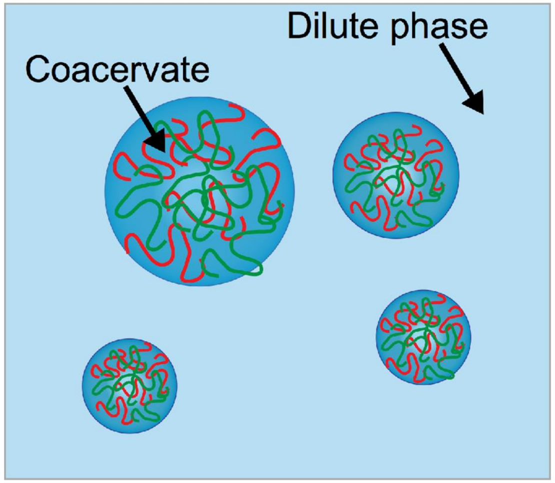
Liquid Liquid Phase Separation and Fibrillization of Intrinsically Disordered Peptides

Joan Shea, Department of Chemistry, UC Santa Barbara

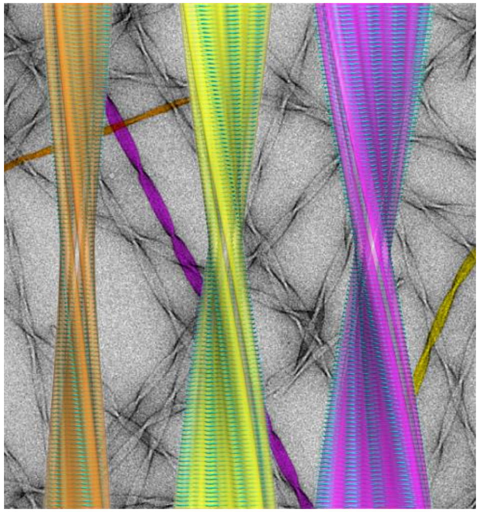
Proteins can assemble in different ways

"Liquid"



Aging

"Solid"

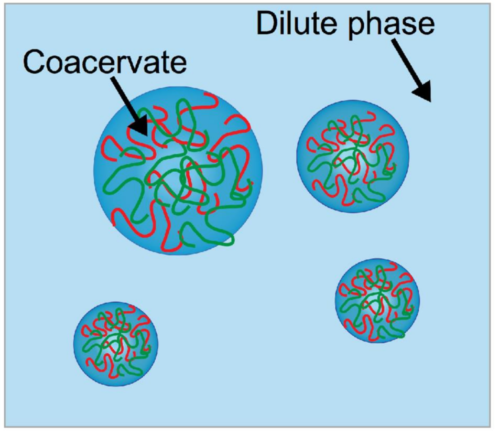


Droplets
Biomolecular condensates
Coacervates

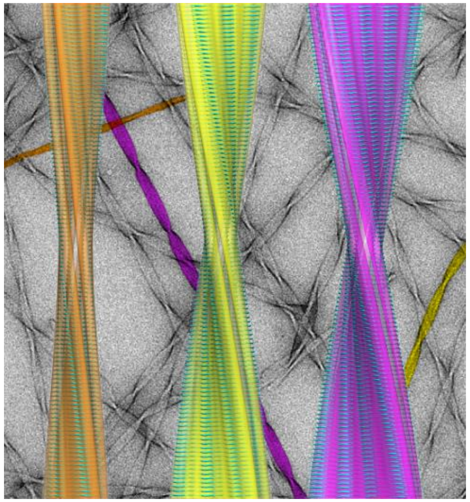
Amyloid Fibrils

Outline

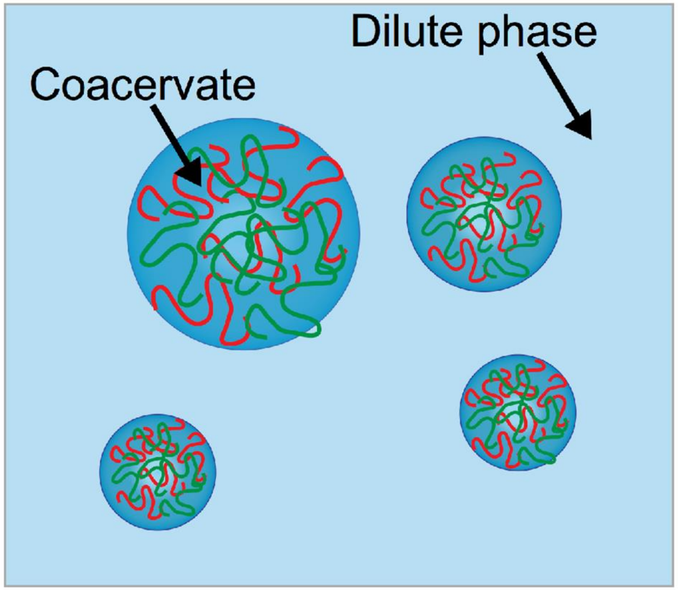
Part 1: Liquid-Liquid Phase Separation: From Model Systems to Tau

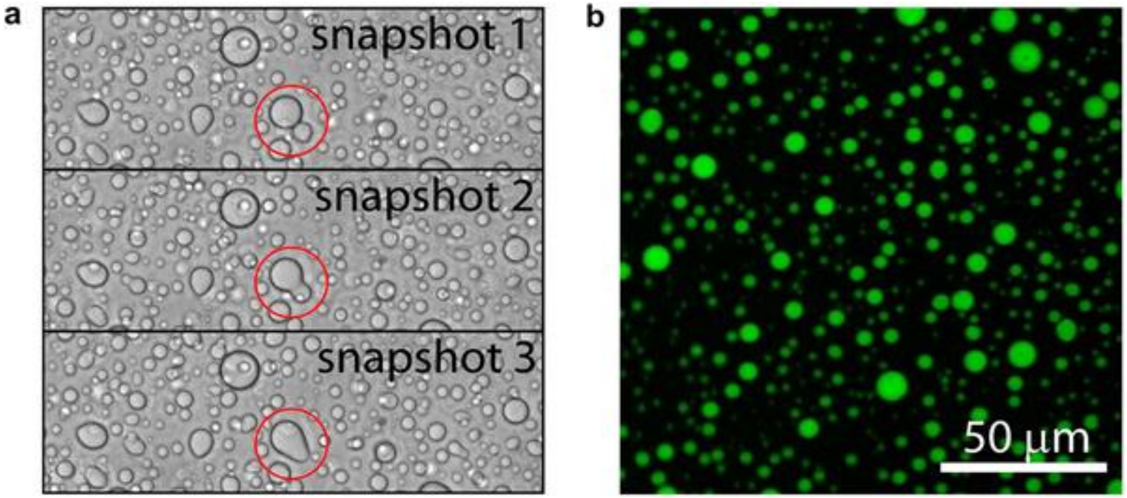


Part 2: Aggregation of the Tau Protein



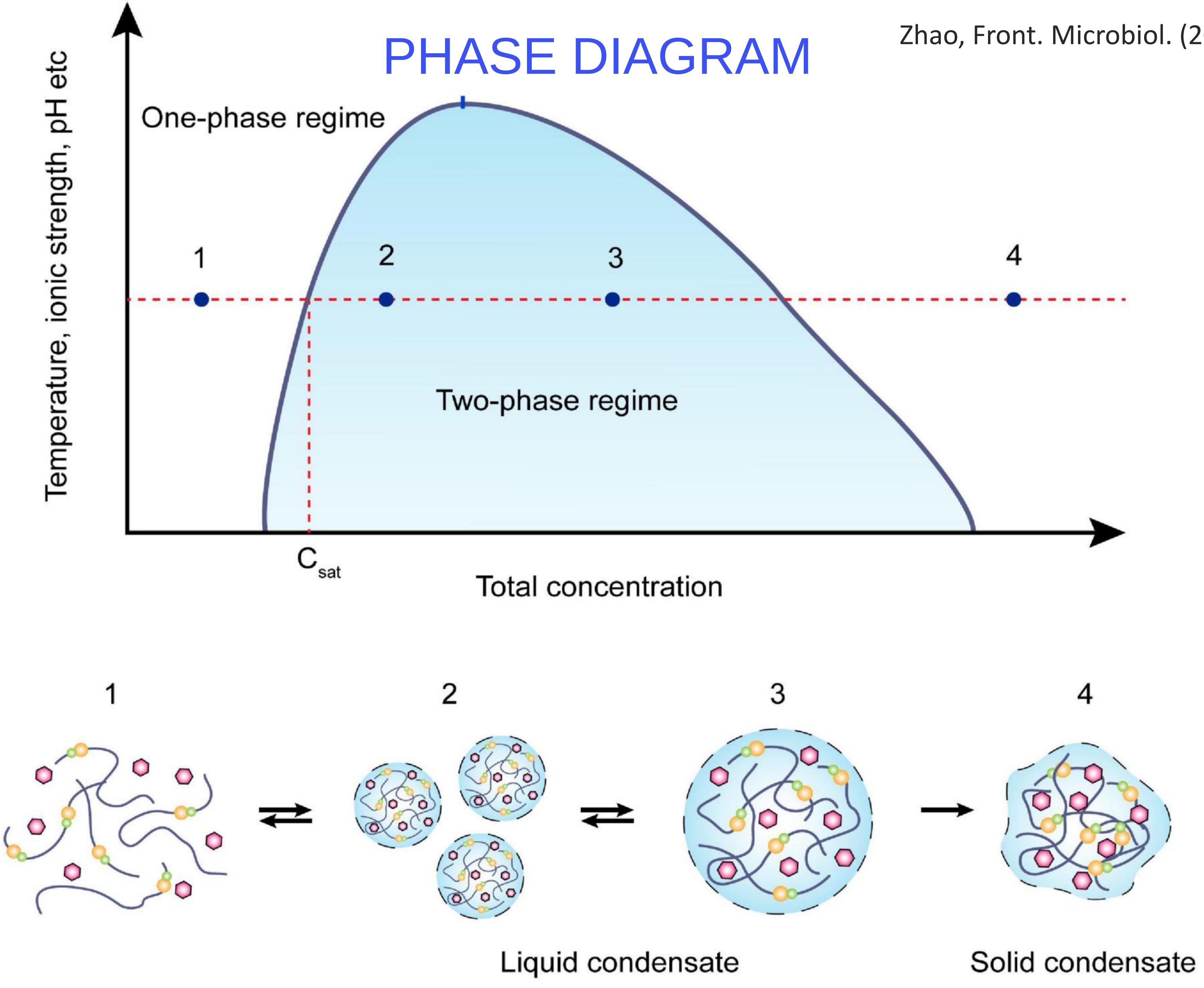
Coacervation = liquid liquid phase separation
= formation of droplets
= formation of biomolecular condensates





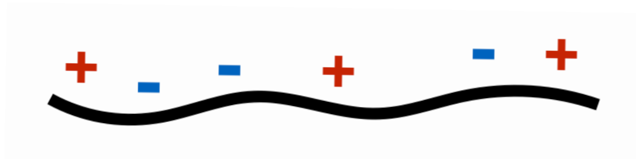
PHASE DIAGRAM

Zhao, Front. Microbiol. (2022)



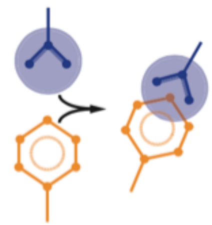
What drives phase separation?

Charge pattern

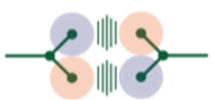


Chain charge density

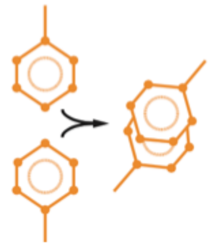
Short-ranged interactions



cation - π



dipole - dipole



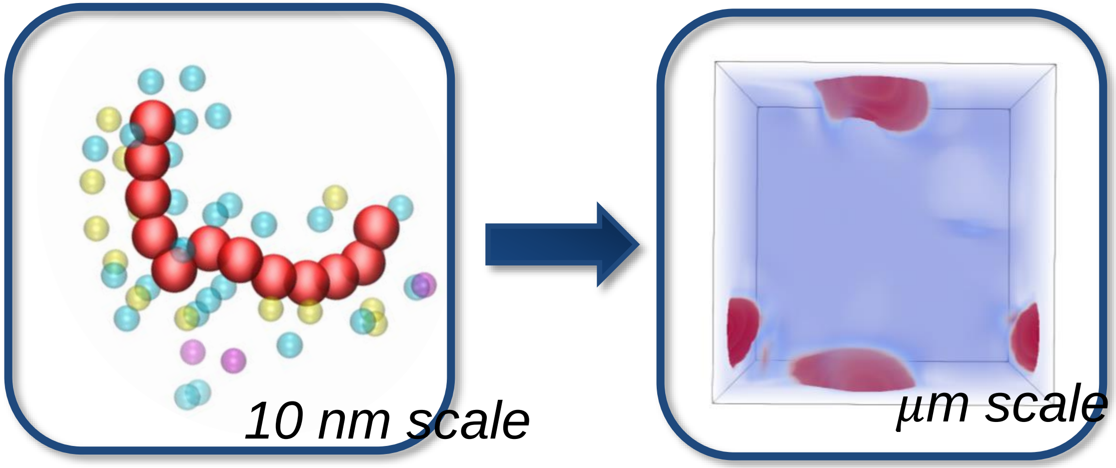
$\pi - \pi$ stacking

Brangwynne, Tompa, Pappu, *Nature Physics*, 11, (2015)

Computational Approaches

Coarse-grained (CG) MD

Field theoretic simulations (FTS)

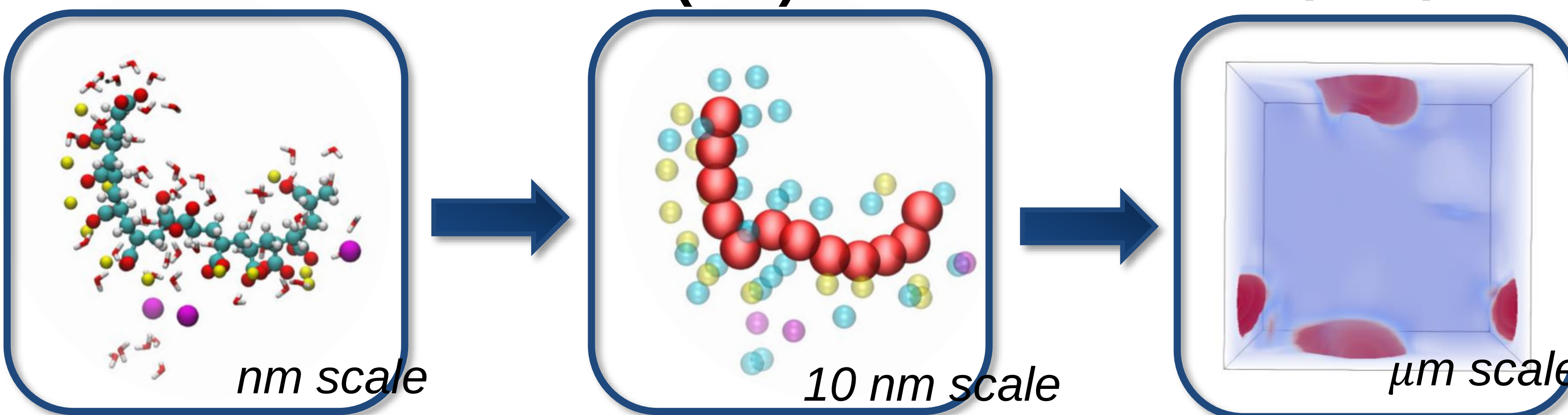


Computational Approach

All-atom (AA) MD

Coarse-grained (CG) MD

Field theoretic simulations (FTS)



chemistry

macroscopic properties

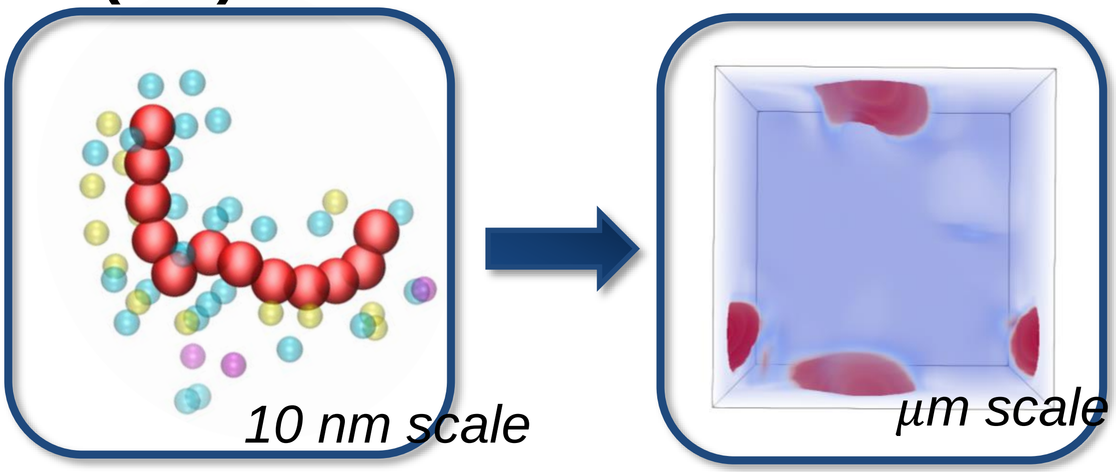
- Clear connection to chemical details
- Short time/length scale
- Dilute conditions

- Can probe long time and length scales
- Efficient for high concentrations

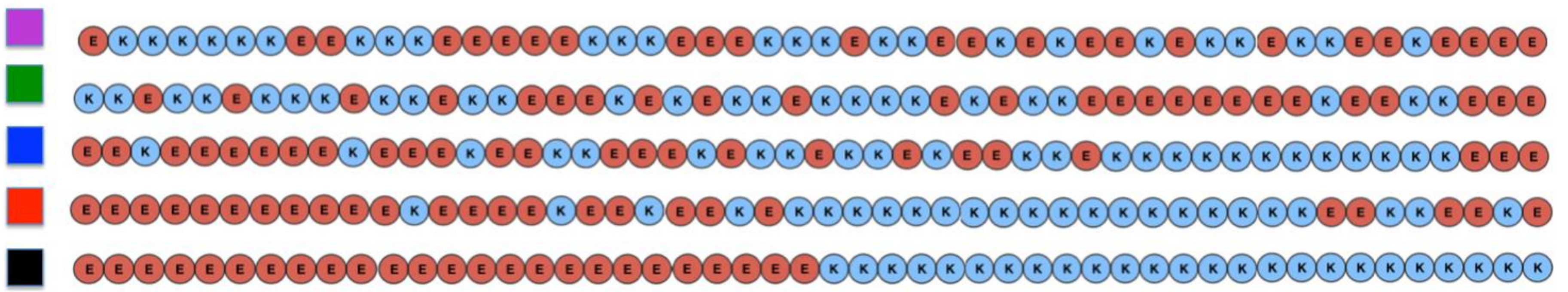
Computational Approaches

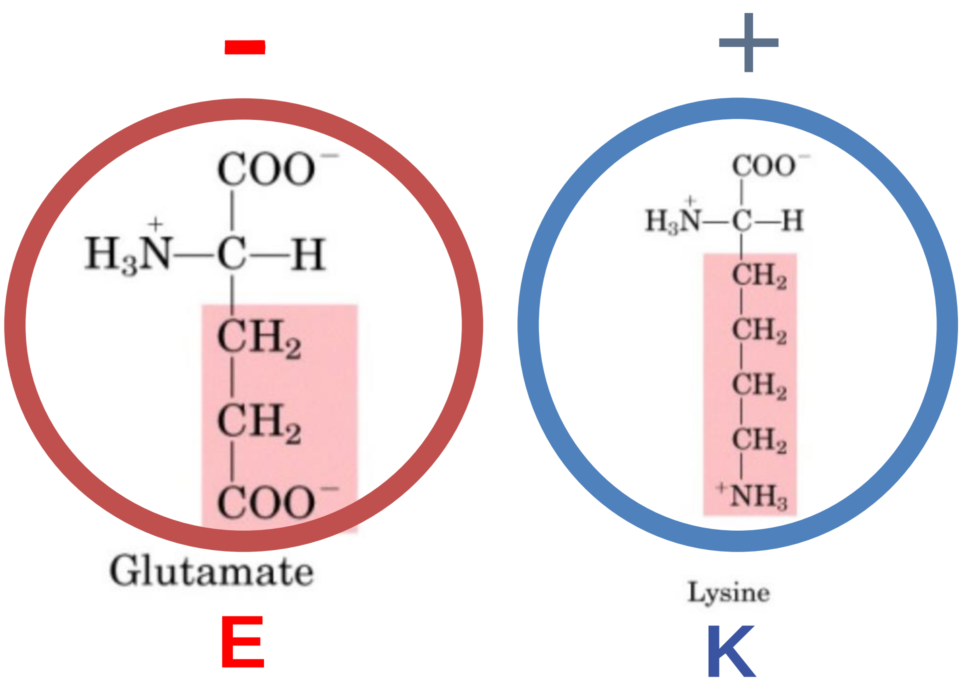
Coarse-grained (CG) MD

Field theoretic simulations (FTS)



