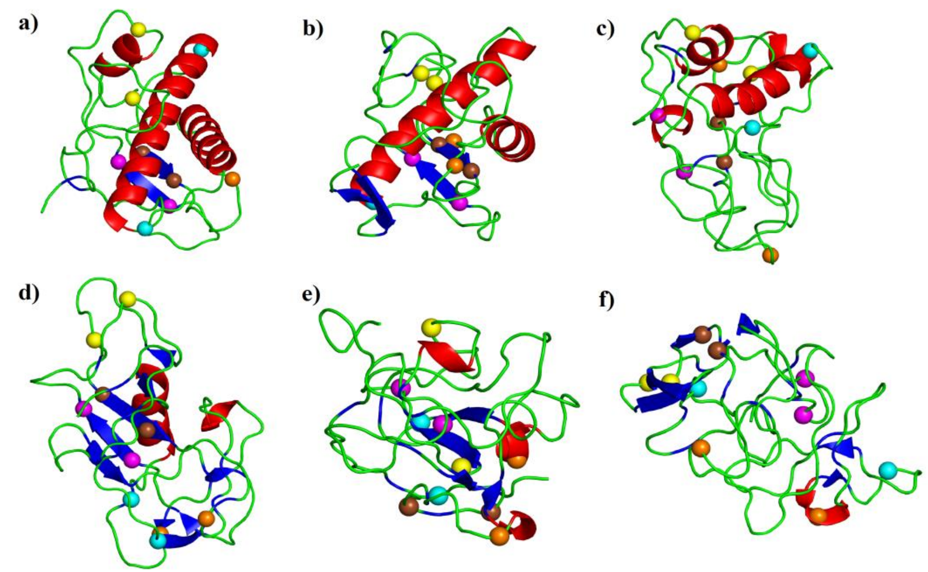
- **Lower RMSD**
- **Lower number of native contacts**

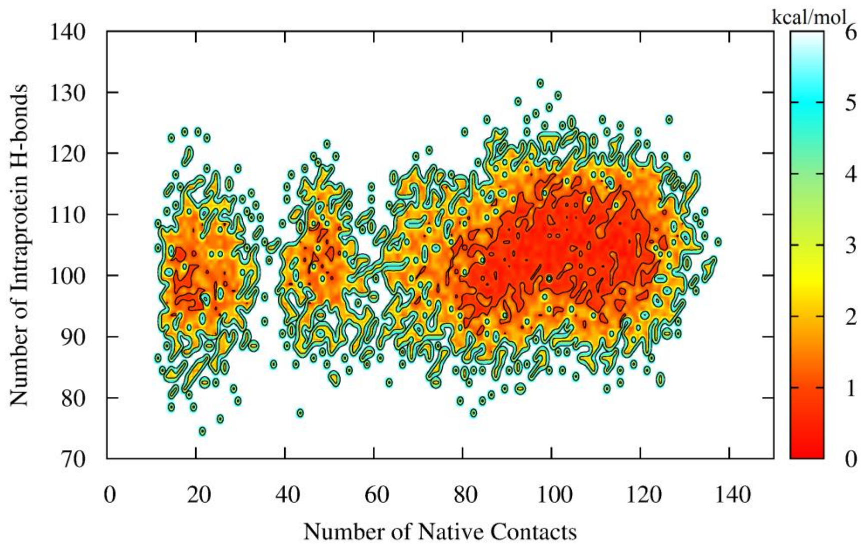
- **Similar R_g**
- **Similar number of contacts**