Model System: EK sequence

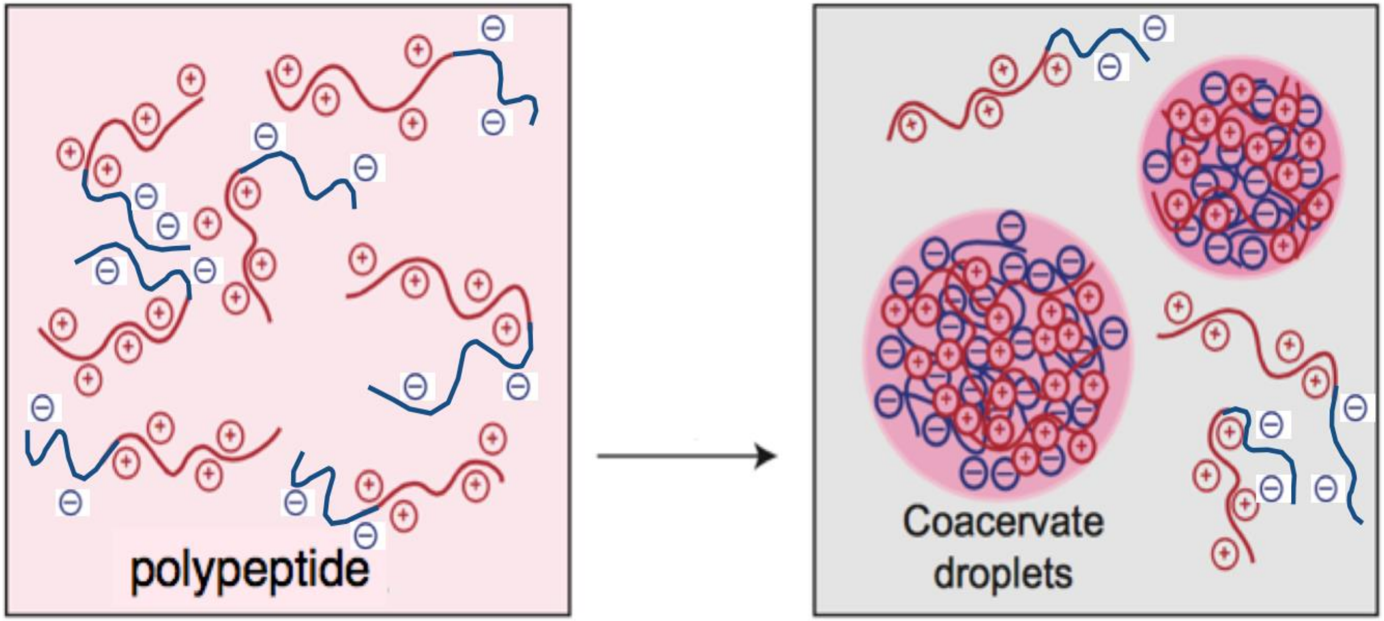


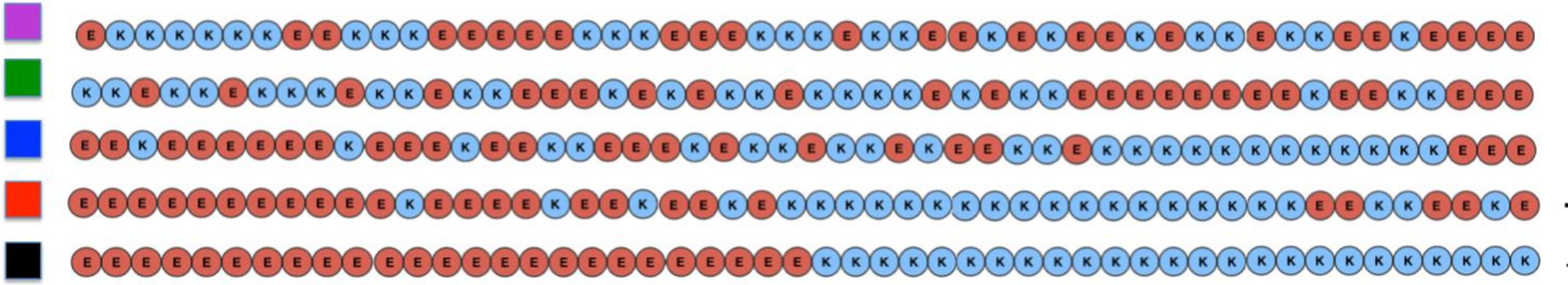


net charge = 0

(equal fractions of + and -)

Simple Coacervation

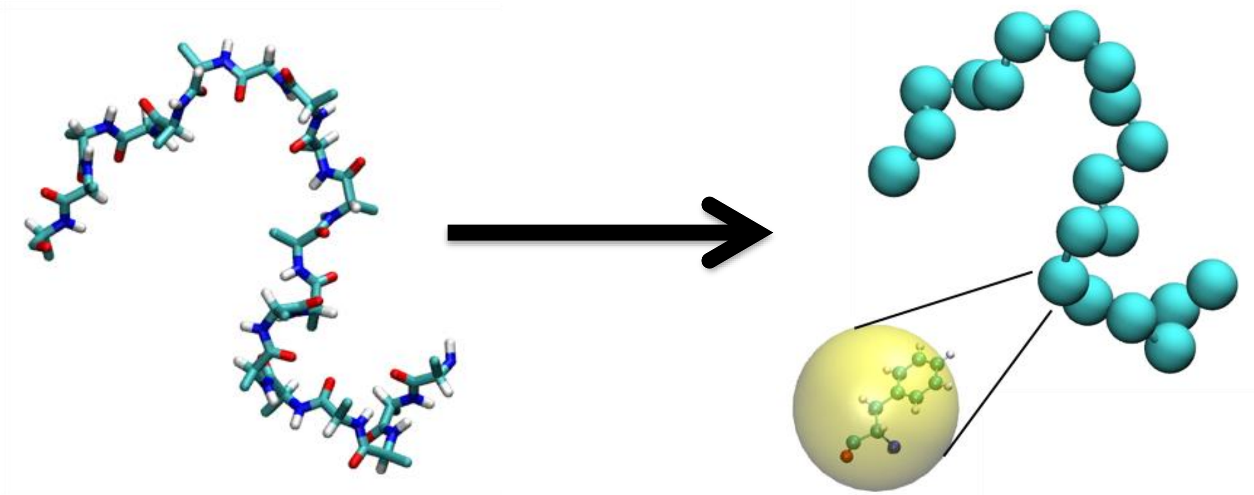




Coarse-Grained Model

Coarse-grained peptide chain

Each amino acid represented by a single site

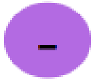


polypeptide

$$\sigma_i = \begin{cases} +1 & \textbf{Lys} \\ -1 & \textbf{Glu} \\ 0 & \text{X} \end{cases}$$

Implicit solvent

Explicit salt ions

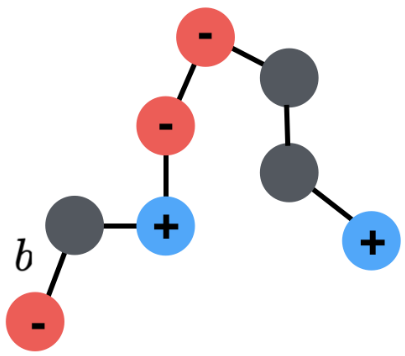


$$\sigma_{s-} = -1$$

$$\sigma_{s+} = +1$$

counter ions

Discrete Gaussian chain polyelectrolyte model



N_l sites per molecule

σ_i charge on bead i

b segment length

charge sequence

$$\{\sigma_i\} = \{\sigma_1, \sigma_2, \ldots, \sigma_N\}$$

charge density

$$\frac{1}{N}\sum_i |\sigma_i|$$

Formally equivalent representations

Particle MD simulation

Field Theoretic simulation

low polymer density

high polymer density

$$H[w,\psi] = \frac{1}{2B}\int d\mathbf{r}\; w(\mathbf{r})^2 + \frac{1}{2E}\int d\mathbf{r}\; |\nabla\psi|^2 - \sum_l \frac{CV\bar{\phi}_l}{N_l}\ln Q_l$$

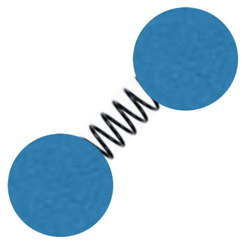
Fluctuating chemical potential field

Fluctuating electrostatic potential field

Single chain partition function for polymers and salt ions

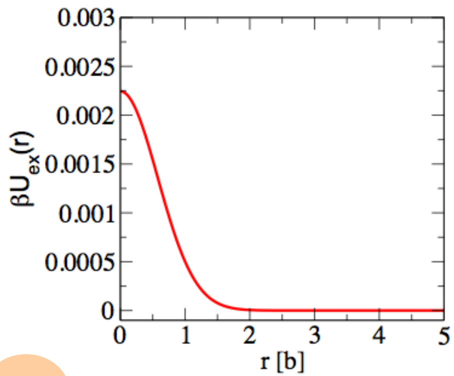
<u>bond potential</u>

$$\beta U_b = \frac{3}{2b^2} r^2$$



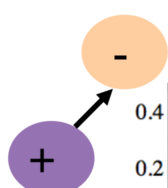
<u>excluded volume parameter</u>

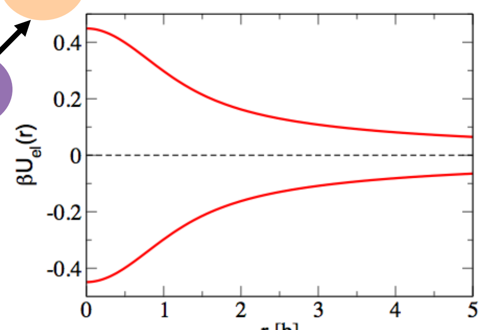
$$\beta U_{ev}(r) = \frac{v}{8\pi^{3/2}a^3} e^{-r^2/4a^2}$$

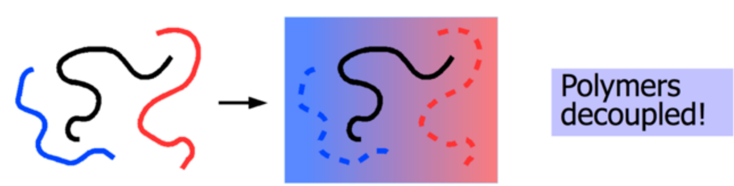


<u>electrostatic potential</u>

$$\beta U_{es} = \frac{l_B z_i z_j}{r}\,\mathrm{erf}\left(\frac{r}{2a}\right)$$





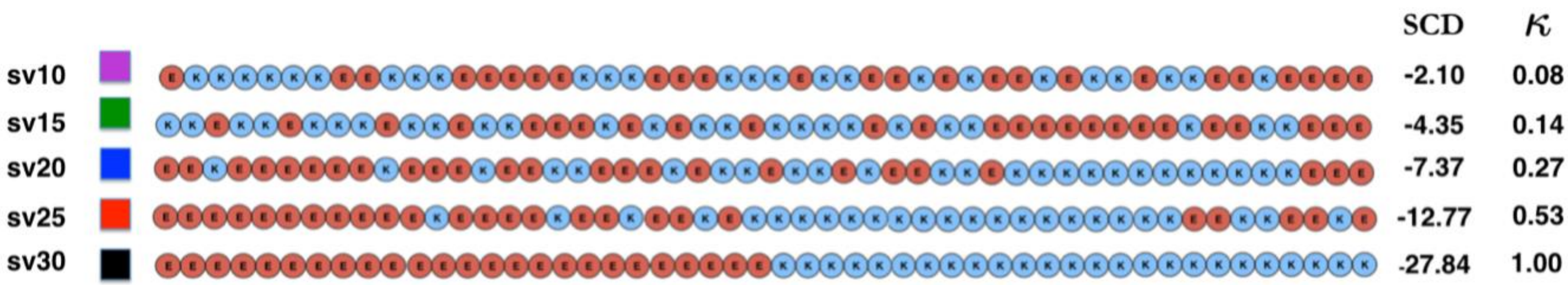


Formally equivalent representations

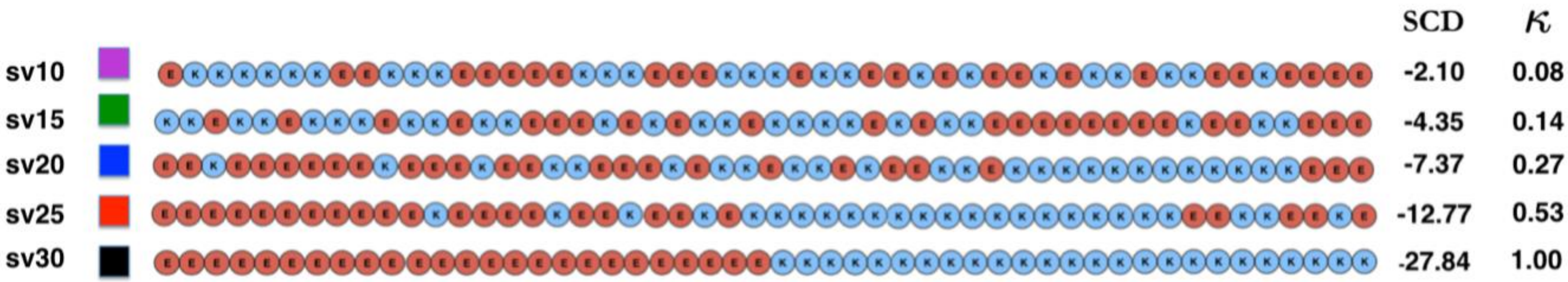
Particle MD simulation ⟷ **Field Theoretic simulation**

low polymer density

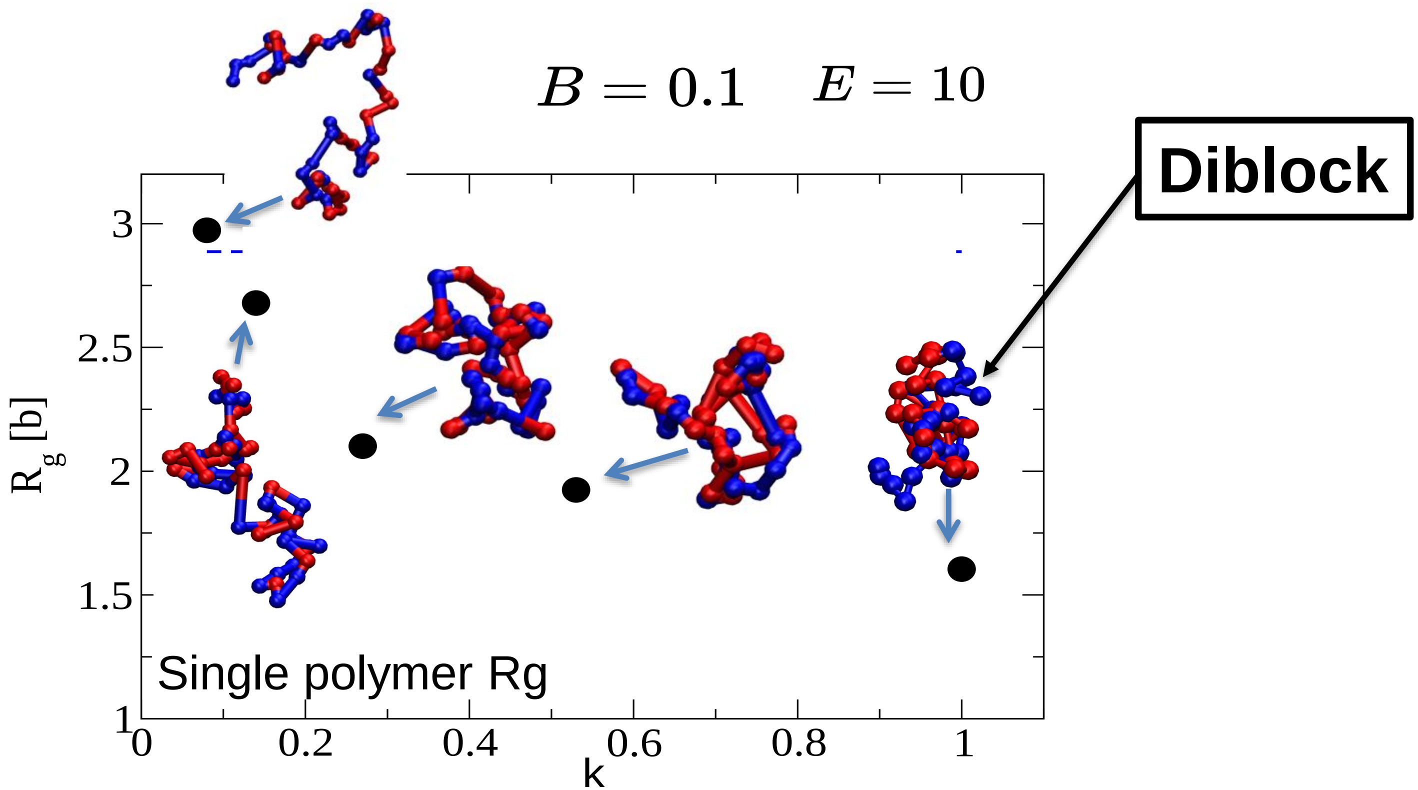
high polymer density



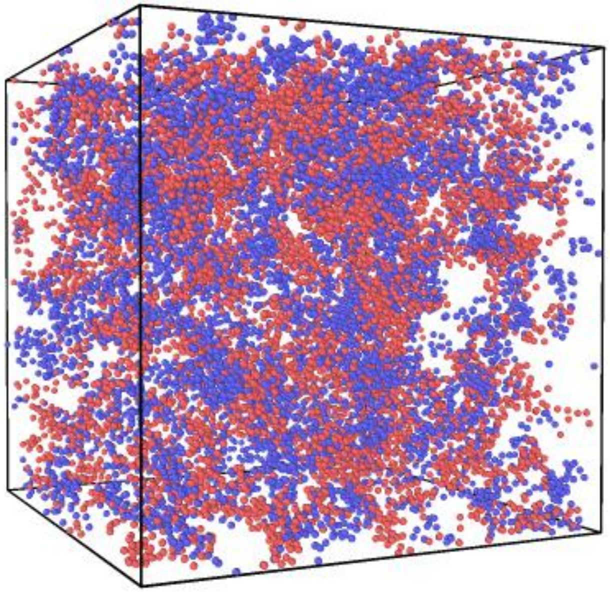
Particle Based Simulations



James Mc Carthy

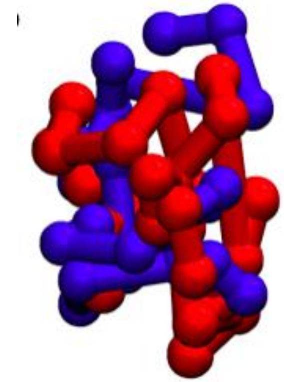


Particle Based Simulations

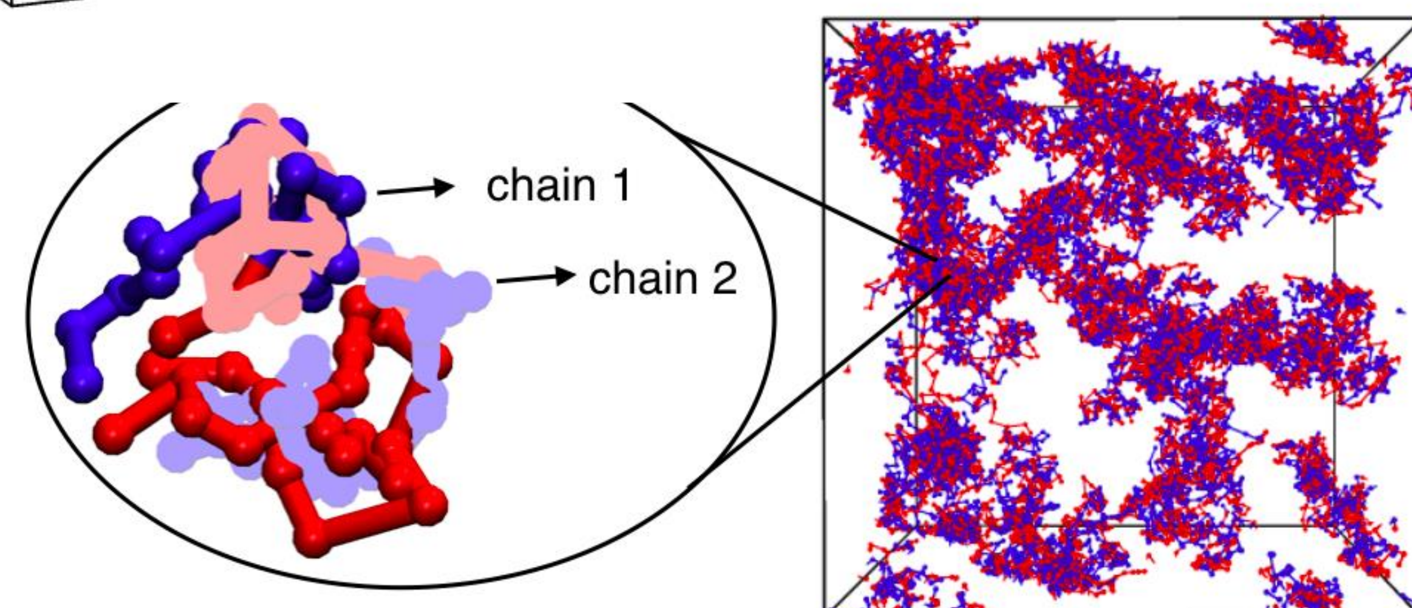


$n = 300$

$N = 50$



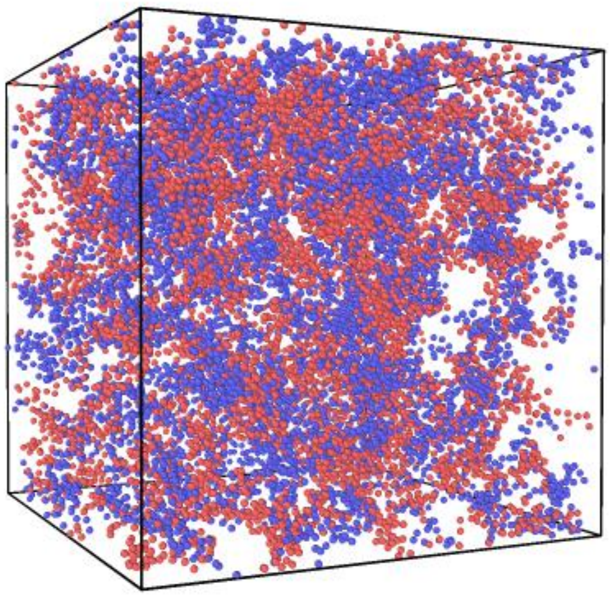
Single Chain

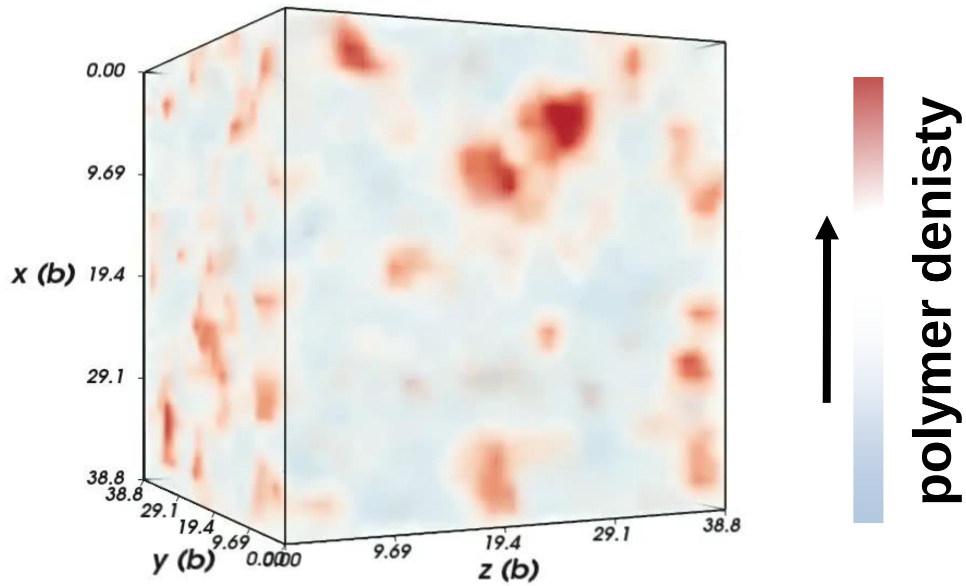


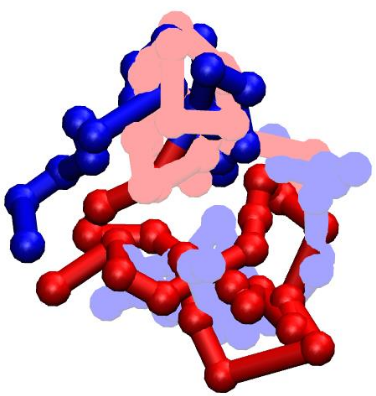
Particle Based versus Field Theoretic Simulations

$n = 300 \qquad N = 50$

$C = 0.015 \qquad B = 0.1$



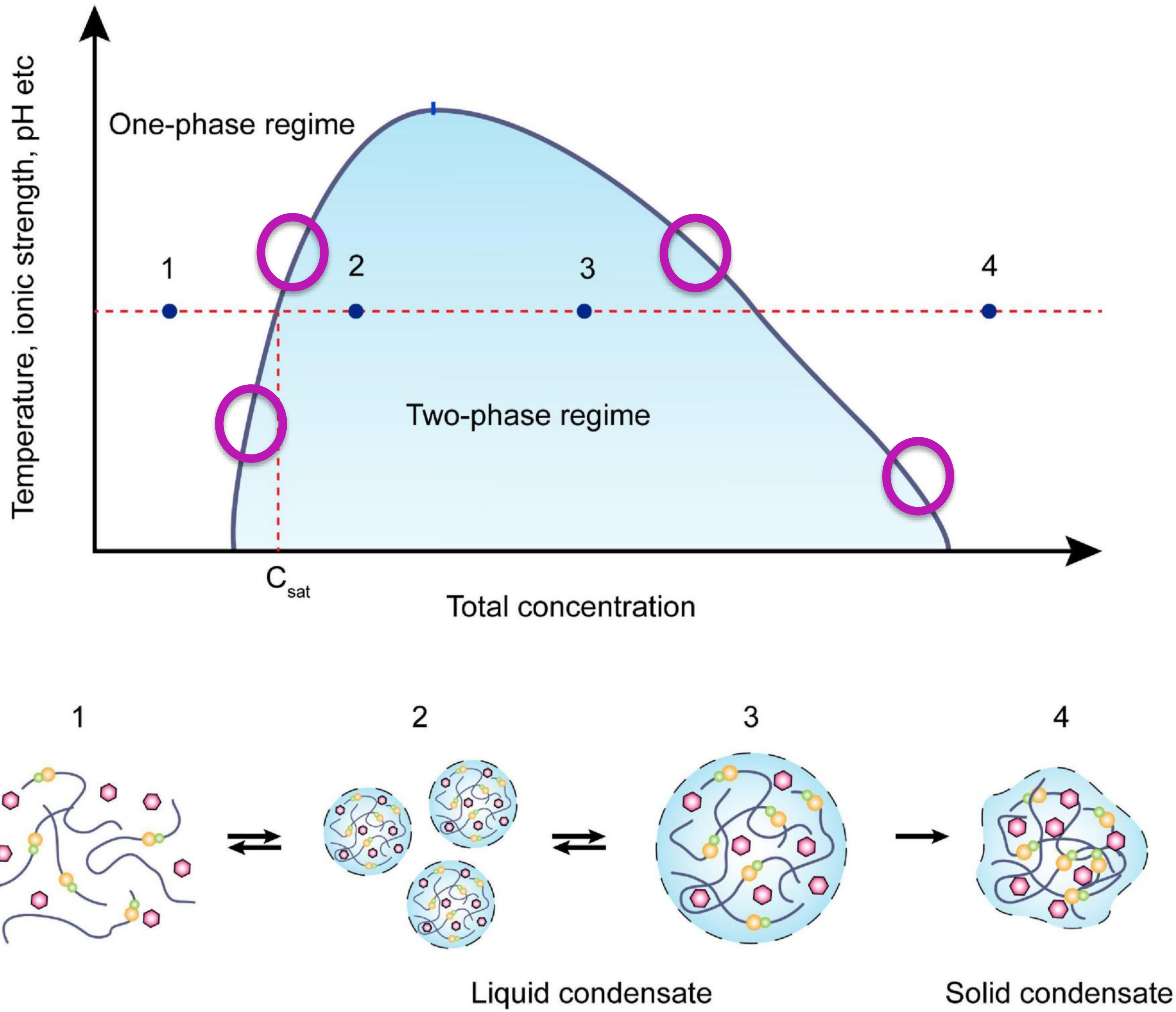




Get a Phase Diagram

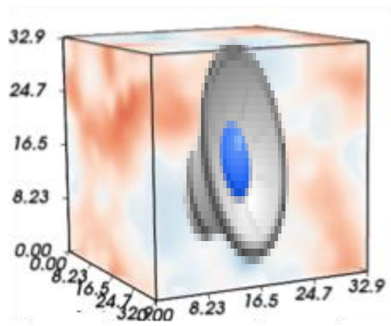
J. Phys. Chem. Lett., 2019, 10 (8), pp 1894–1899

We map the boundaries of the phase diagram



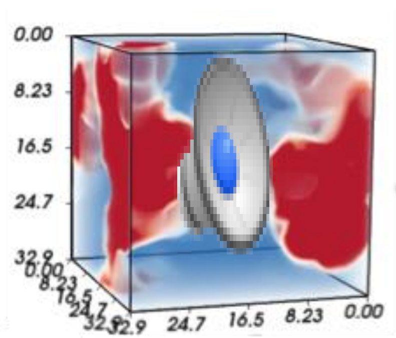
“Blocky” sequences form coacervates more readily

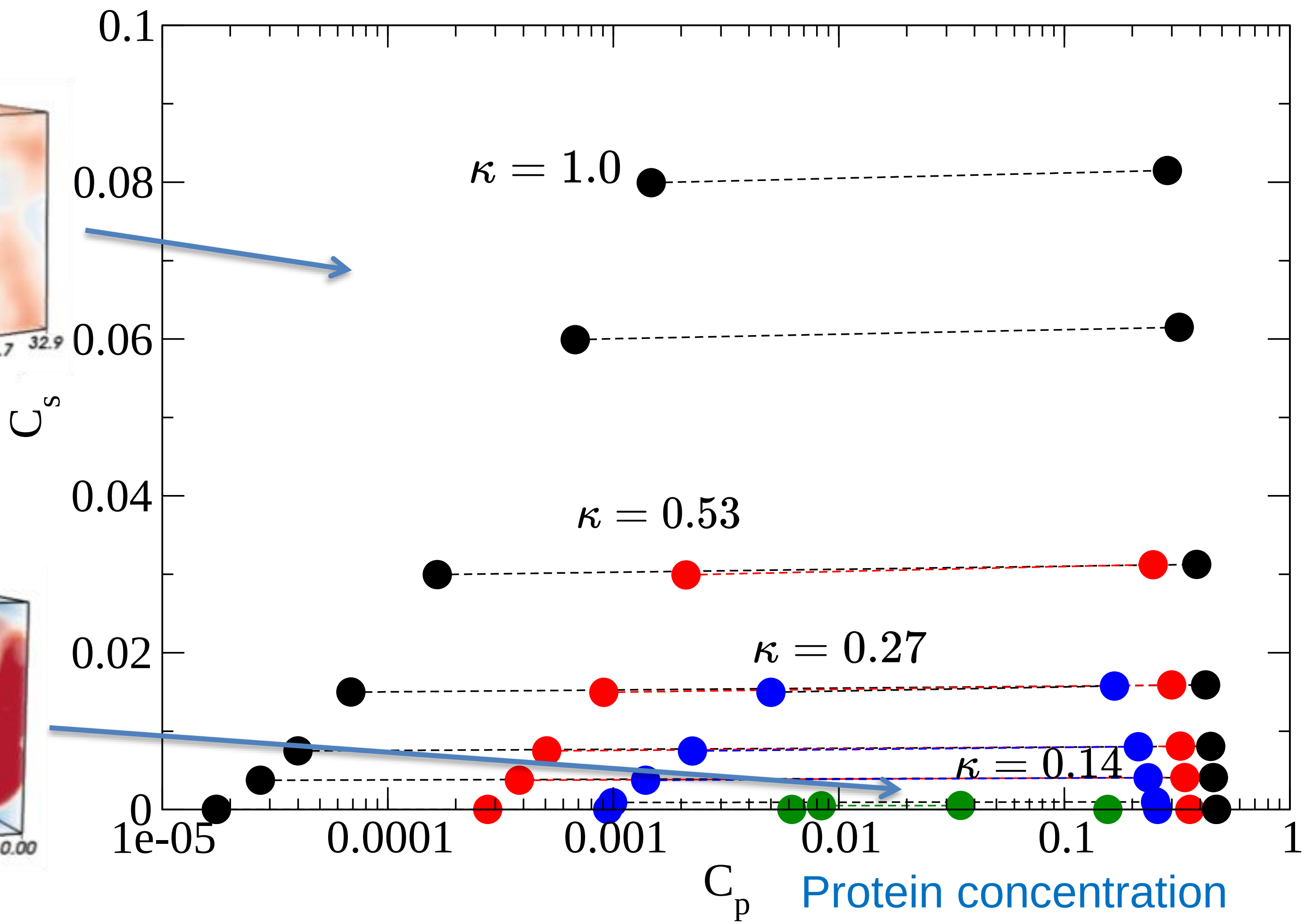
one phase



Salt concentration

2 phases





Protein concentration

sv15 KKEKKEKKKEKKEKKEEEKEKEKKEKKKKEKEKKEEEEEEEEKEEKKEEE

sv20 EEKEEEEEEKEEEKEEKKEEEKEKKEKKEKEEKKEKKKKKKKKKKKKEEE

sv25 EEEEEEEEEEEKEEEEKEEKEEKEKKKKKKKKKKKKKKKKKKEEKKEEKE

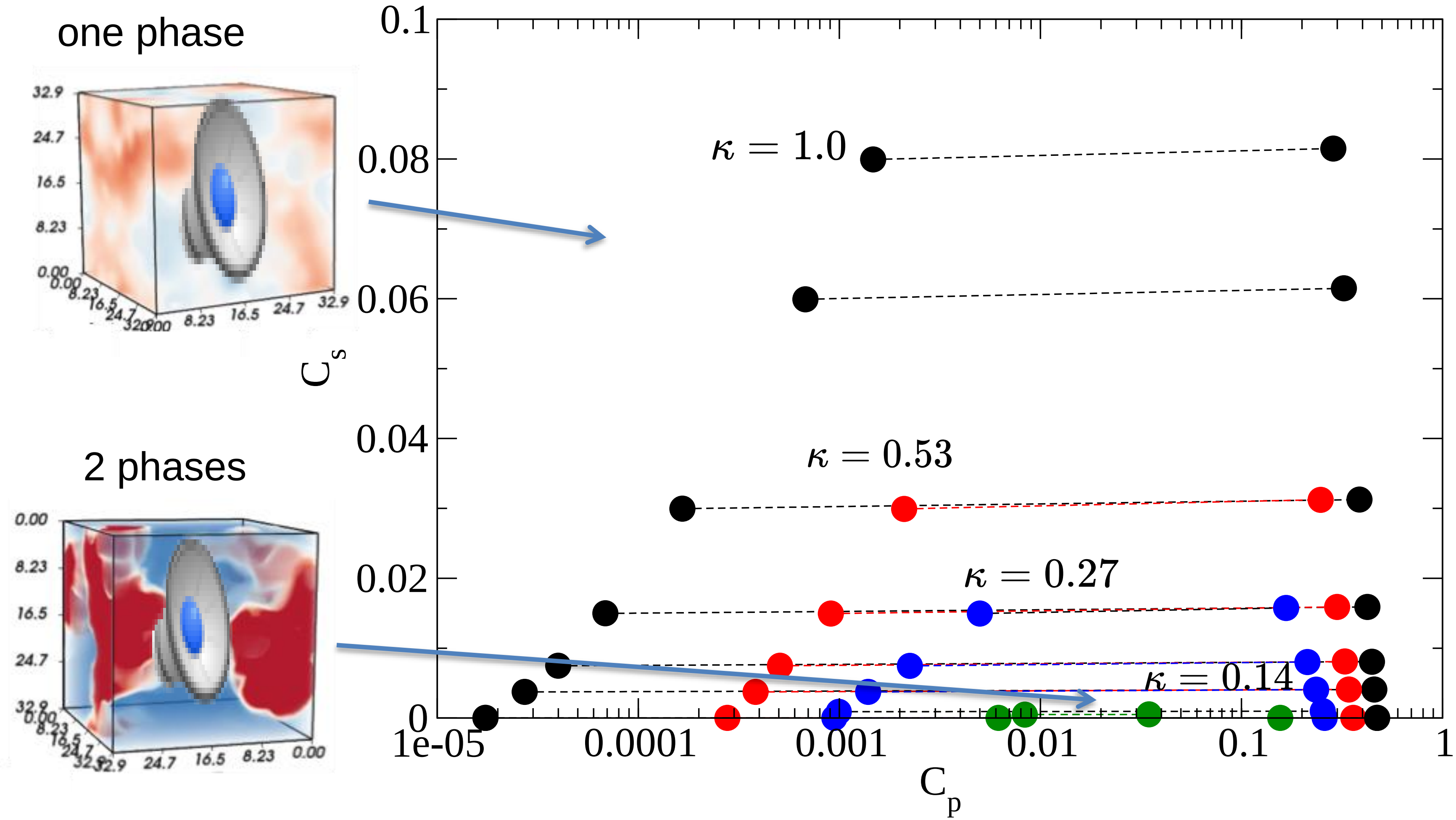
sv30 EEEEEEEEEEEEEEEEEEEEEEEEEKKKKKKKKKKKKKKKKKKKKKKKKK

fixed

$B = 0.1$

$E = 10$

Increasing the salt concentration favors a single phase



sv15 KKEKKEKKKEKKEKKEEEKEKEKKEKKKKEKEKKEEEEEEEEEKEEKKEEE

sv20 EEKEEEEEEEKEEEKEEKKEEEKEKKEKKEKEEKKEKKKKKKKKKKKKKEEE

sv25 EEEEEEEEEEEKEEEEKEEKEEKEKKKKKKKKKKKKKKKKKKEEKKEEKE

sv30 EEEEEEEEEEEEEEEEEEEEEEEEEKKKKKKKKKKKKKKKKKKKKKKKKK

fixed

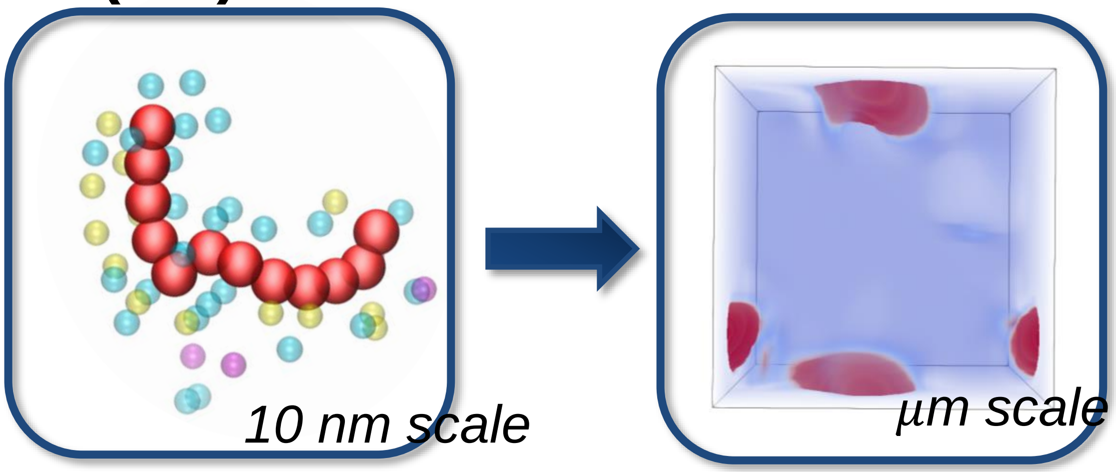
$B = 0.1$

$E = 10$

Computational Approach

Coarse-grained (CG) MD

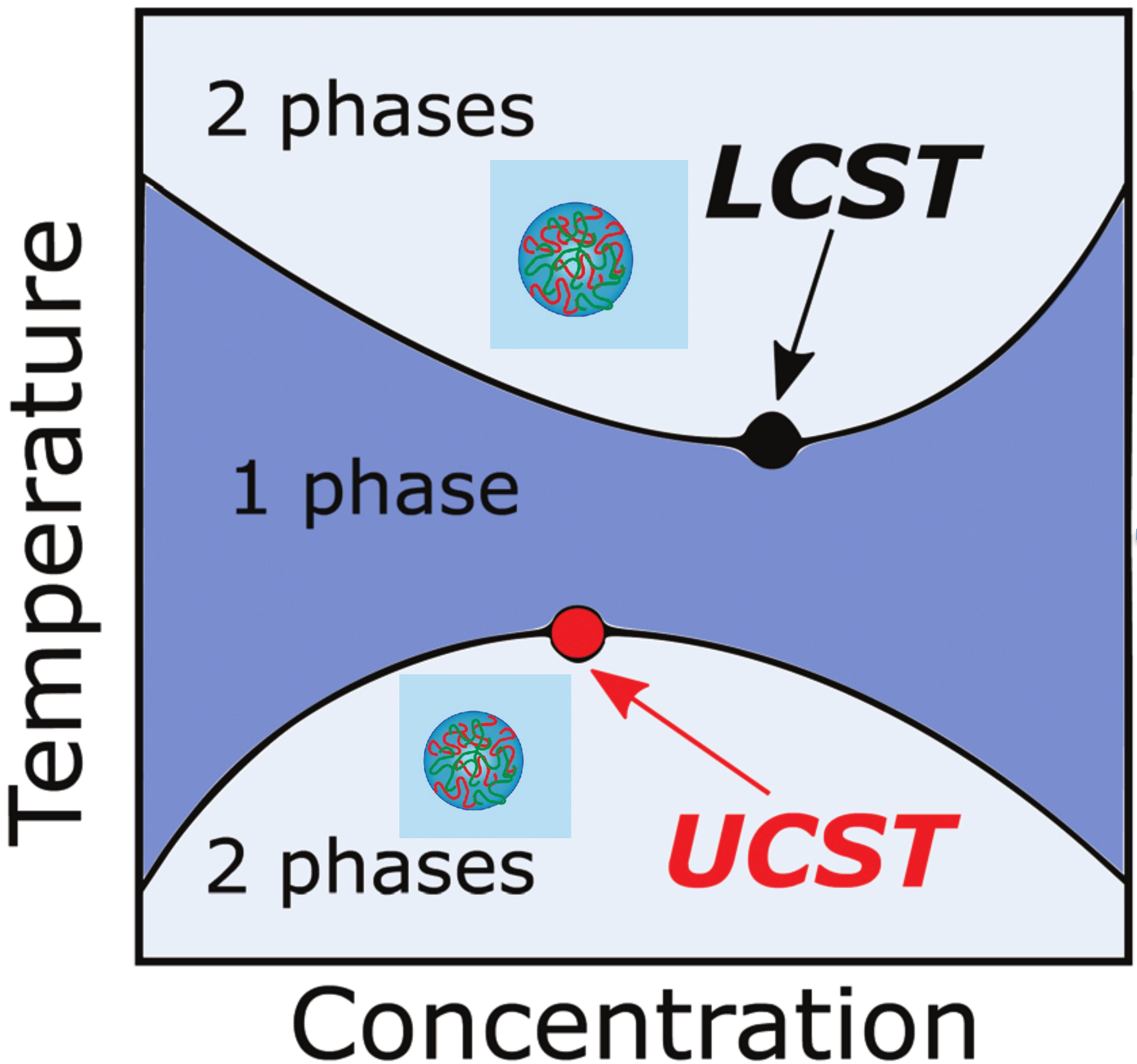
Field theoretic simulations (FTS)