- **Higher β-content**
- **Higher hydrophobic SASA**

❑ Free energy landscape of Prion misfolding

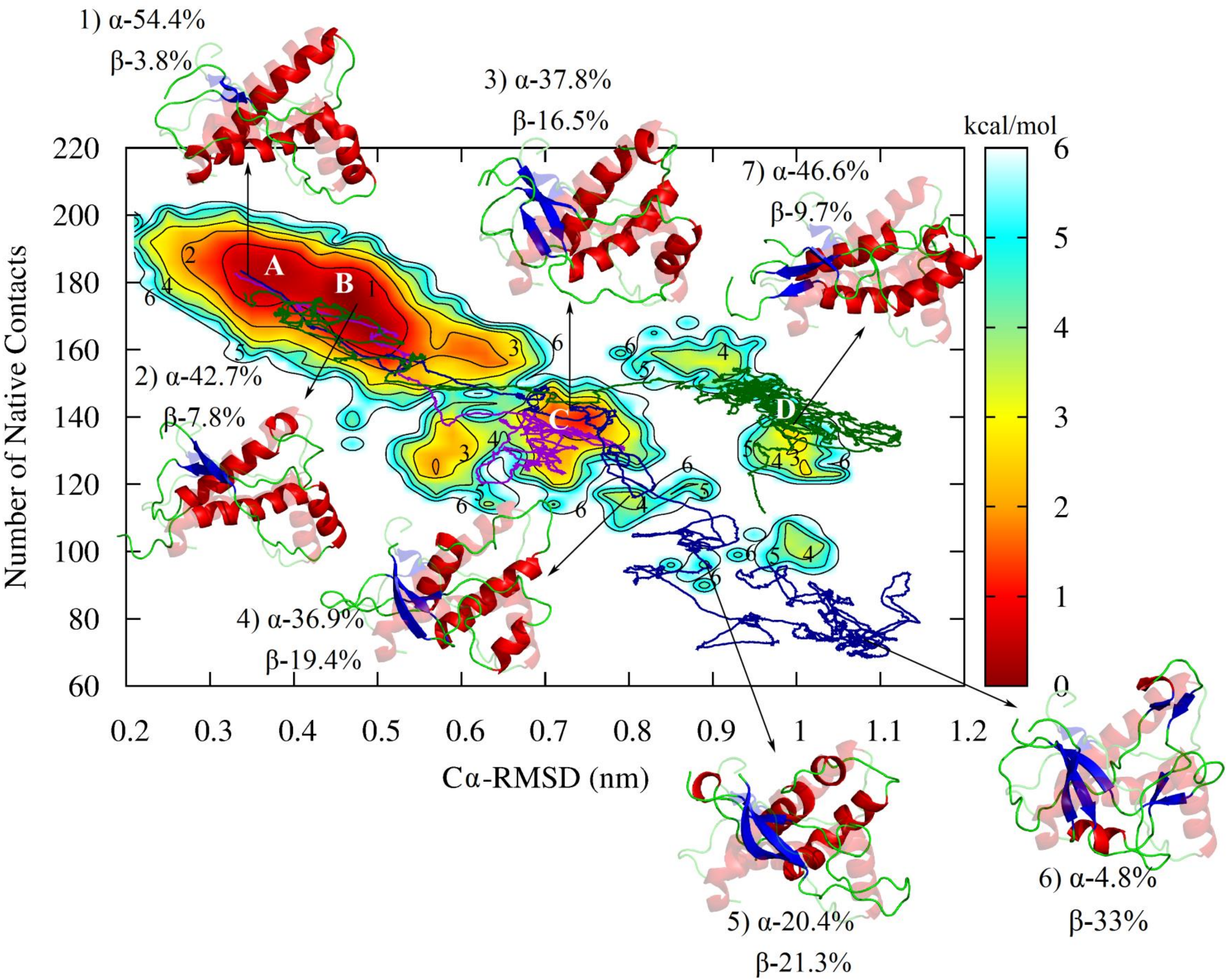




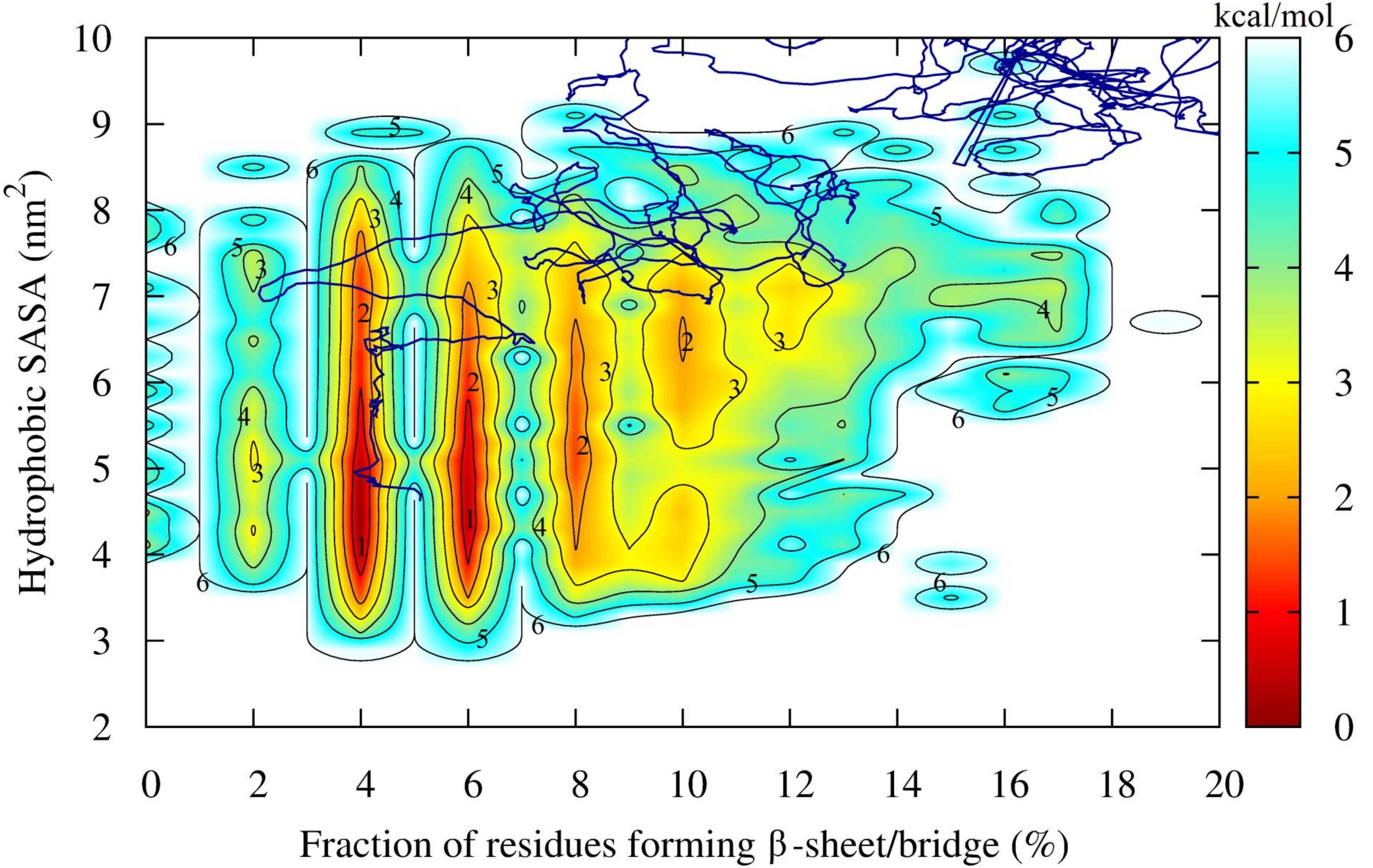


Misfolded states stabilized by non-native hydrogen bonds!