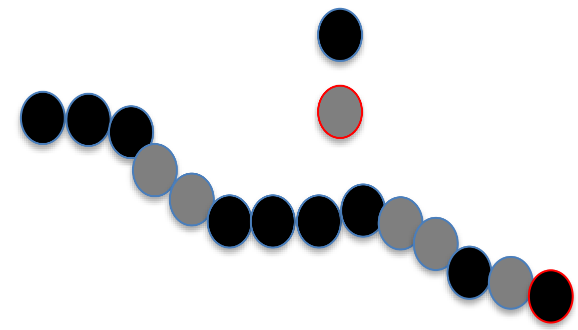
Limitations

- No amino acid specificity
- Implicit solvent

Upper (UCST) and Lower (LCST) critical solution temperature

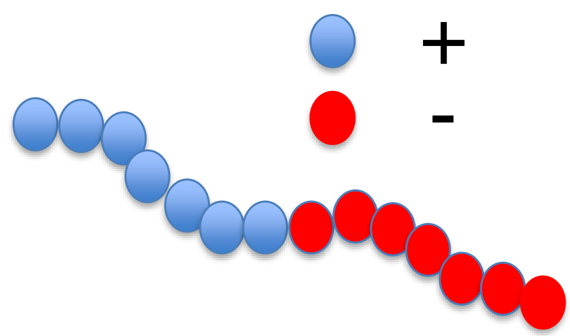


Hydrophobic amino acids



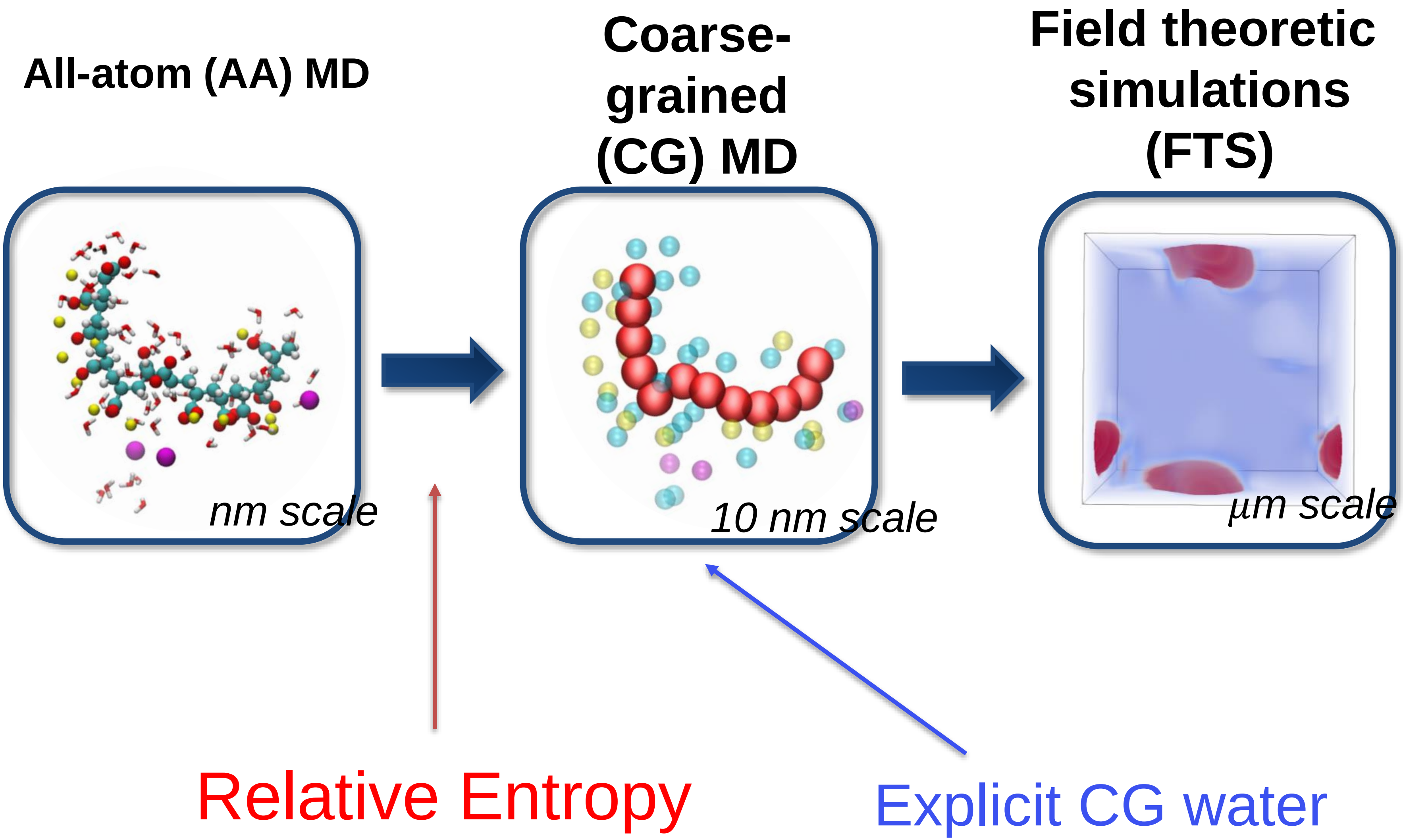
Release of water

Charged amino acids

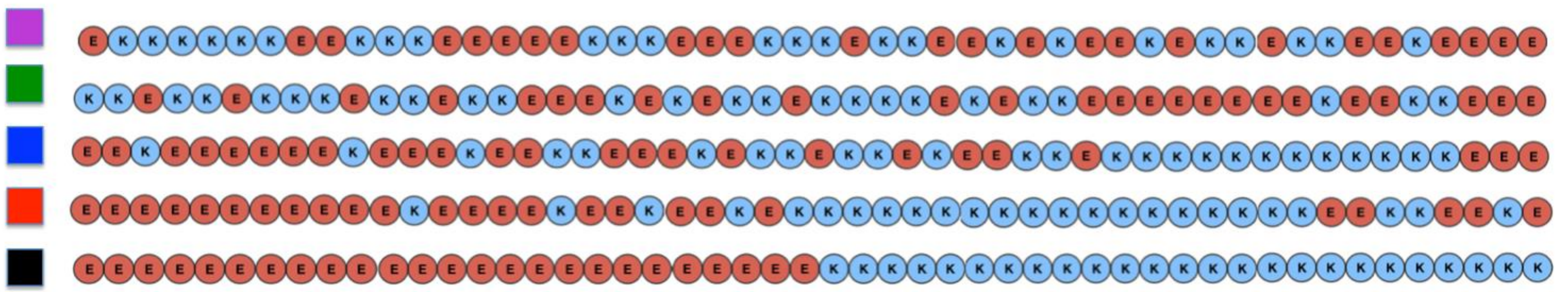


Mittag, Biochemistry. 2018; 57(17): 2478–2487.

Computational Approach



OLD: Model System: KE (Lys/Glu)sequence



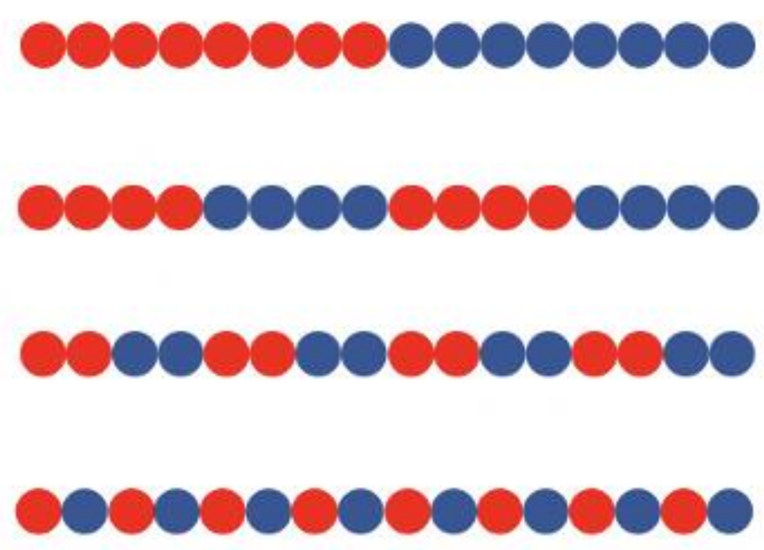
NEW: Model System: RE(Arg/Glu)sequence

RE1: Poly-Arg/Glu

RE2: Poly-Arg/Glu

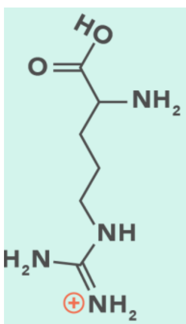
RE3: Poly-Arg/Glu

RE4: Poly-Arg/Glu

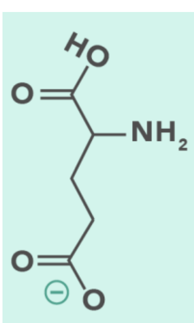


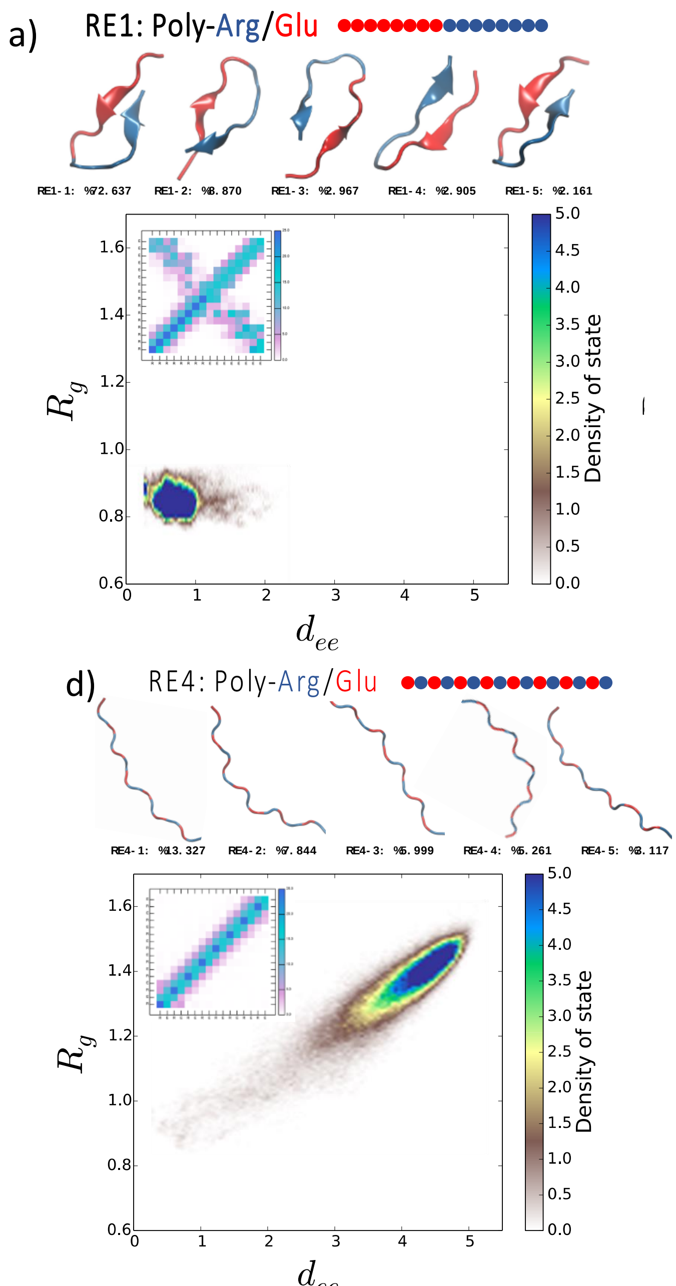
All atom replica exchange MD reference simulations

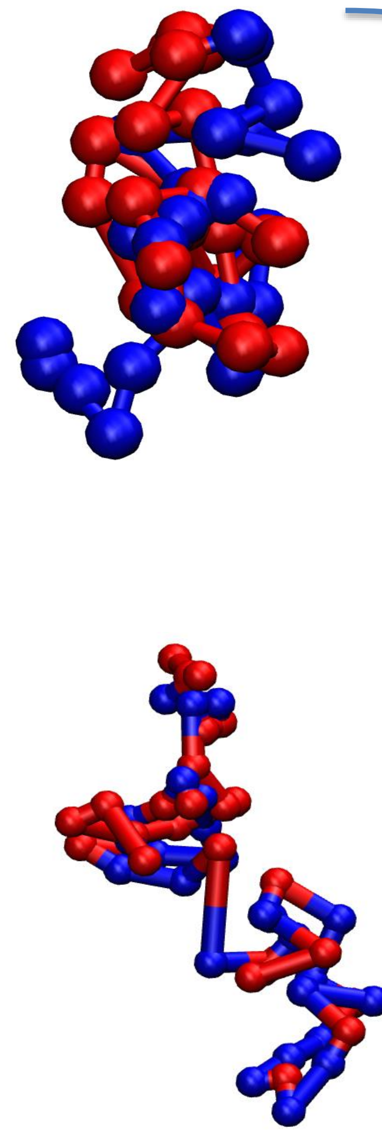
Arg



Glu







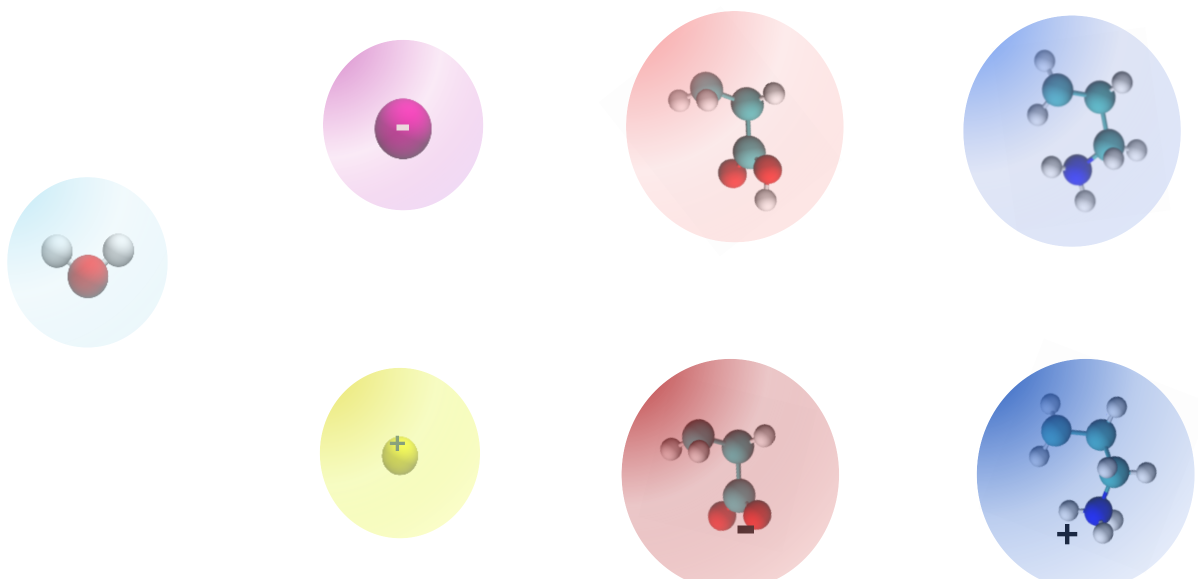
Original CG

Relative Entropy Coarse-graining

Minimizing the relative entropy:

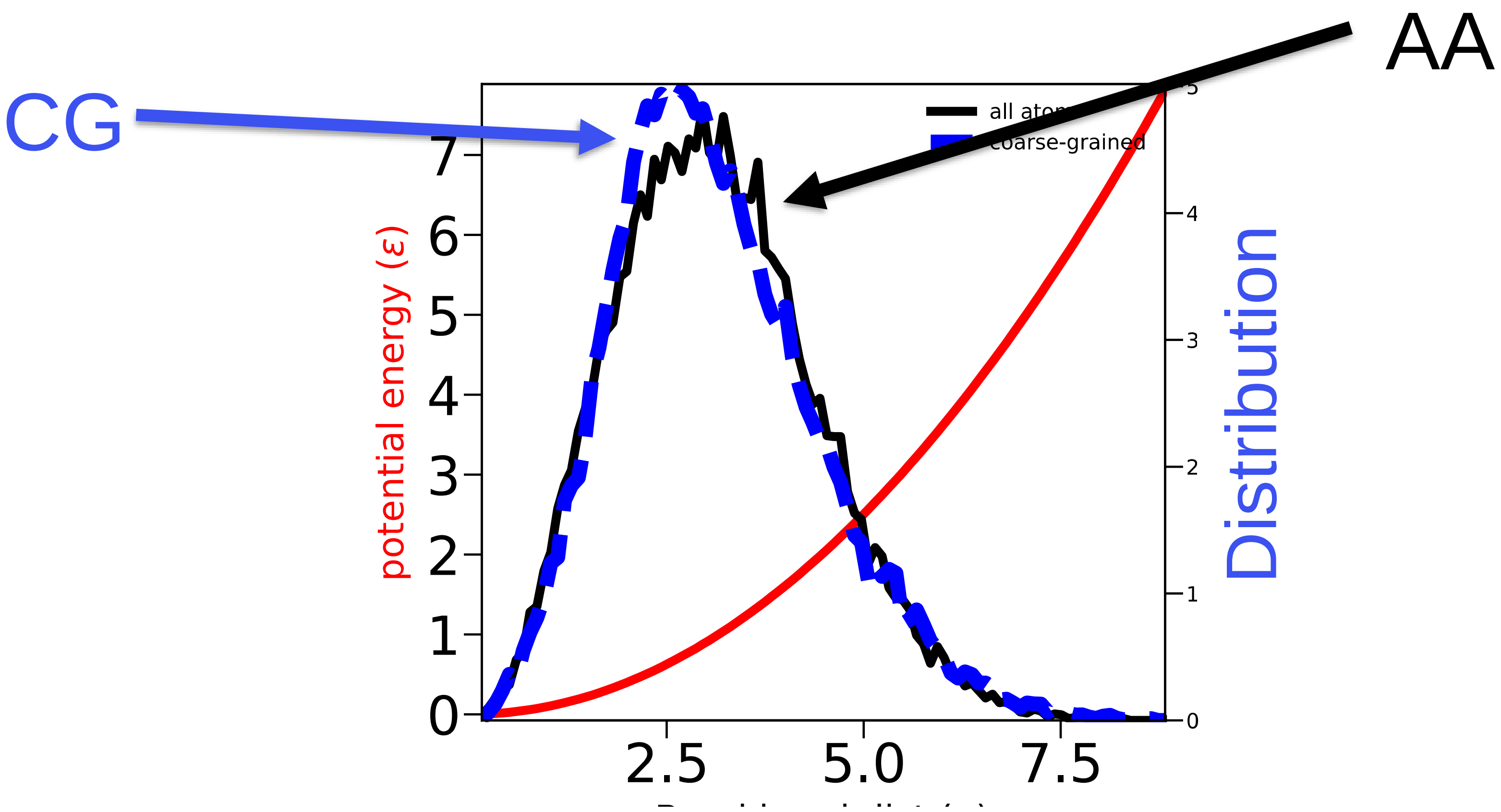
$$S_{rel} = \int\int \wp_{AA}(\boldsymbol{r}) \; \ln\left(\frac{\wp_{AA}(\boldsymbol{r})}{\wp_{CG}(\boldsymbol{R})}\right) \delta(\boldsymbol{M}(\boldsymbol{r}) - \boldsymbol{R}) d\boldsymbol{r} \, d\boldsymbol{R}$$

Scott Shell



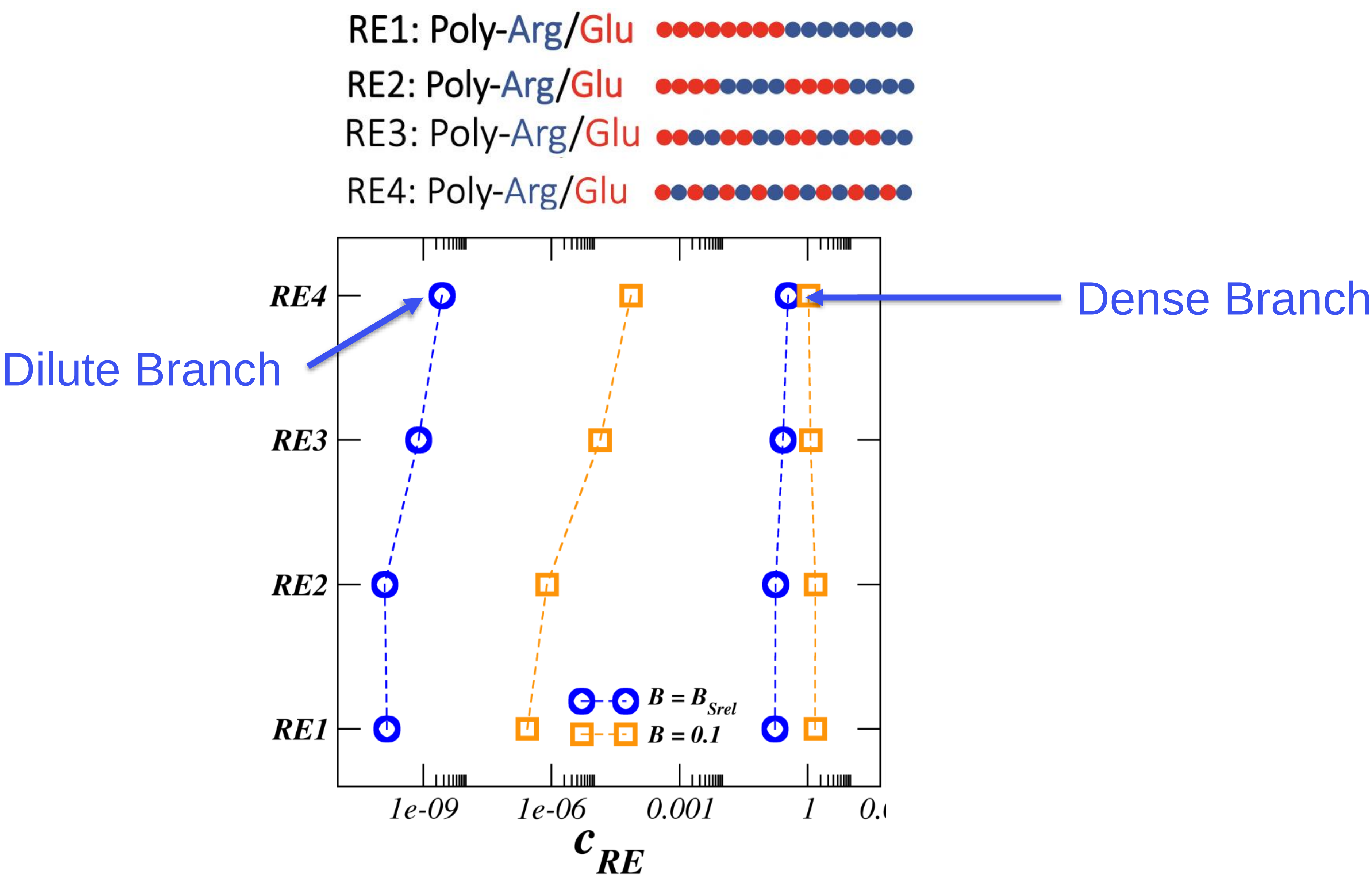
Water ions Amino acids

Relative Entropy Parameterization

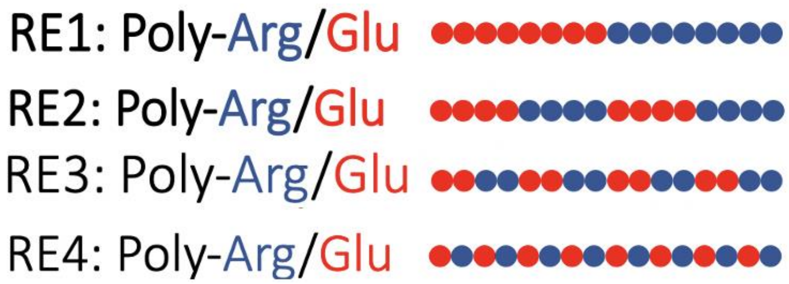


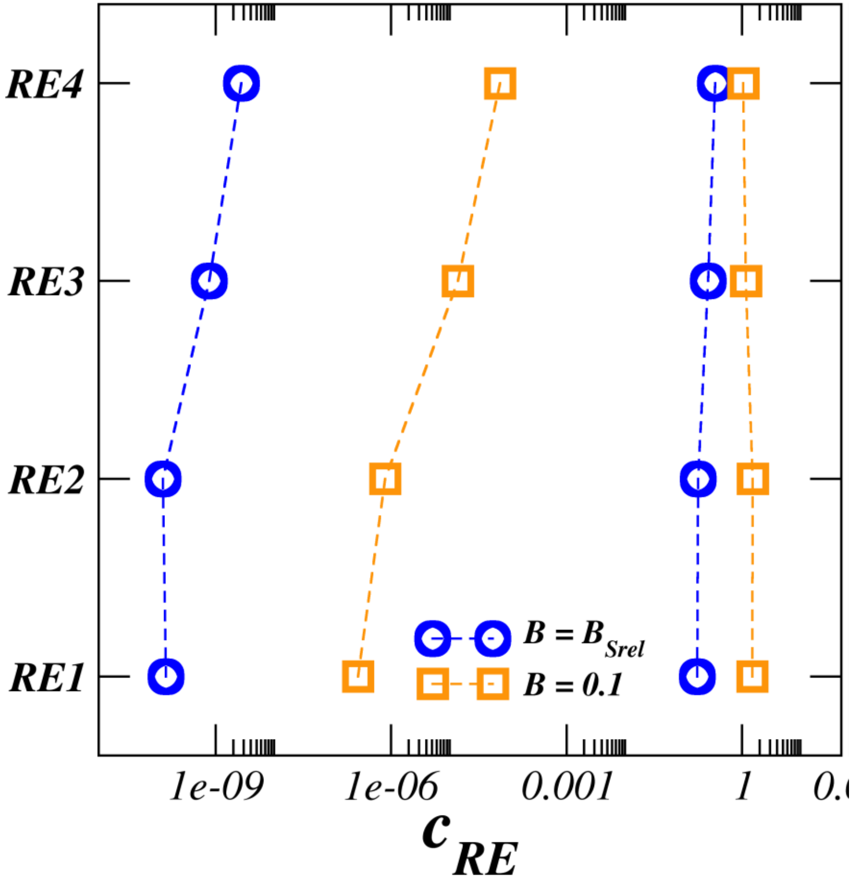
Bond Bond Distance (σ)