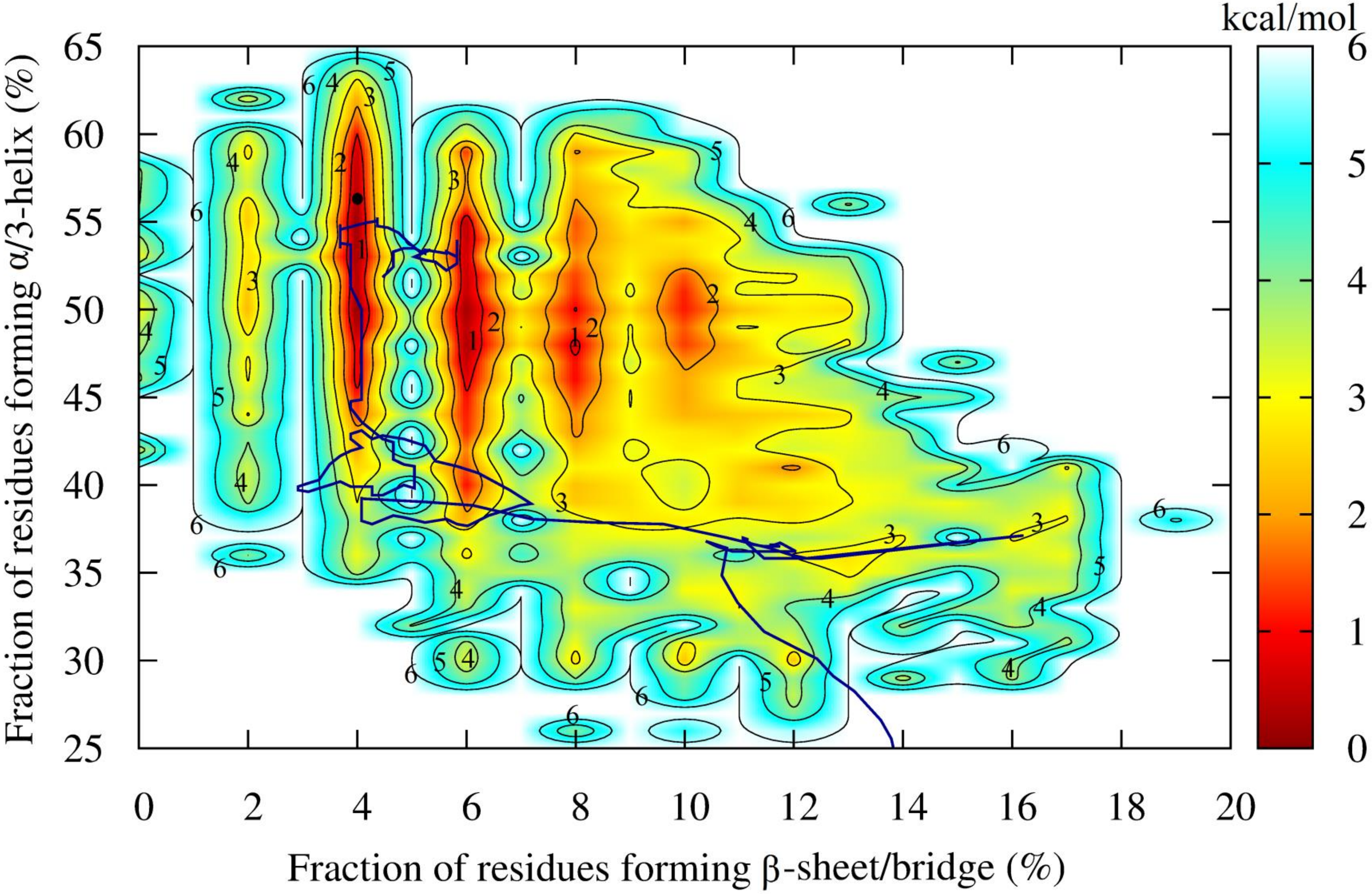
❑ Free energy landscape of Prion misfolding