RE peptides phase behavior

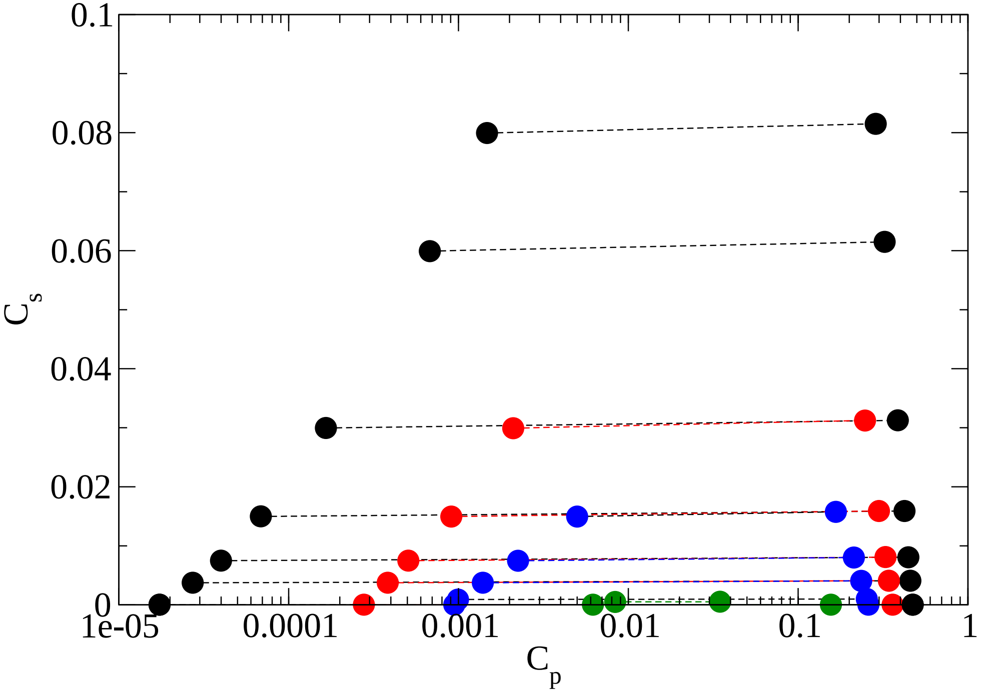


RE peptides phase behavior



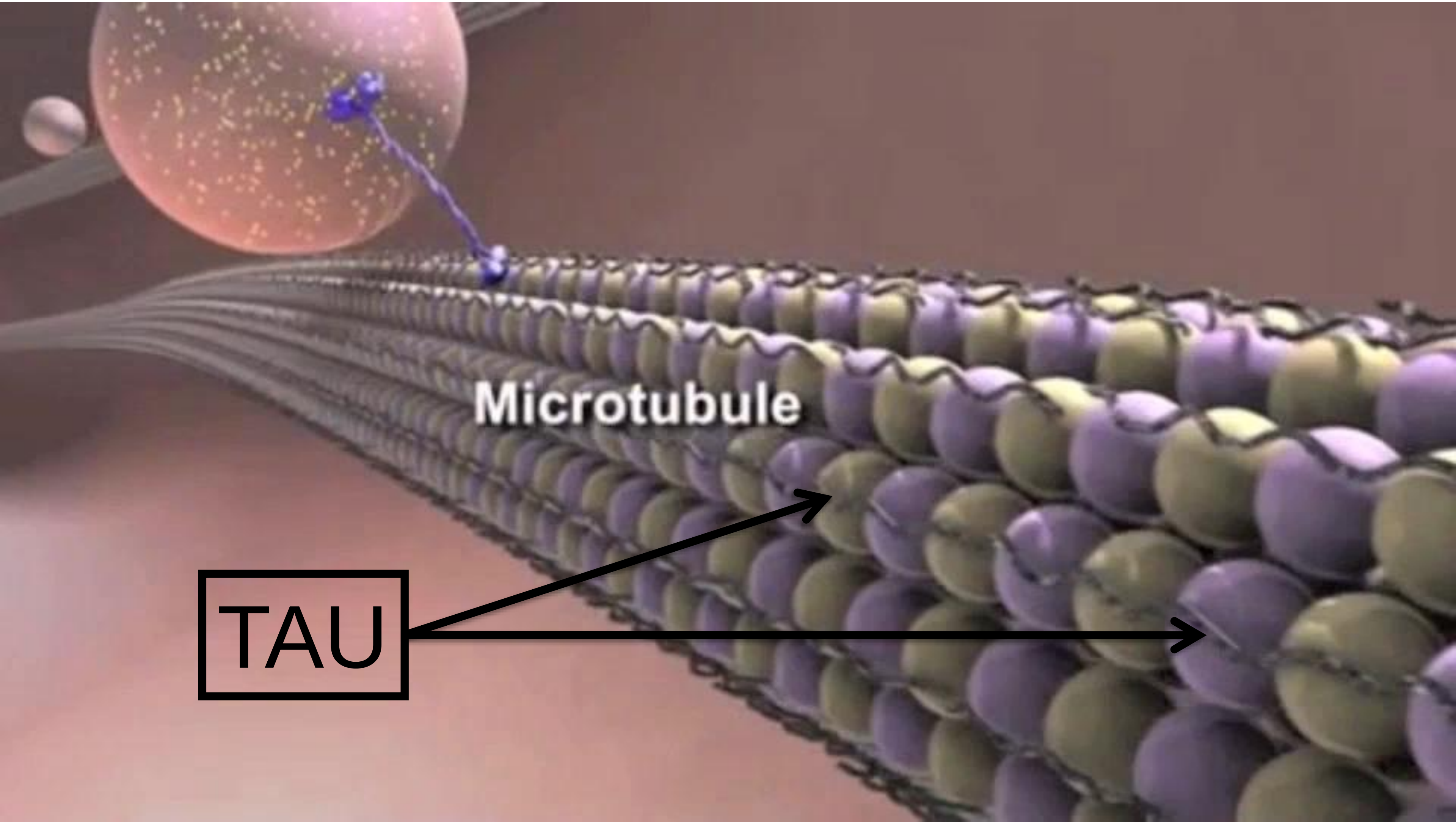


"OLD" FTS

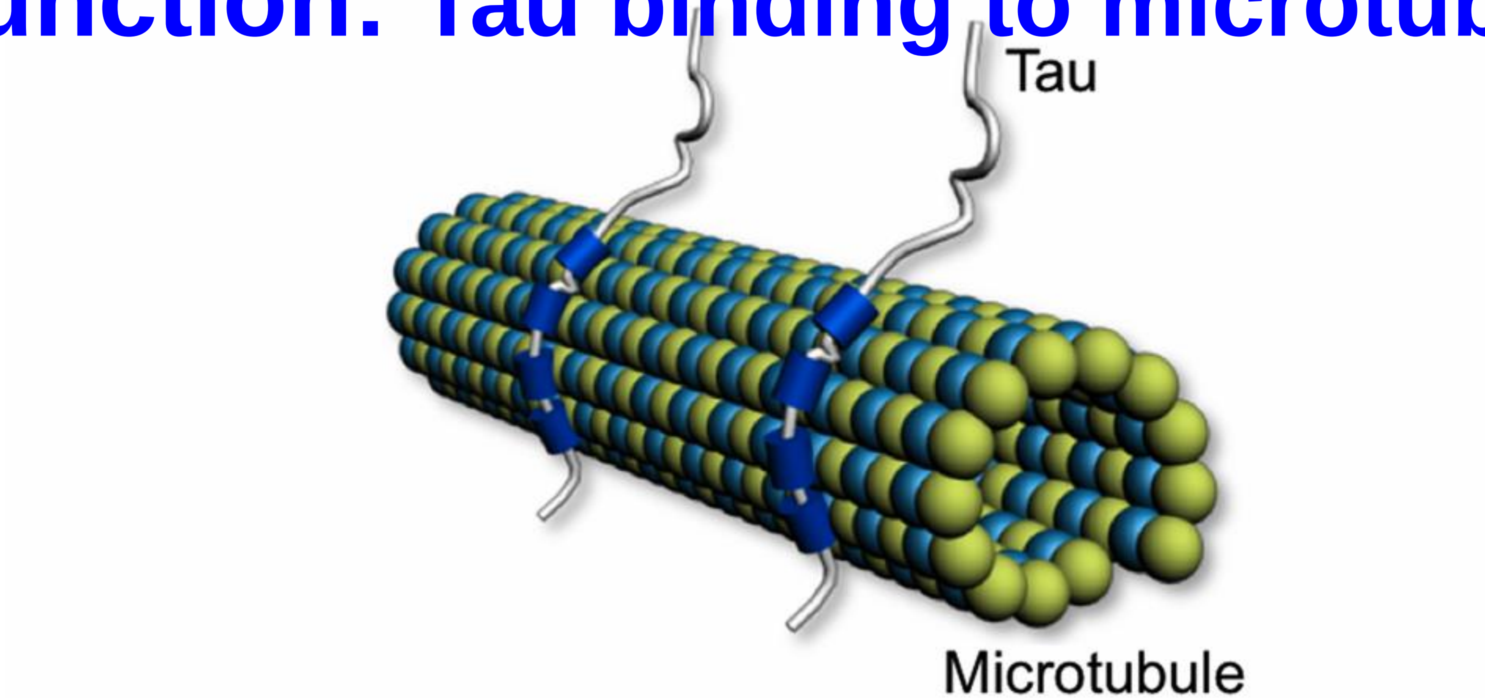


Tau Protein
Liquid Liquid Phase Separation and Fibrillization

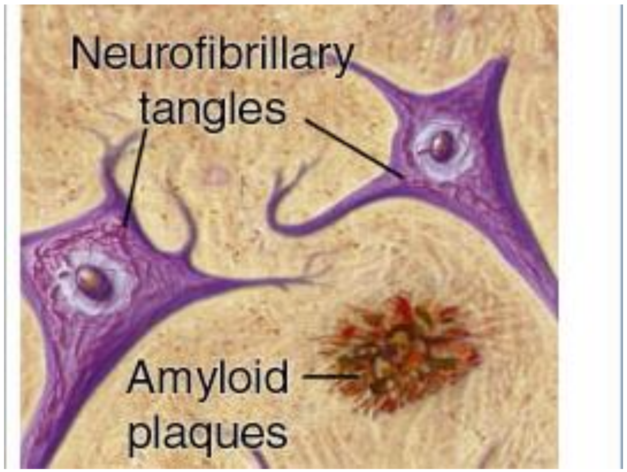
MICROTUBULES ARE STABILIZED BY TAU PROTEINS

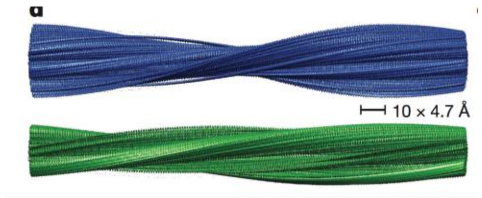


Function: Tau binding to microtubule

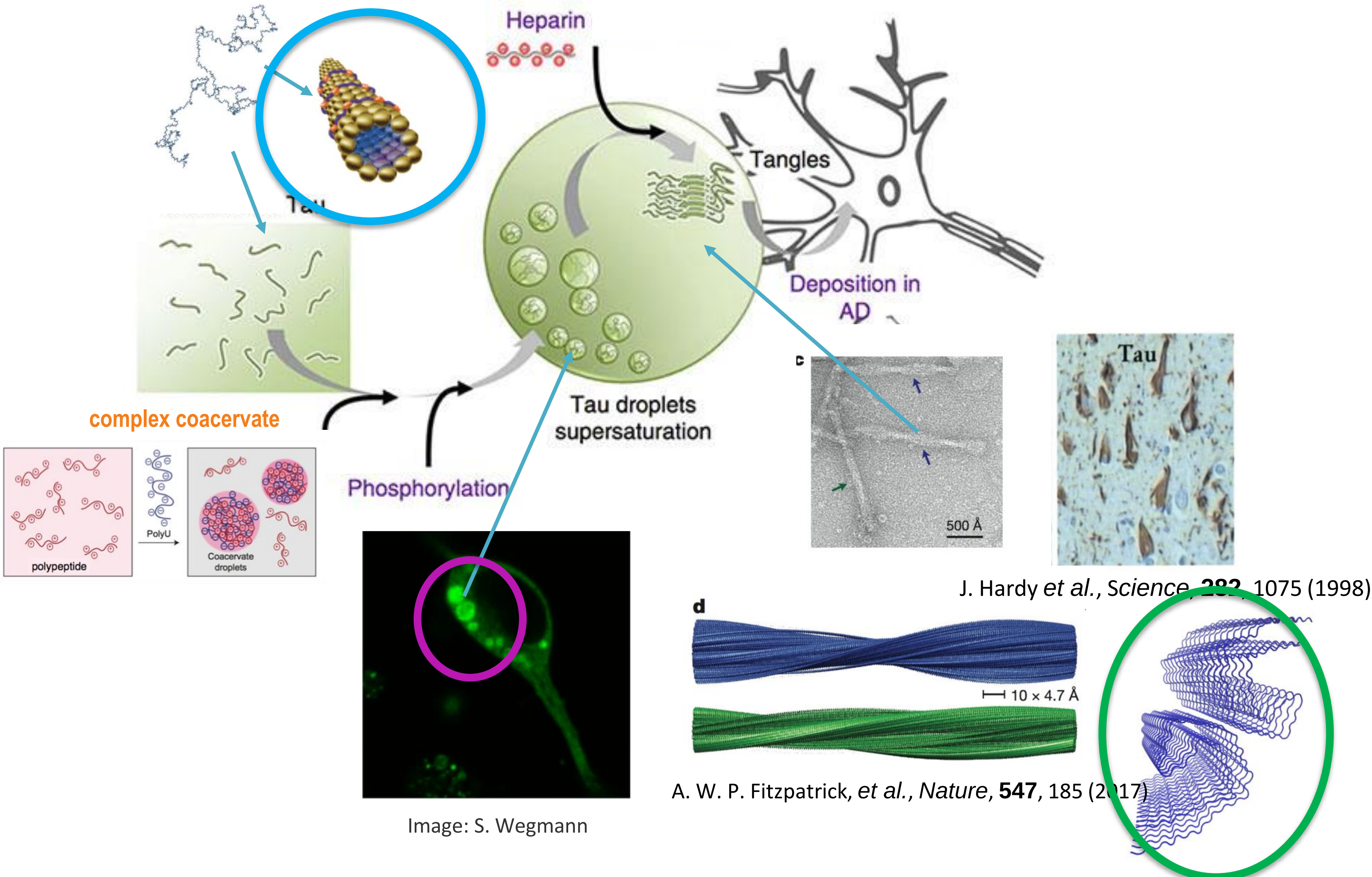


Pathology:Tau and Aggregation





Liquid-Liquid Phase Separation of Tau and Fibril Formation

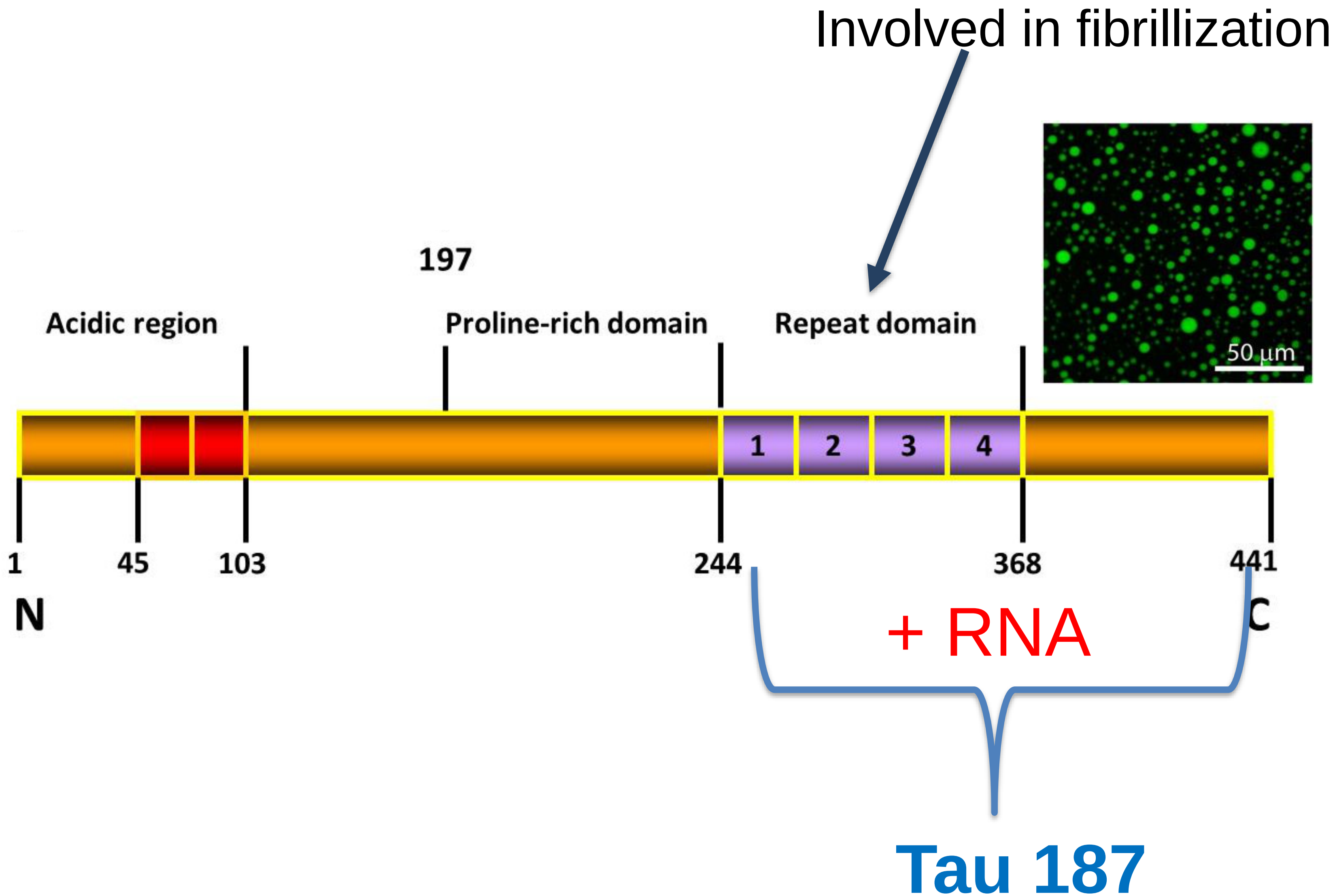


J. Hardy *et al.*, *Science*, **282**, 1075 (1998)

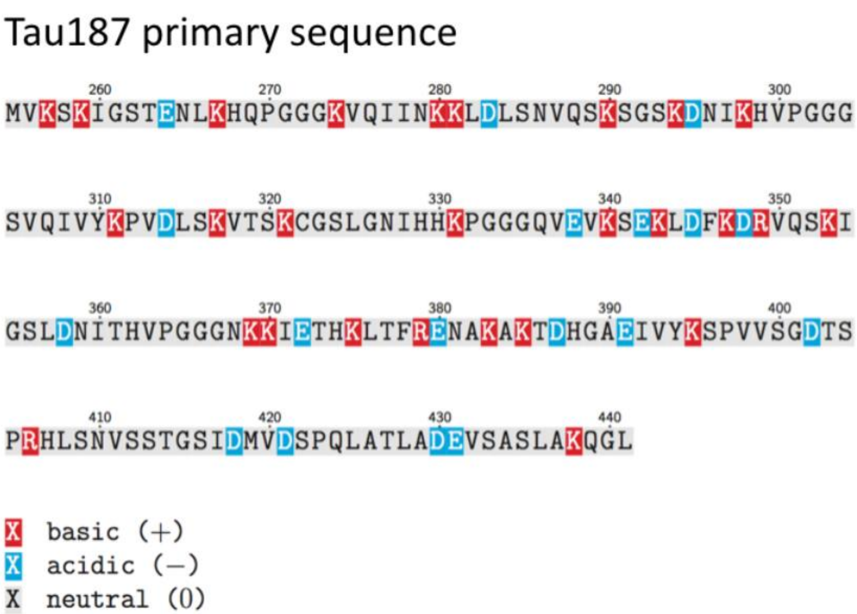
A. W. P. Fitzpatrick, *et al.*, *Nature*, **547**, 185 (2017)