❑ Hydrophobicity vs. β-content

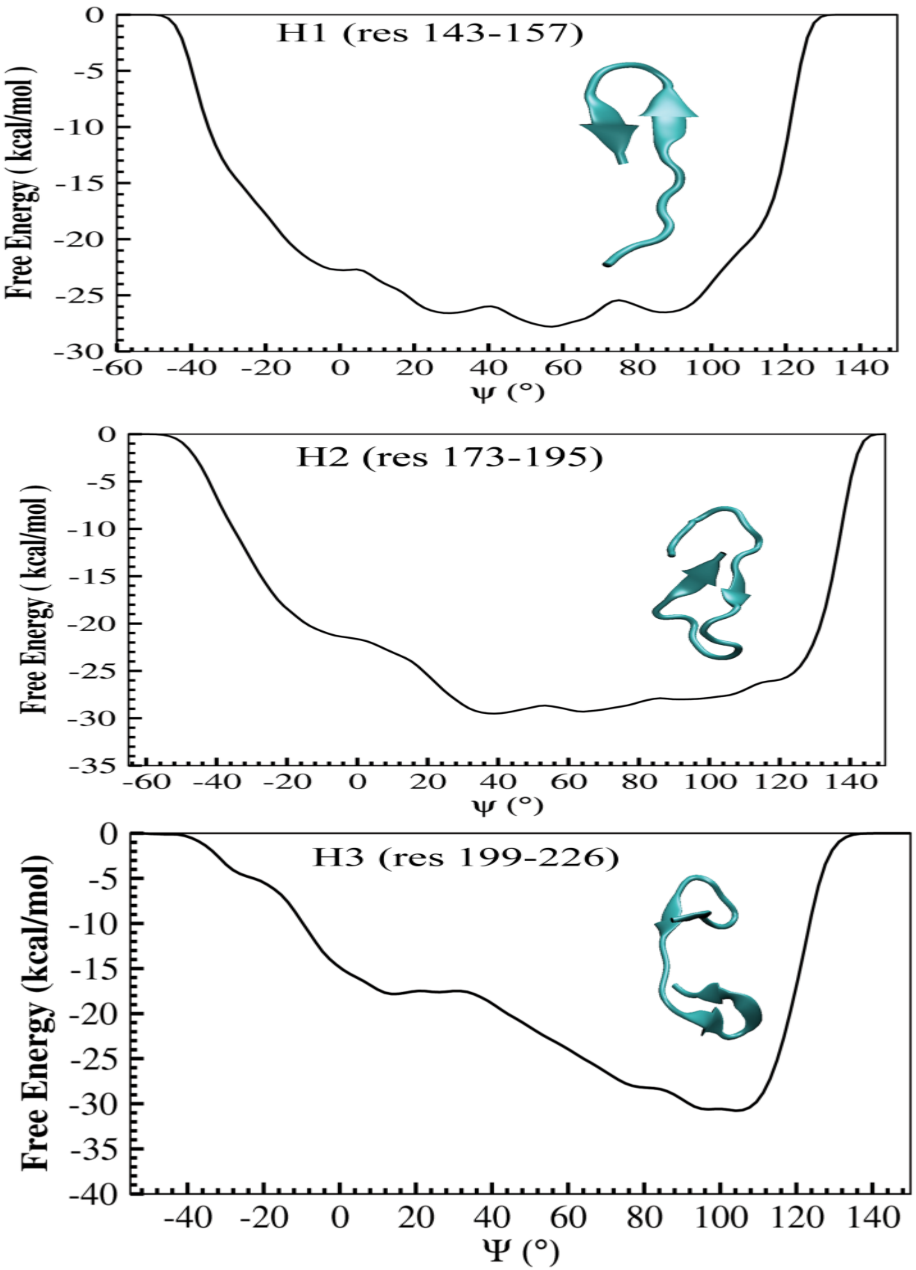


❑ Helicity vs. β-content



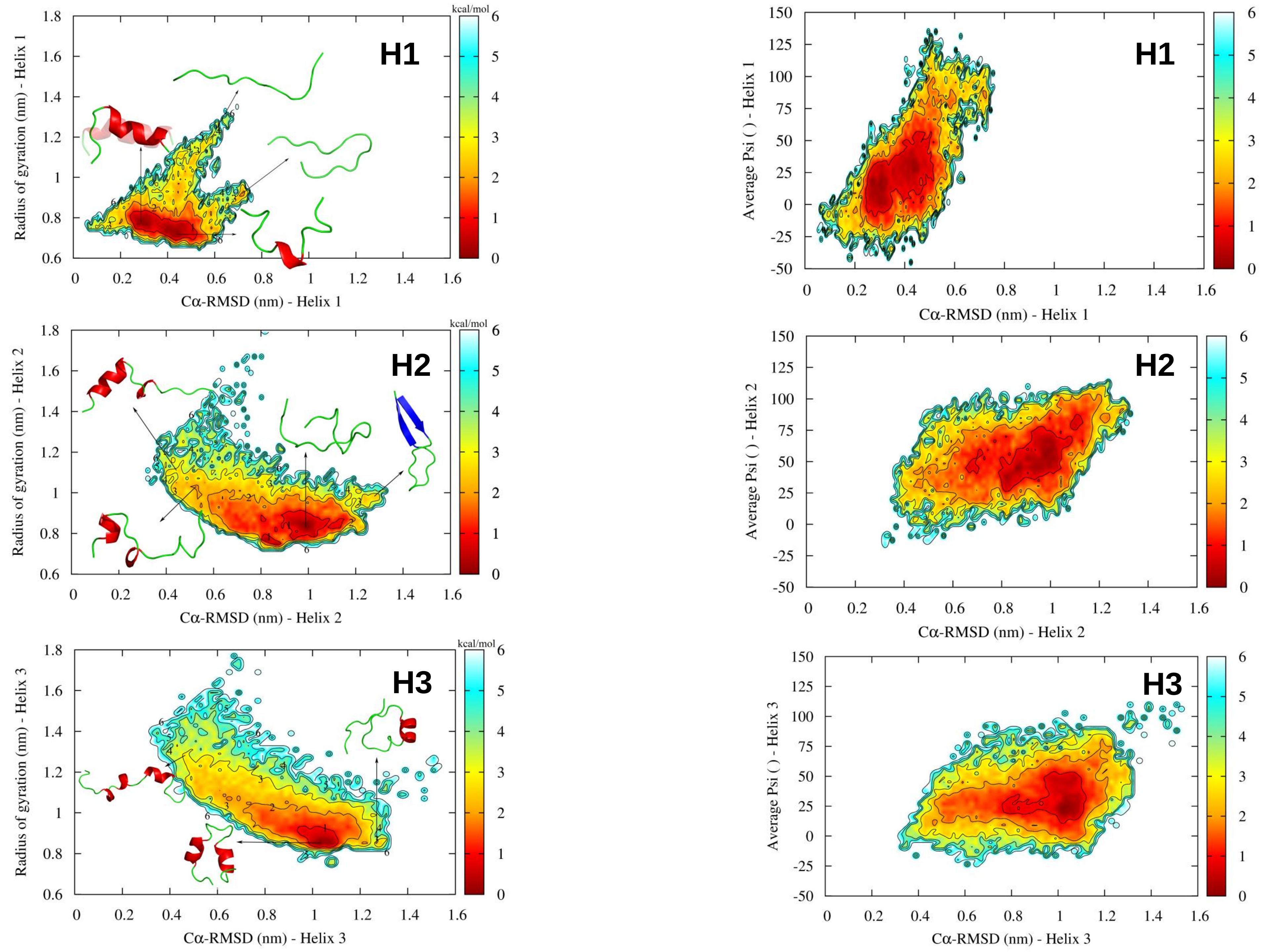
What is the Origin of Marginal Stability of Prion protein?

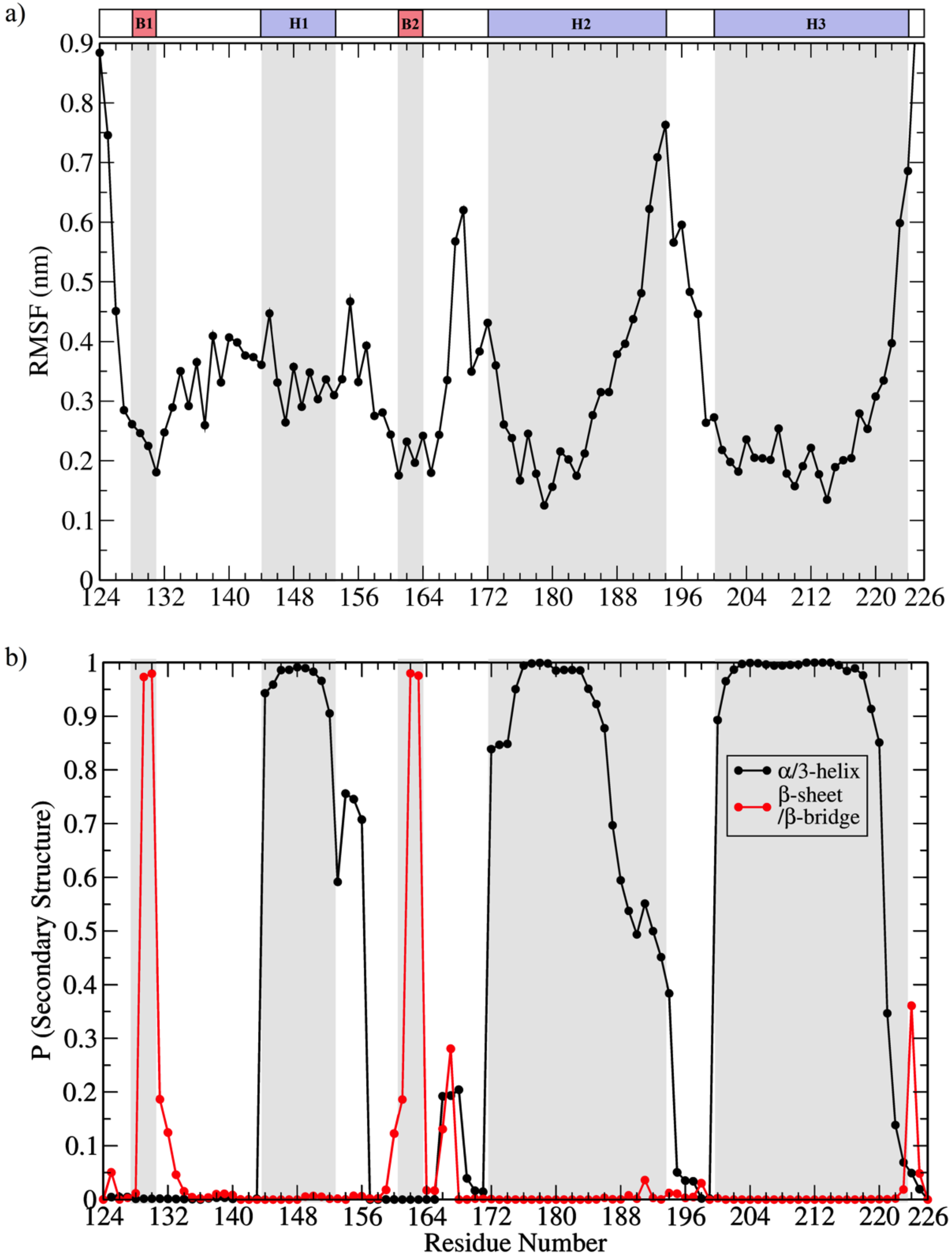
❑ Free energy surfaces of chopped helices:



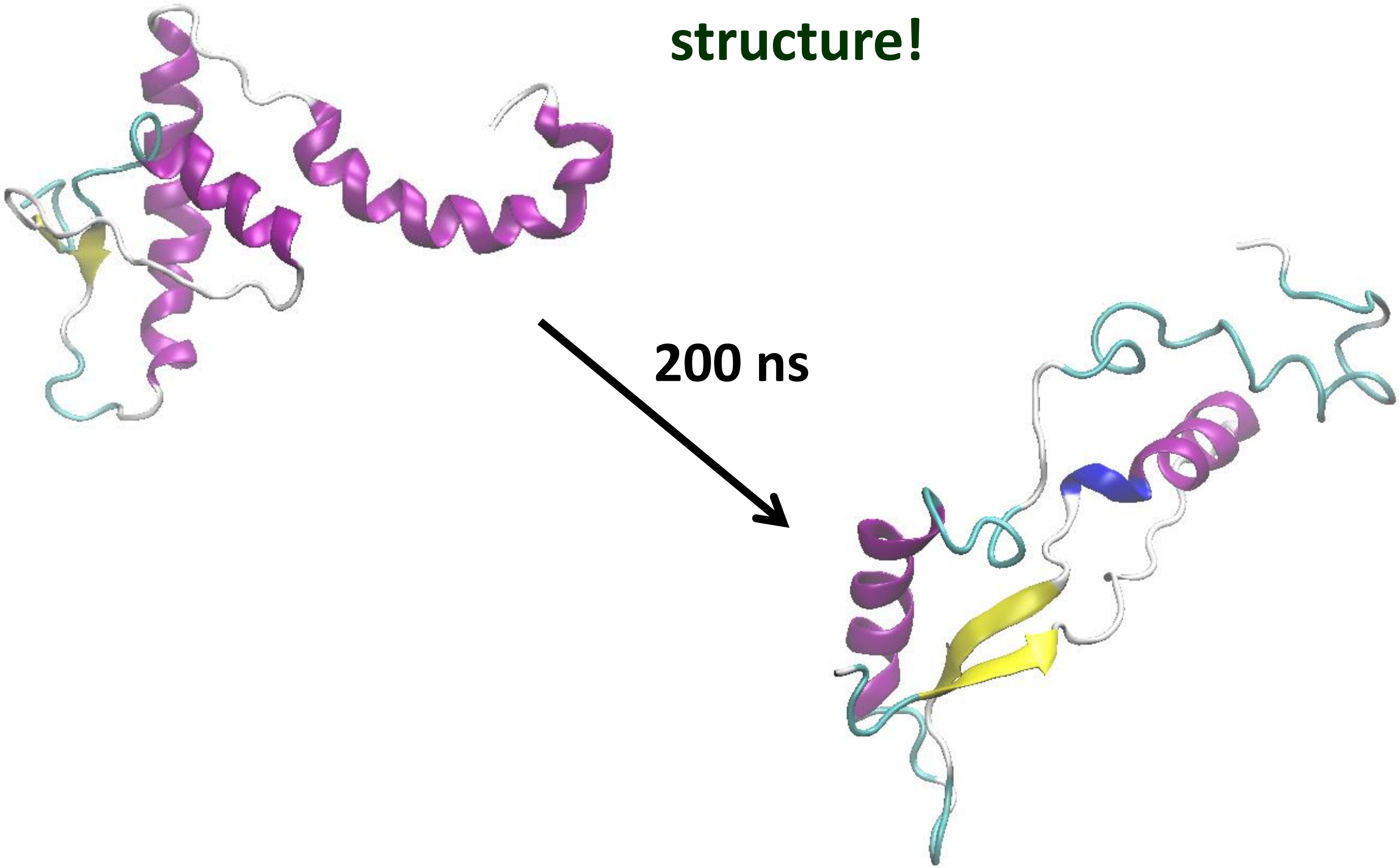
- ✓ Three different helices have been simulated
- ✓ Metadynamics has been used for enhanced sampling