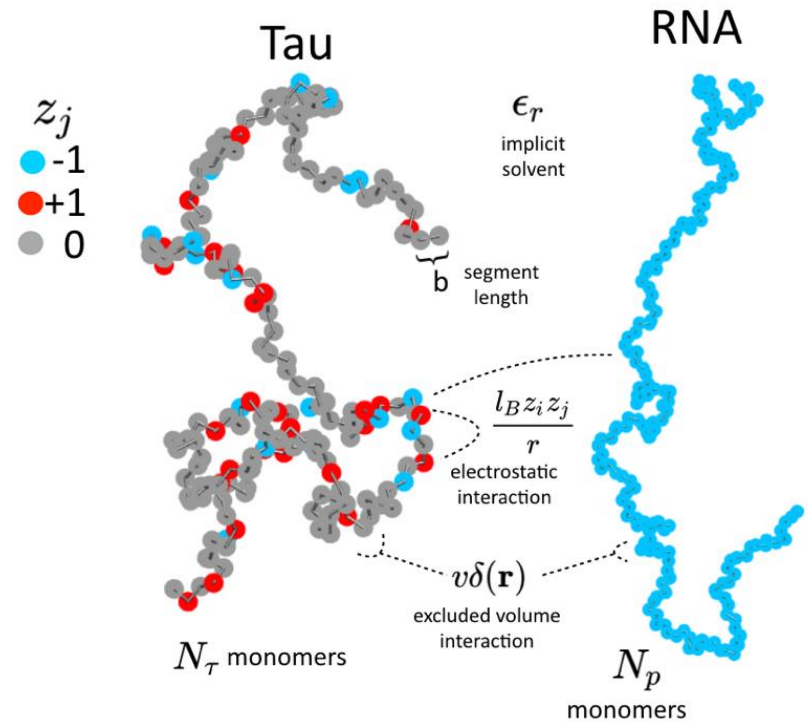
Image: S. Wegmann

Tau sequence



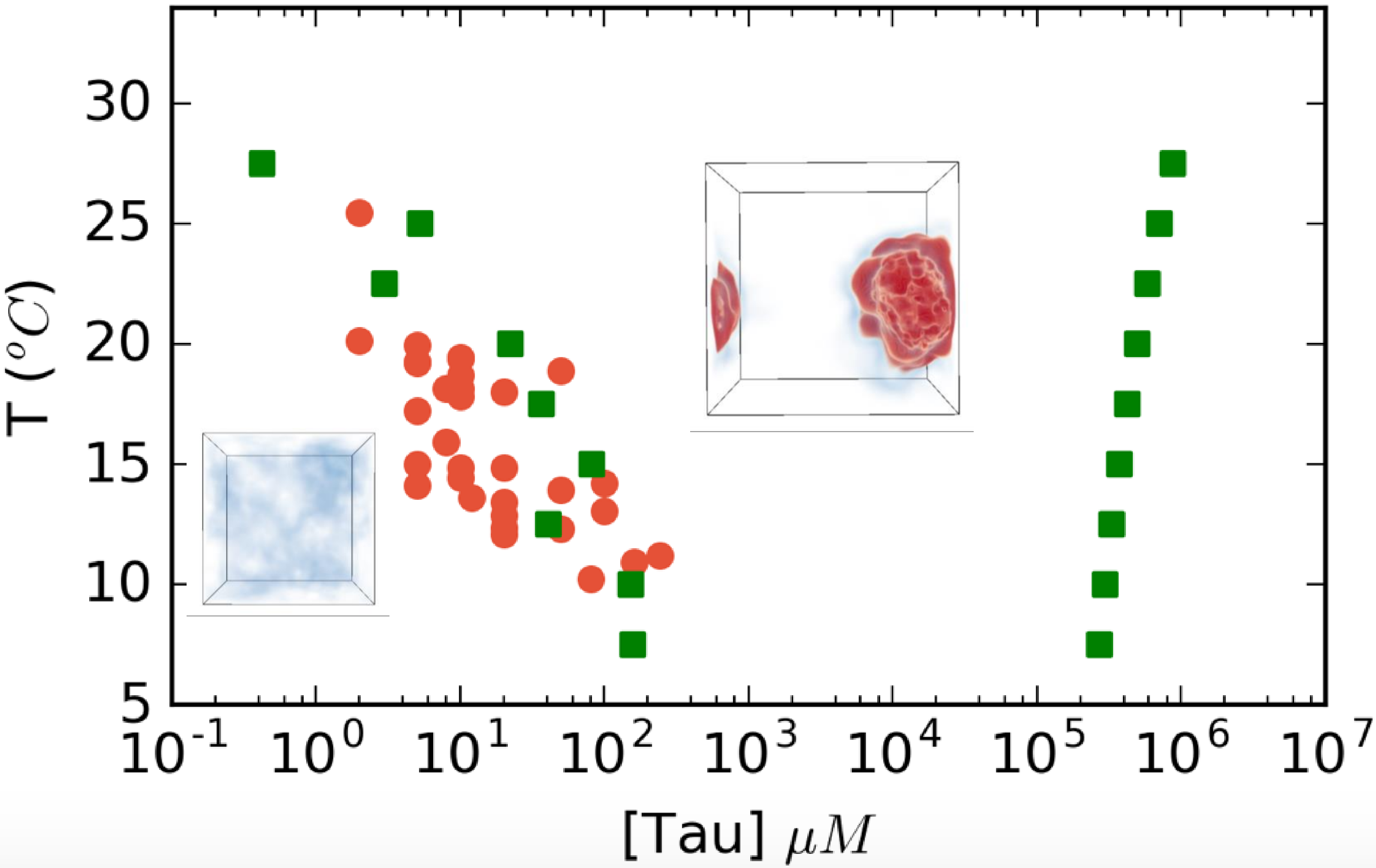
Field Theory Modeling of Tau-RNA complex coacervation



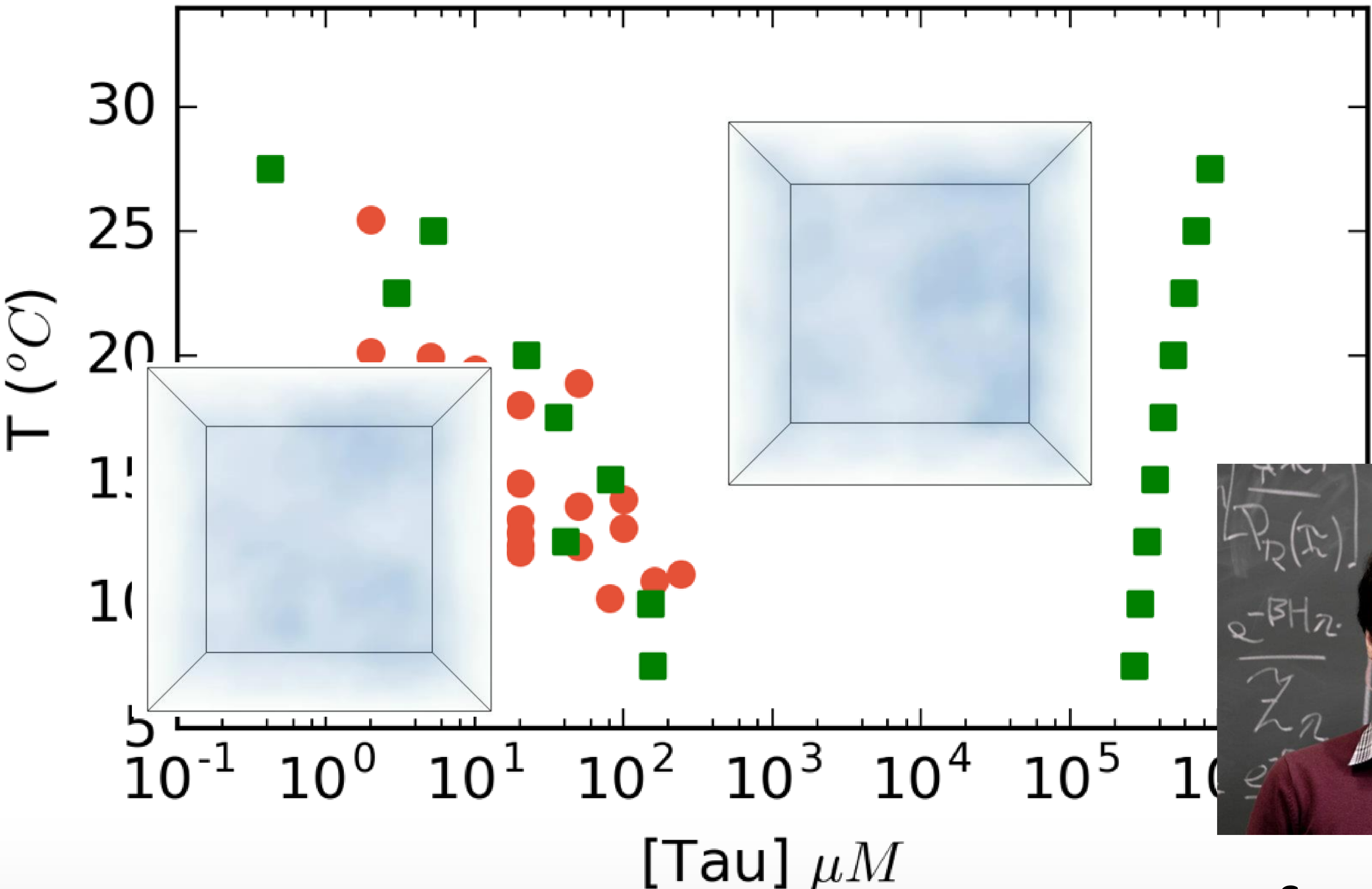


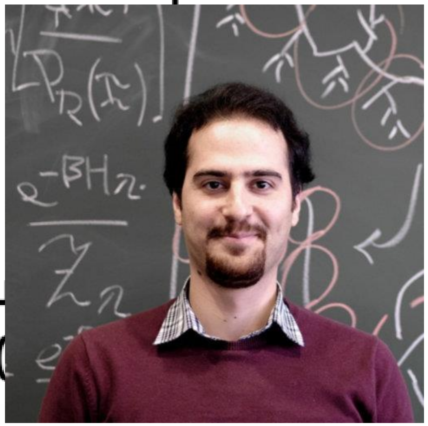
Fit excluded volume (B) and Bjerrum length (E) to experimental values for Tau

Field Theory can map out the entire phase diagram of Tau-RNA Complex Coacervation



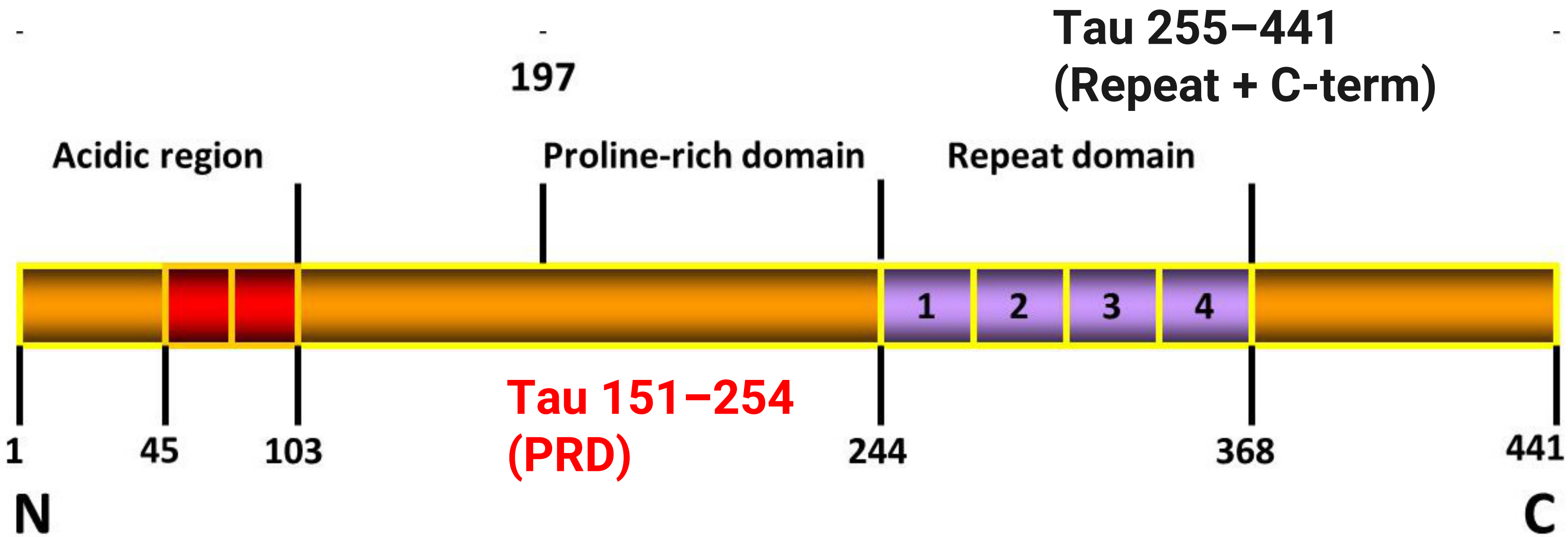
Field Theory can map out the entire phase diagram of Tau-RNA Complex Coacervation





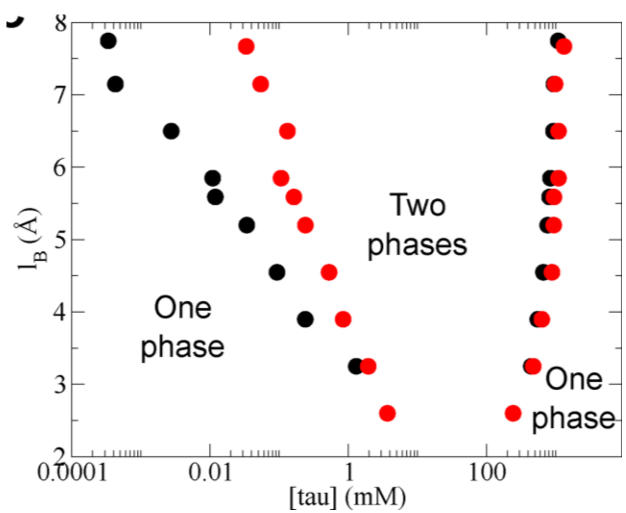
Saeed Najafi

Tau sequence: The Proline-Rich Domain Liquid-Liquid Phase Separates in vitro

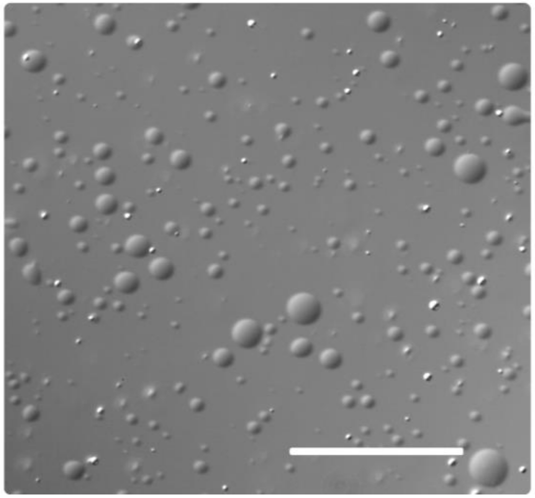


Tau 255−441
(Repeat + C-term)

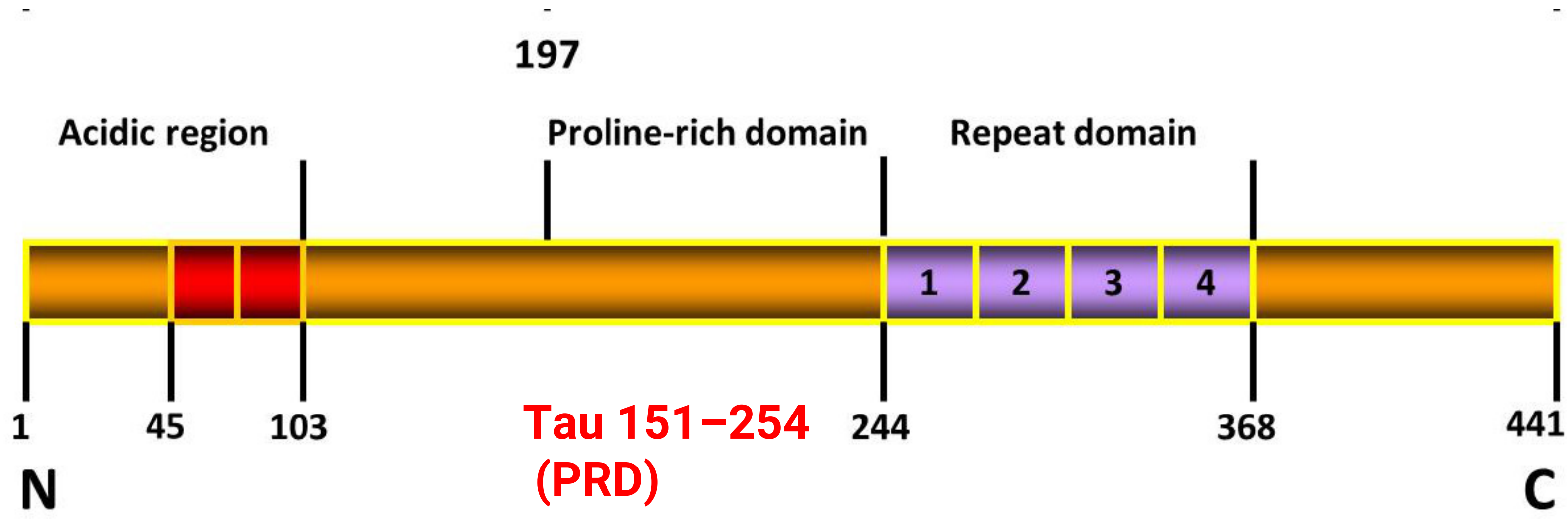
Tau 151−254
(PRD)

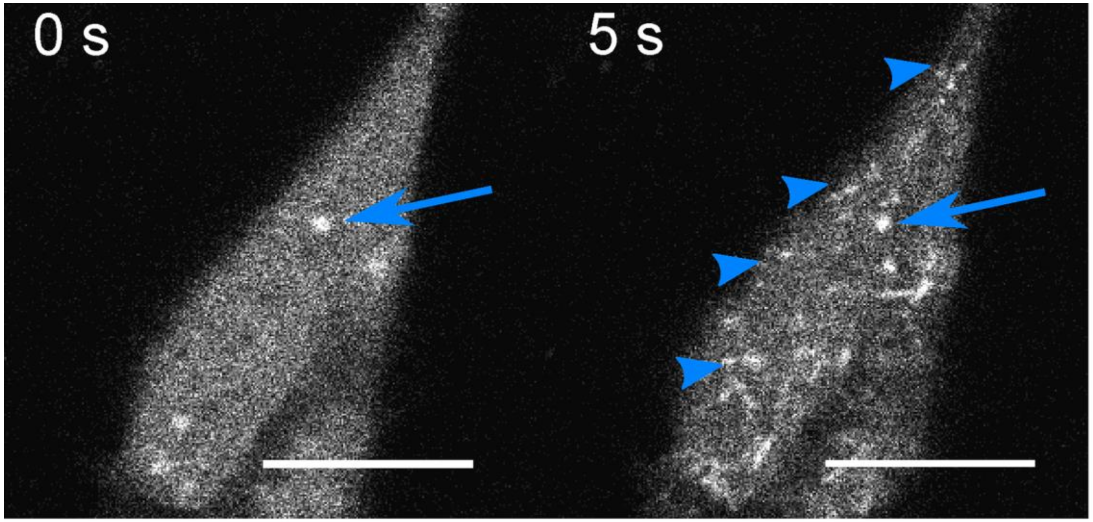


In Vitro



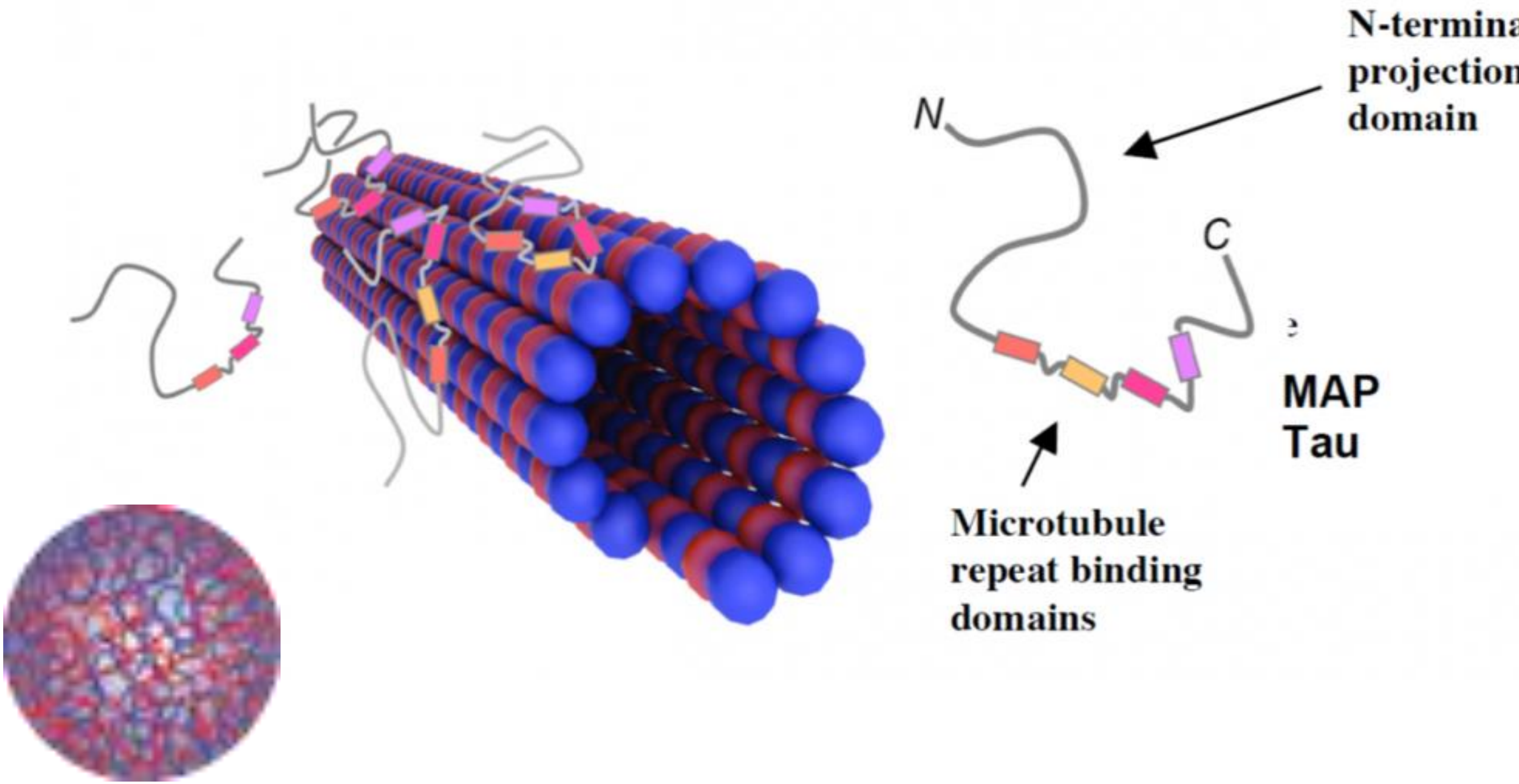
The Proline-Rich Domain Condensates promote Tau association with MTs