Free energy profile shows that inherently the helices lack stability and tend to form beta sheet and random coil


H1
Radius of gyration (nm) - Helix 1
Cα-RMSD (nm) - Helix 1
kcal/mol
H1
Average Psi ( ) - Helix 1
Cα-RMSD (nm) - Helix 1
H2
Radius of gyration (nm) - Helix 2
Cα-RMSD (nm) - Helix 2
kcal/mol
H2
Average Psi ( ) - Helix 2
Cα-RMSD (nm) - Helix 2
H3
Radius of gyration (nm) - Helix 3
Cα-RMSD (nm) - Helix 3
kcal/mol
H3
Average Psi ( ) - Helix 3
Cα-RMSD (nm) - Helix 3

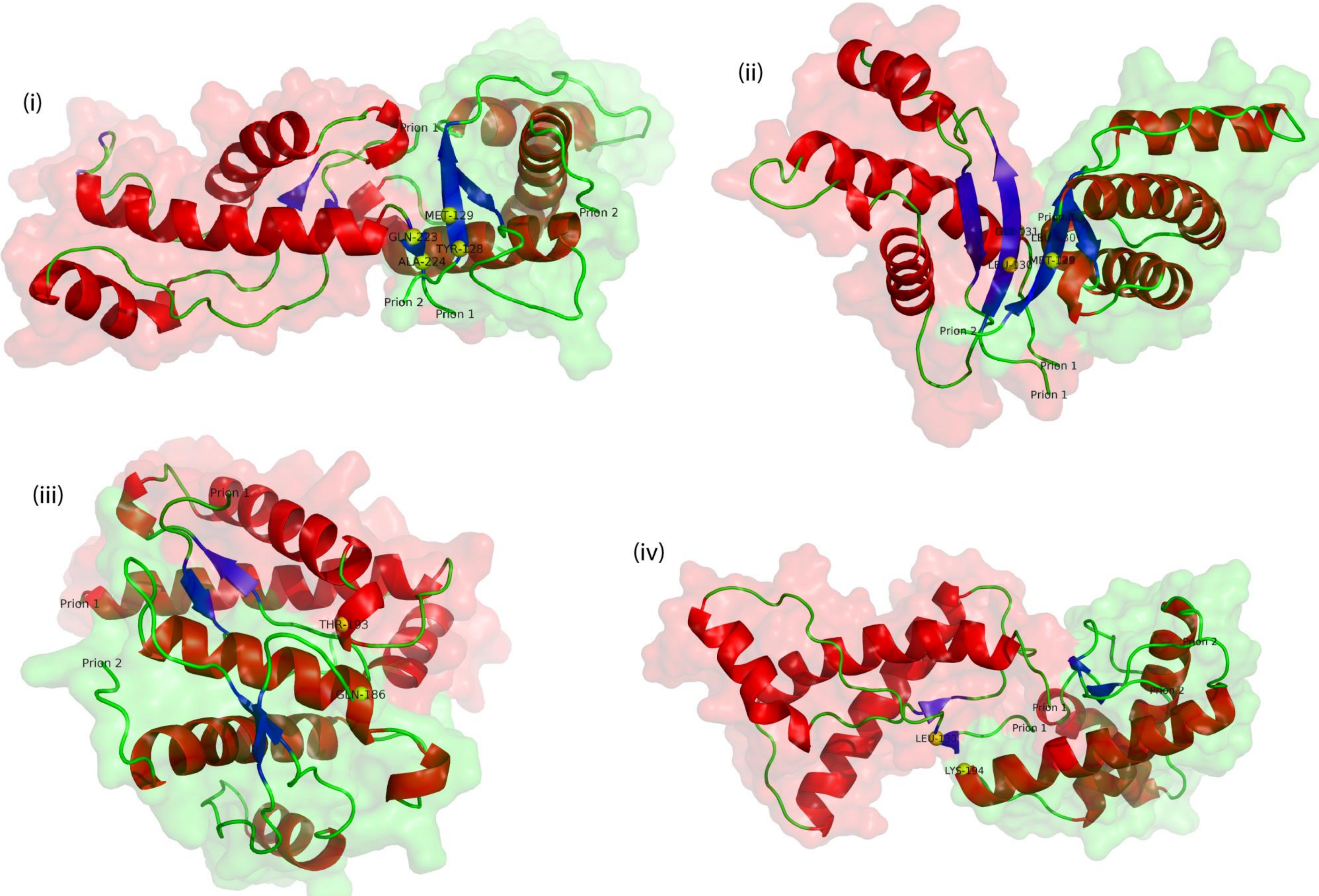

a)
B1
H1
B2
H2
H3
RMSF (nm)
b)
P (Secondary Structure)
α/3-helix
β-sheet
/β-bridge
Residue Number

Tertiary structure seems to stabilize the secondary structure!



Dimerization pathways and effect on conformational stability

c.



(i)
Prion 1
MET-129
GLN-223
TYR-128
ALA-224
Prion 2
Prion 2
Prion 1
(ii)
LEU-130
Prion 2
Prion 1
Prion 1
(iii)
Prion 1
Prion 1
THR-193
Prion 2
GLN-186
(iv)
Prion 2
Prion 2
Prion 1
Prion 1
LYS-194

Summary:

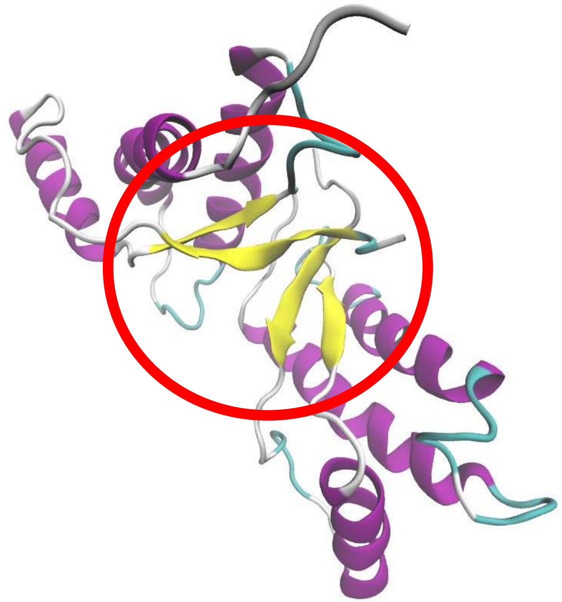
- PrP^{C} form is weakly stable with shallow FES, and multiple low-lying misfolded states

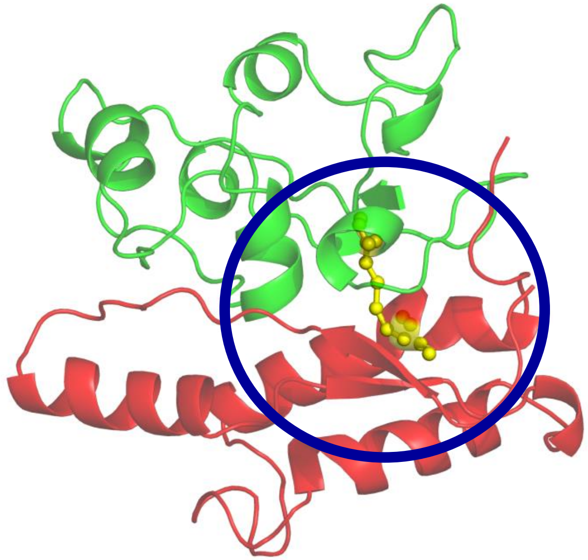
- Misfolded β-sheet rich structures are more hydrophobic and aggregation prone

- H2 and H3 are inherently unstable; Initiation sites of misfolding

- Evidence of cross β-sheet formation in dimers

- Intermolecular disulphide bond is a possible pathway for fibril formation

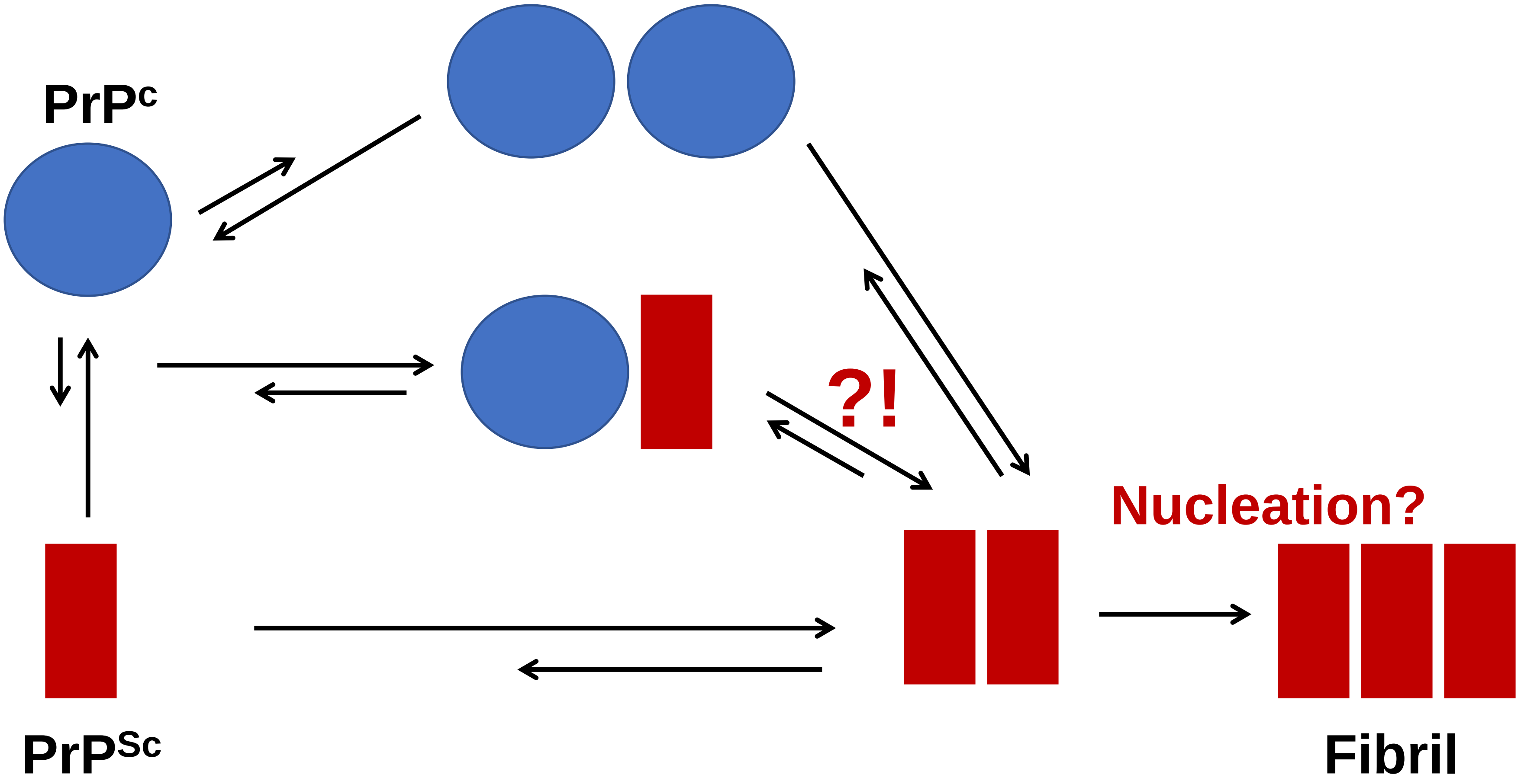




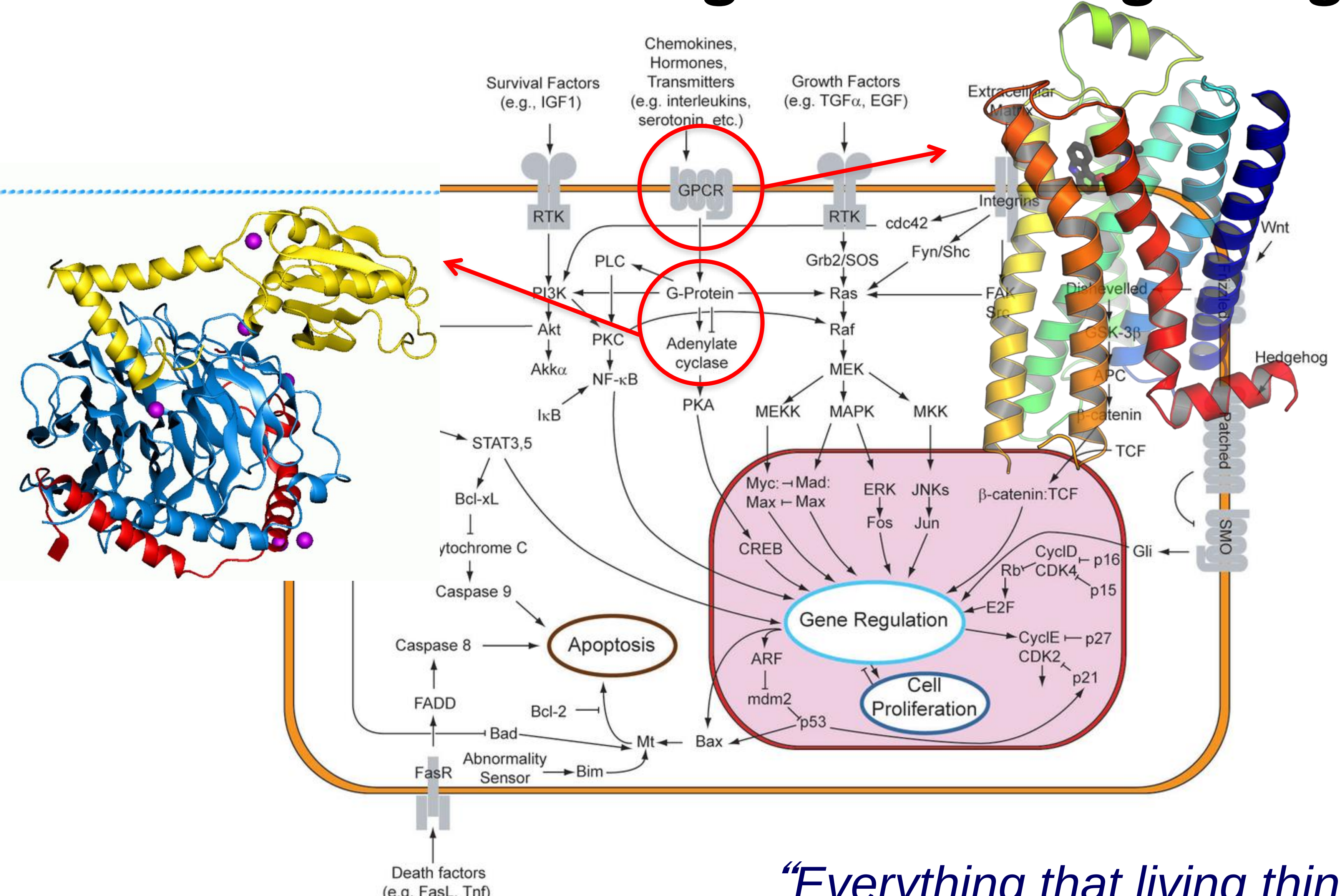
Challenges:

- Anti-parallel β-sheets
- Nucleation pathway towards fibril formation in oligomers