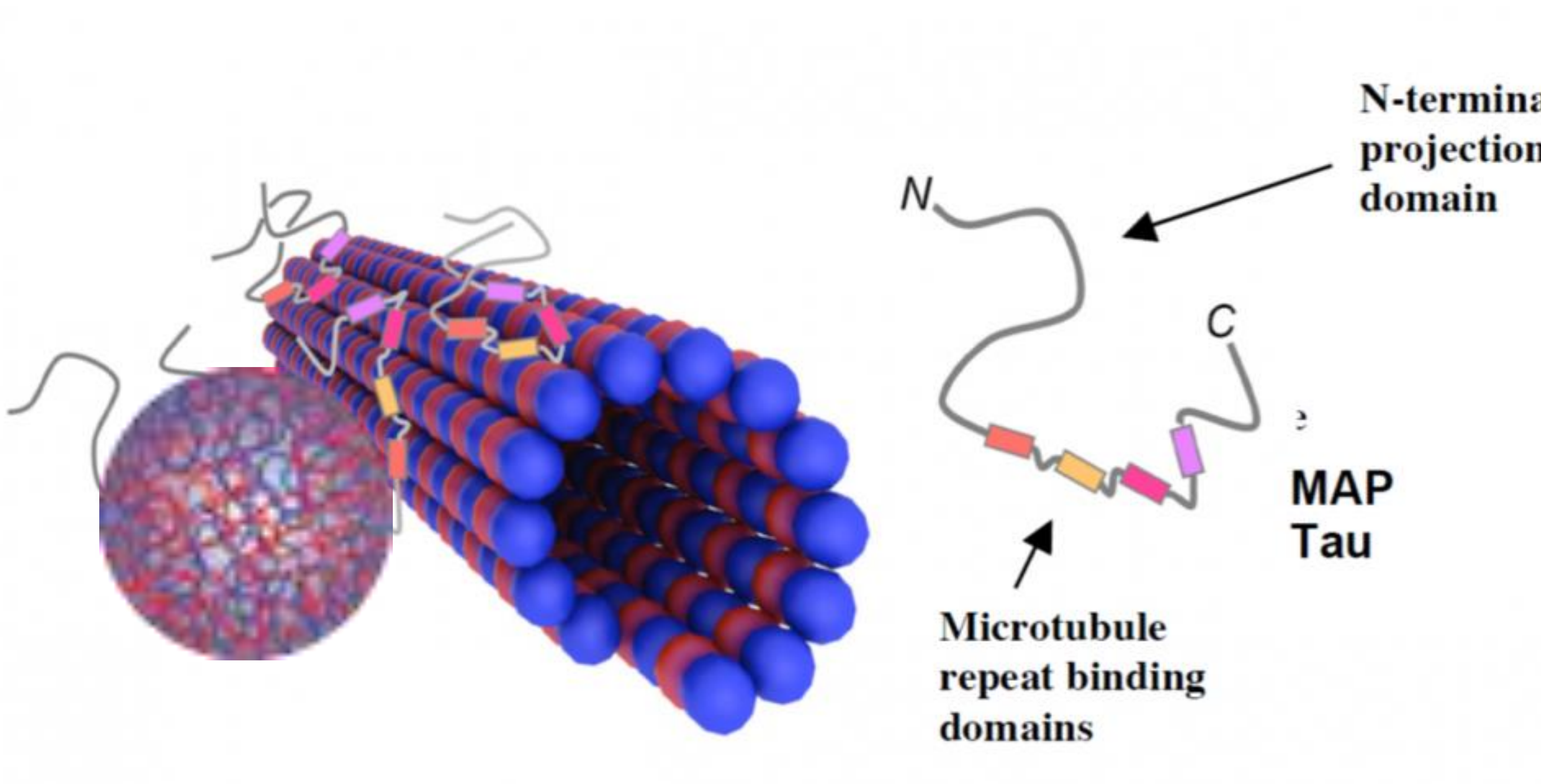
PRD condensates align along the MTs

MICROTUBULES ARE STABILIZED BY TAU PROTEINS



LLPS CONCENTRATES TAU AND FACILITATES BINDING

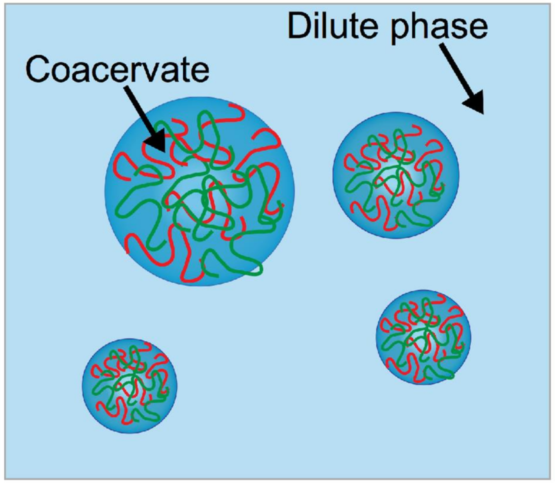
MICROTUBULES ARE STABILIZED BY TAU PROTEINS



LLPS CONCENTRATES TAU AND FACILITATES BINDING

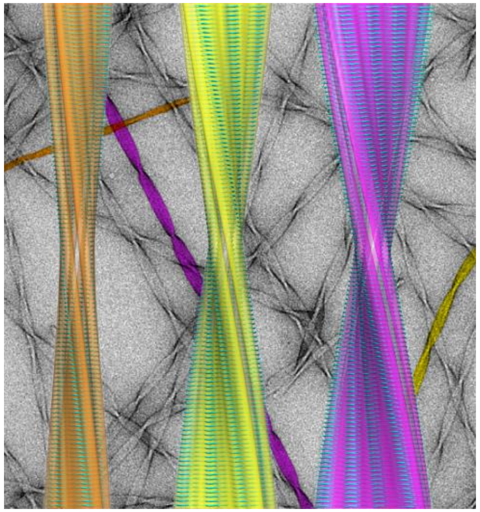
Proteins can assemble in different ways

"Liquid"



Aging →

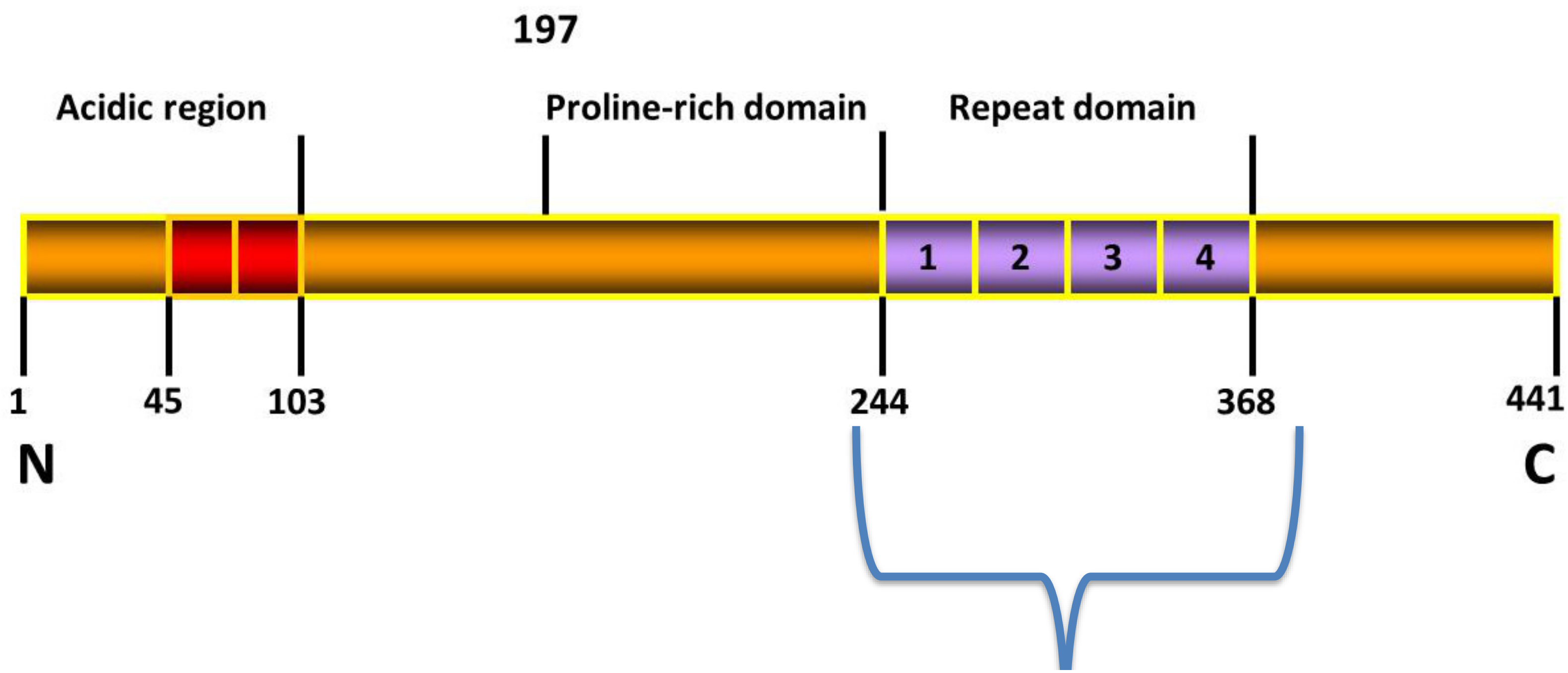
"Solid"



Droplets
Biomolecular condensates
Coacervates

Amyloid Fibrils

Tau Aggregation: Repeat Domain makes up the core of Tau Fibrils



272 GGK 274 — R1

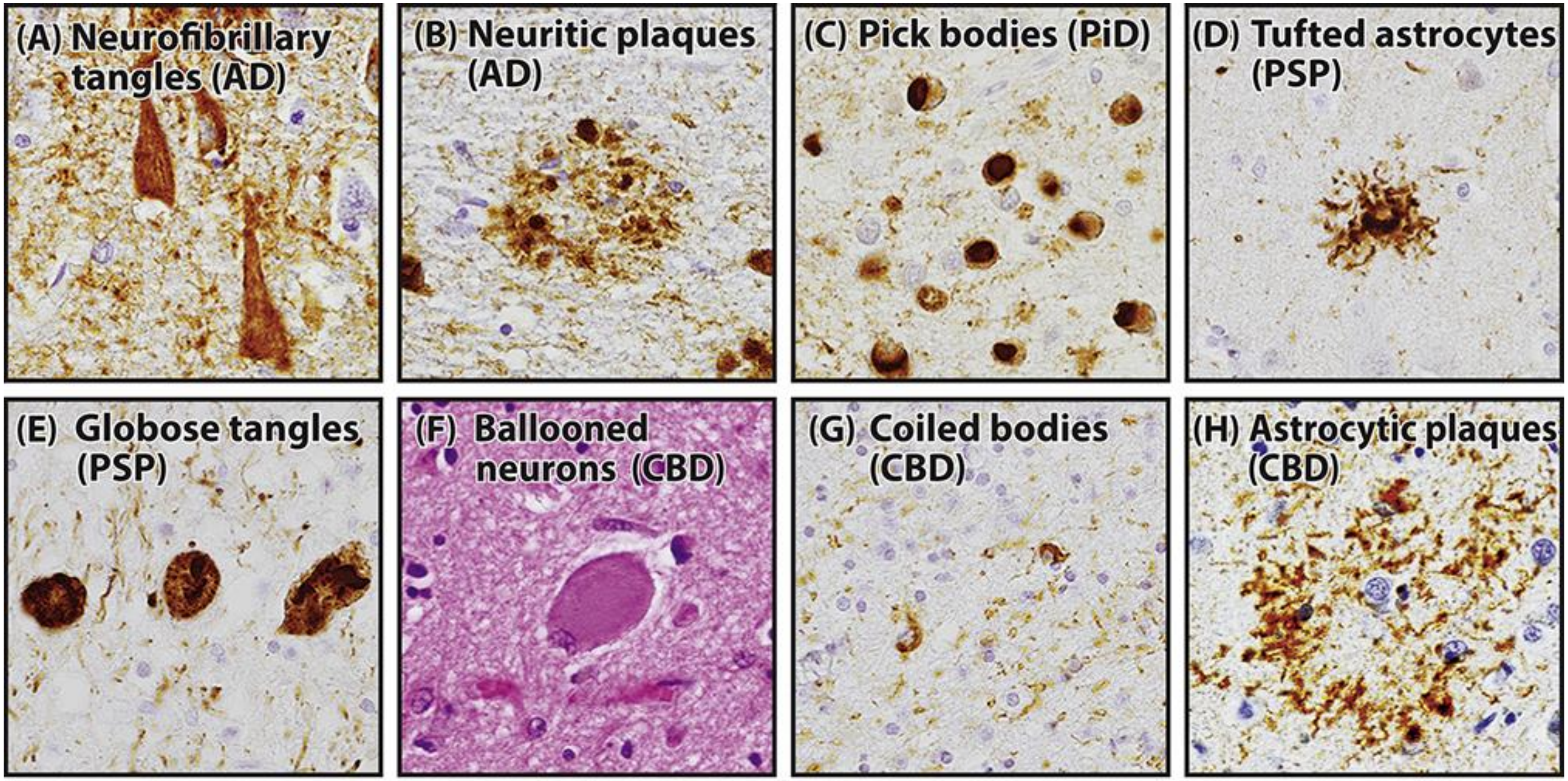
275 VQIINKKLDLS-NVQSKCGSKDNIKHVPGGGS 305 — R2

306 VQIVYKPVDLS-KVTSKCGSLGNIHHKPGGGQ 336 — R3

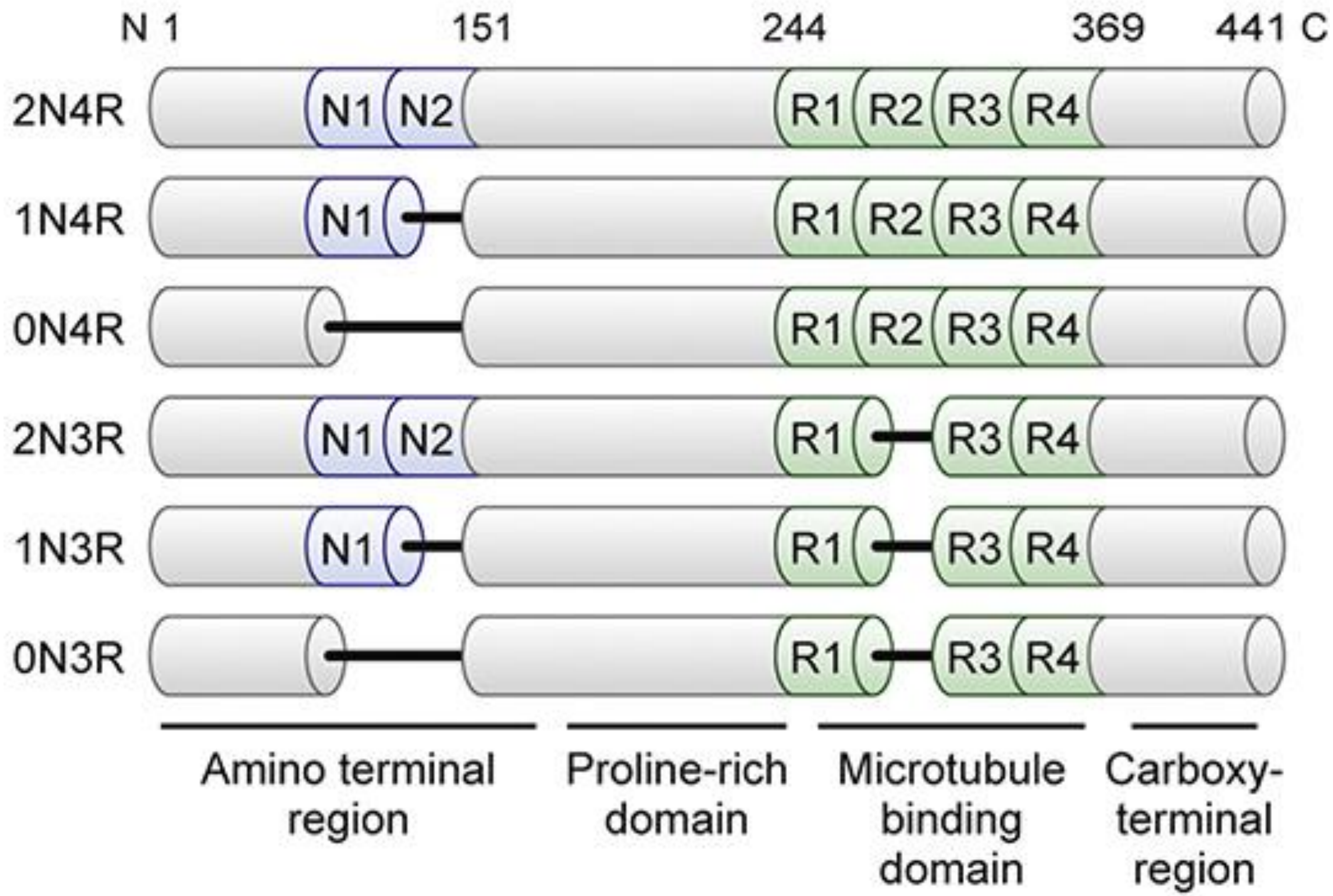
337 VEVKSEKLDFKDRVQSKIGSLDNITHVPGGGN 368 — R4

369 KKIETHKLTFRENAKAKTD 387 — C

There are many neurodegenerative diseases associated with Tau

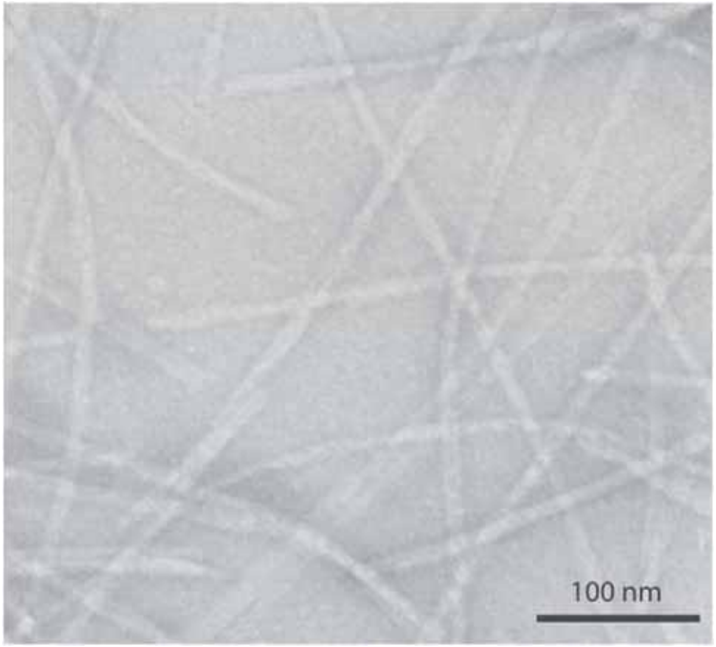


There are many forms of Tau



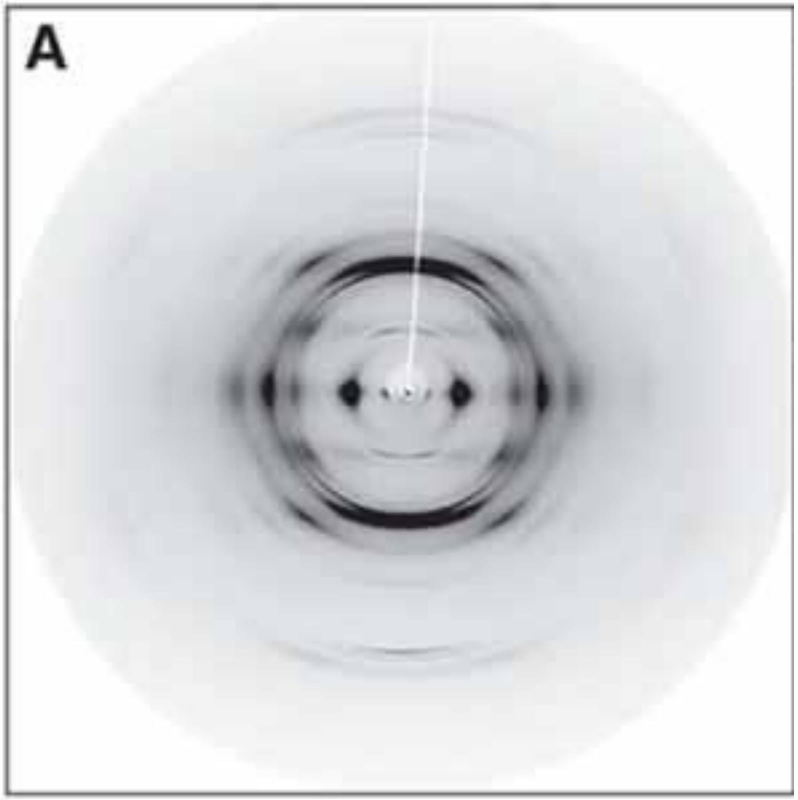
At first glance they all look the same

Transmission Emission Microscopy (TEM)