- Mechanism of poisoning

- Thermodynamics and kinetics of various competing pathways of Prion aggregation:



Biomolecular Recognition and Signaling



"Everything that living things do can be understood in terms of the jiggling and wiggling of atoms"

-- Richard Feynman (1963)

➢ Thanks to:

Students:
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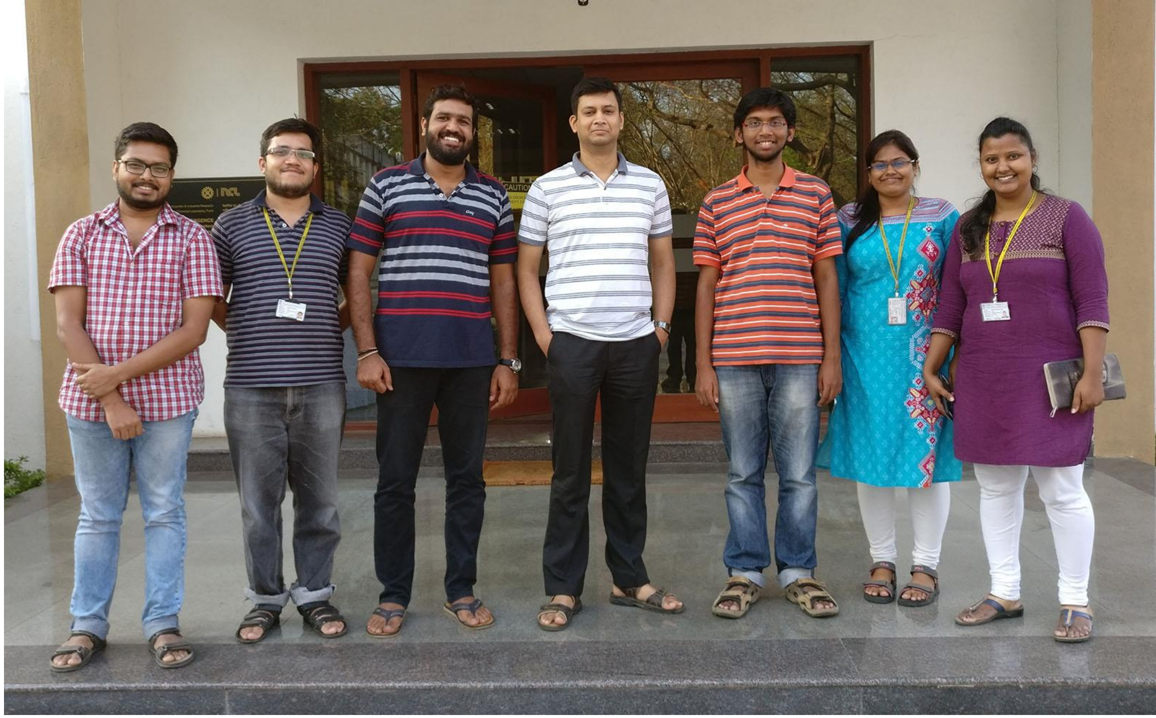

Thank you!

Further questions?
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