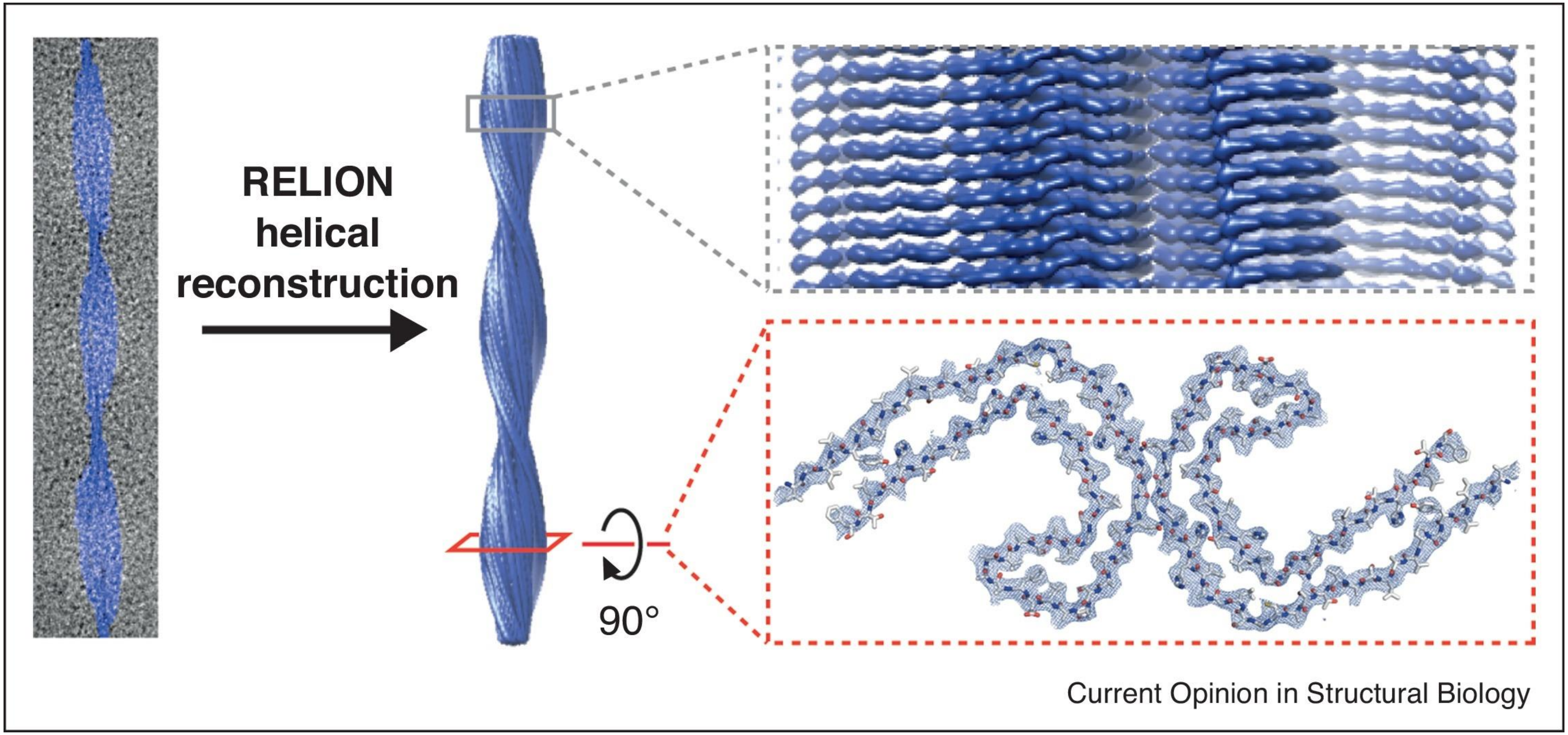
Fibrils

X-Ray diffraction

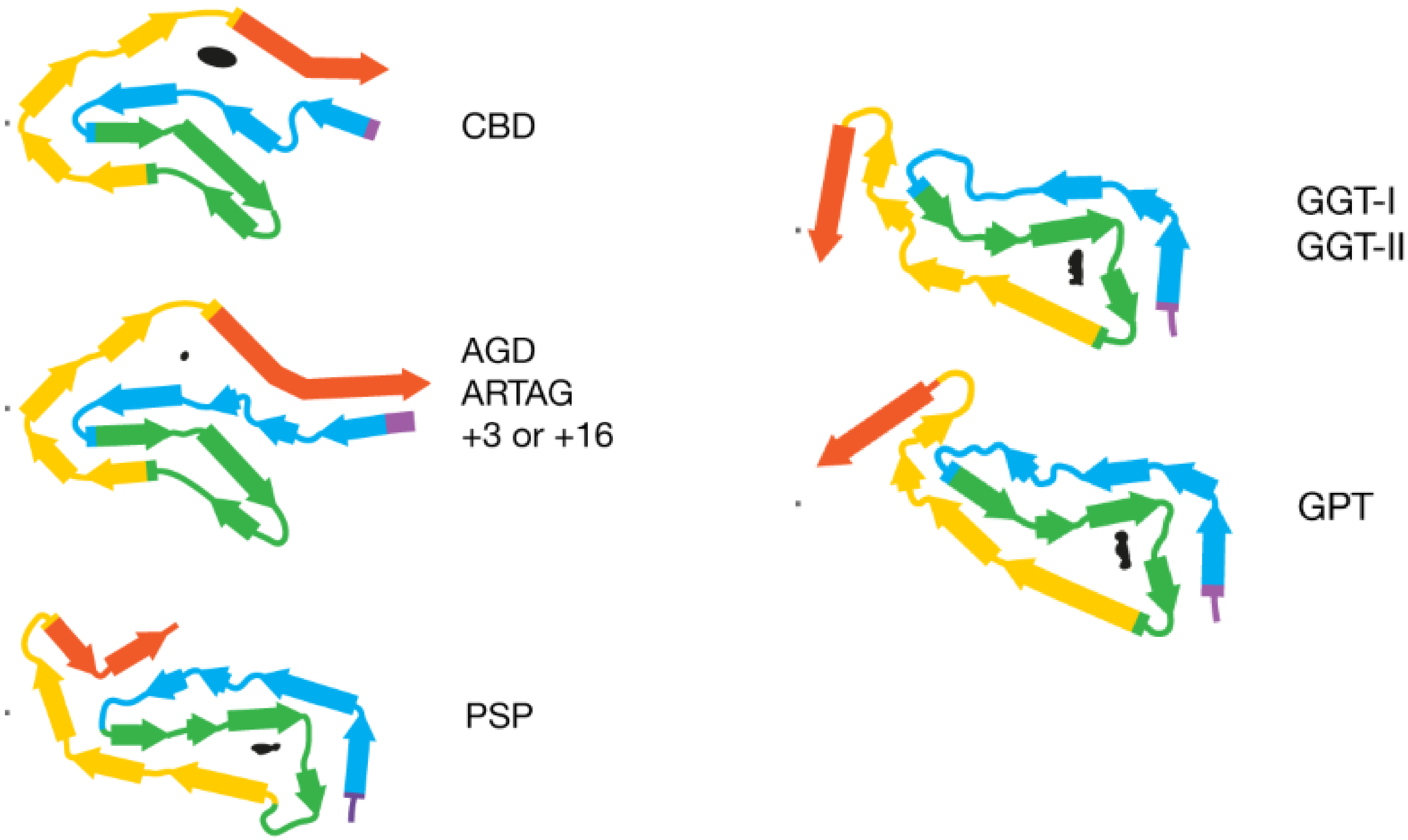


Cross-beta structure

But Cryo-EM shows (subtle) differences



The specific fibril shape is a signature of a specific disease



COMMON STRAND-LOOP-STRAND MOTIF

272 GGK 274 — R1

275 VQIINKKLDLS-NVQSKCGSKDNIKHVPGGGS 305 — R2

PHF6 — 306 VQIVYKPVDLS-KVTSKCGSLGNIHHKPGGGQ 336 — R3

337 VEVKSEKLDFKDRVQSKIGSLDNITHVPGGGN 368 — R4

369 KKIETHKLTFRENAKAKTD 387 — C

CBD

AGD

Created: 19 Amino-acid Peptide

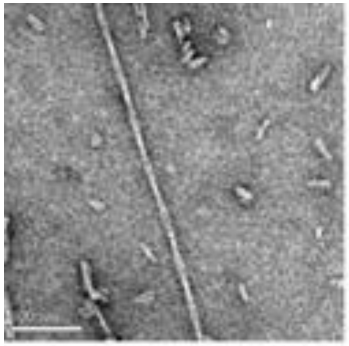
WILD TYPE: jR2R3

MUTANT: jR2R3-P301L

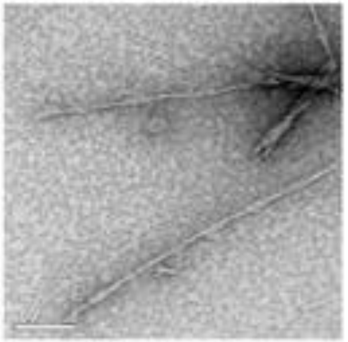
jR2R3-P301L mutant aggregates faster than jR2R3 and shows more fibril morphologies

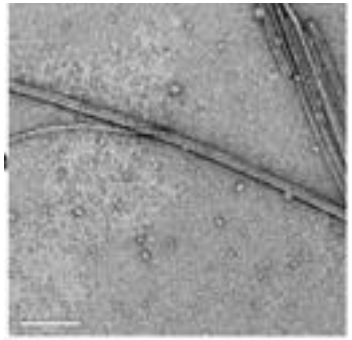
jR2R3

jR2R3-P301L

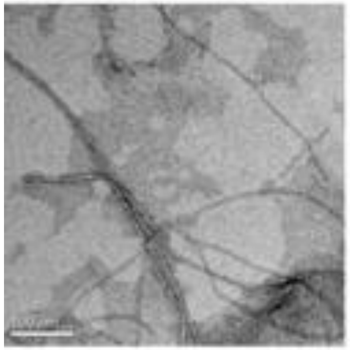


PHF

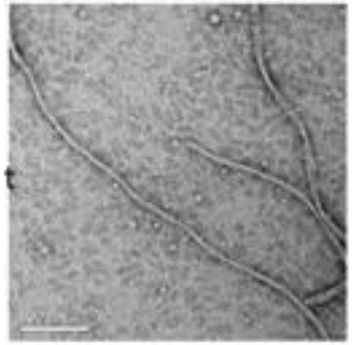


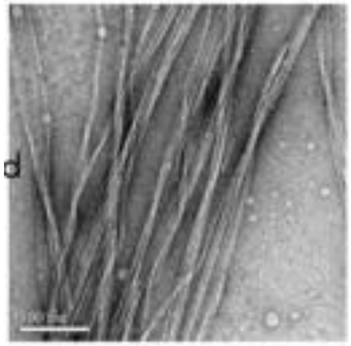


Ribbon



Straight

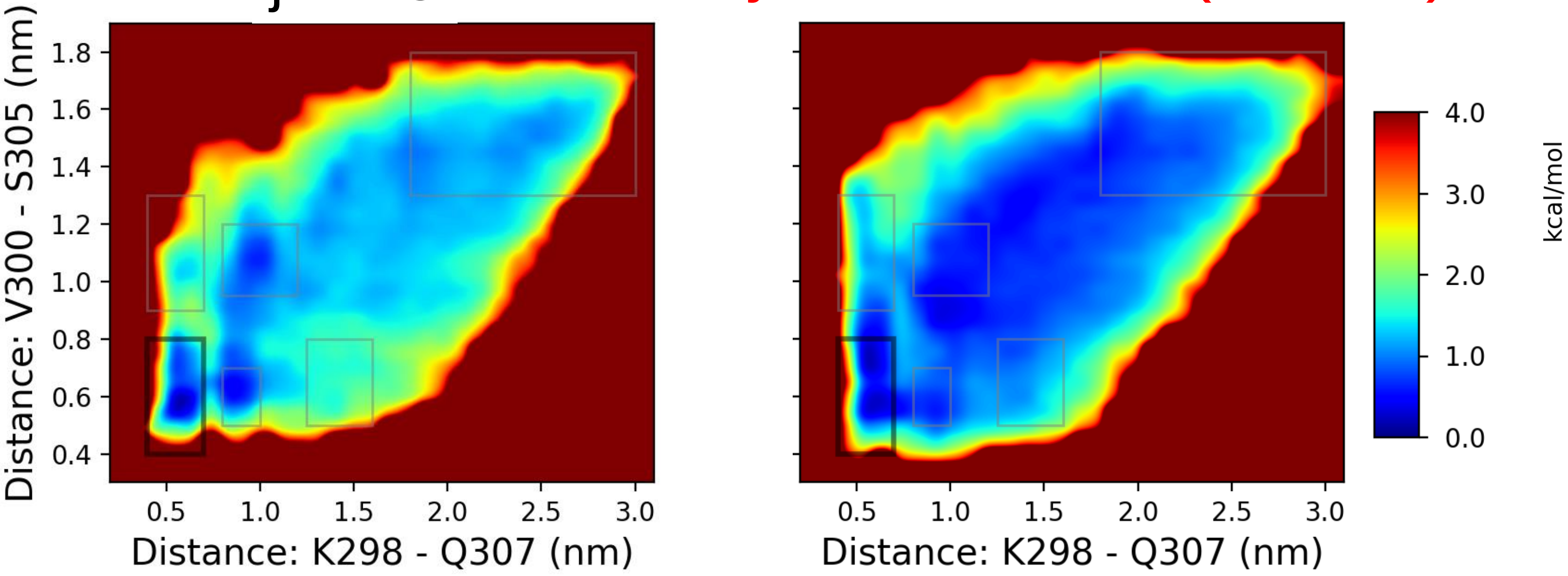




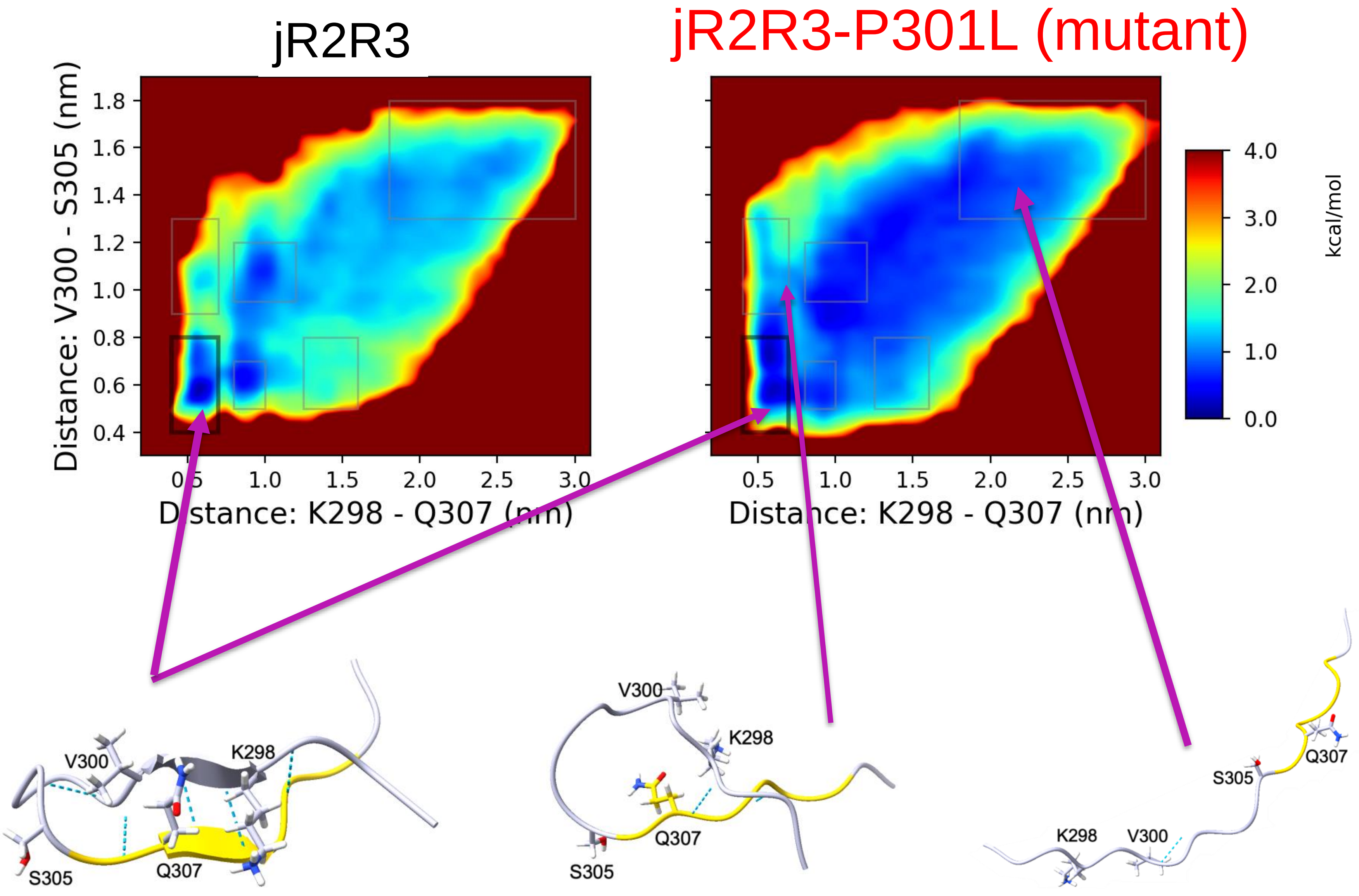
Bundle

jR2R3-P301L can explore more conformational space

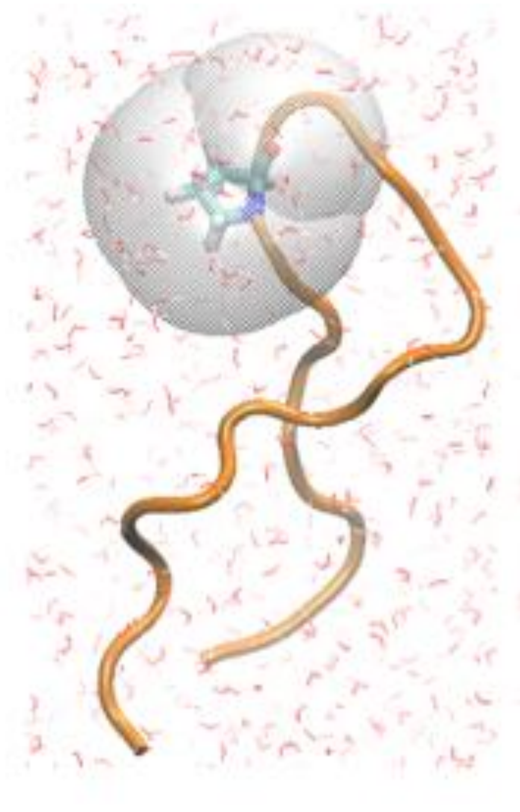
jR2R3 | jR2R3-P301L (mutant)

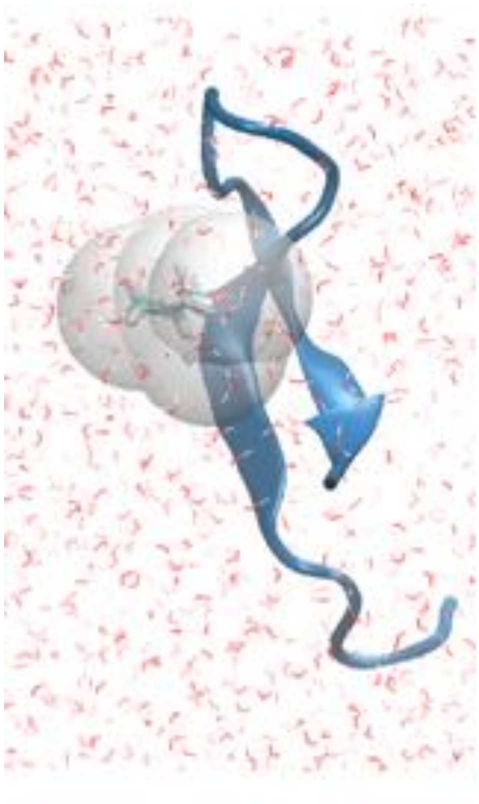


jR2R3-P301L can get out of a ”fibril protective” hairpin



Overhauser Dynamic Nuclear Polarization (ODNP) experiments show a reduction in hydration water dynamics around 301 site for the P→L mutant (jR2R3-P301L)





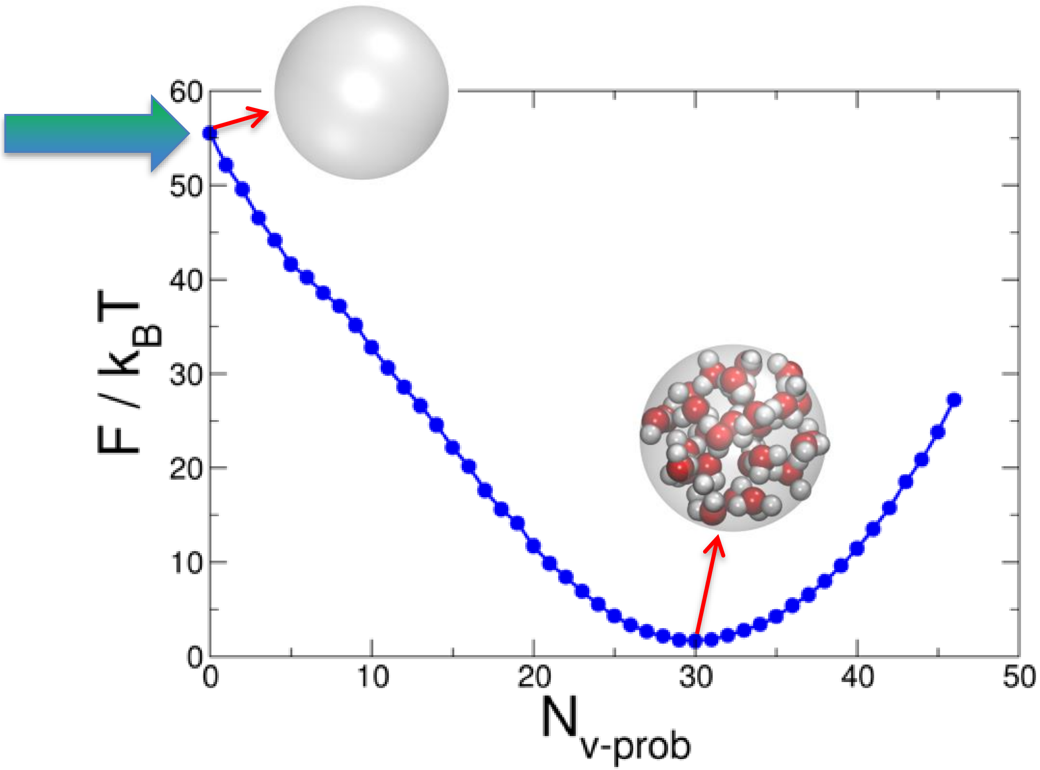
Increased ordering of water around mutation site

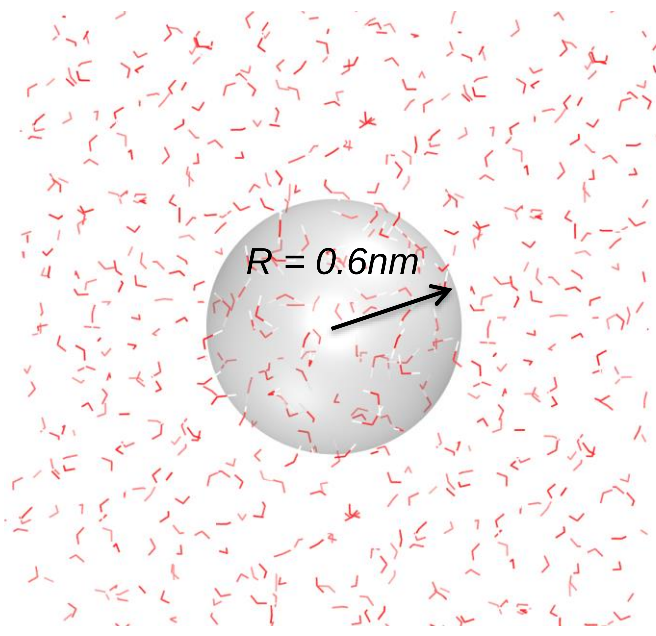
→ locally more hydrophobic

Probing Hydrophobicity Computationally through Umbrella Sampling (INDUS)

Free energy of dewetting a spherical volume in bulk

$$\mu_{ex} = F(0) = -\ln P_v(0)$$

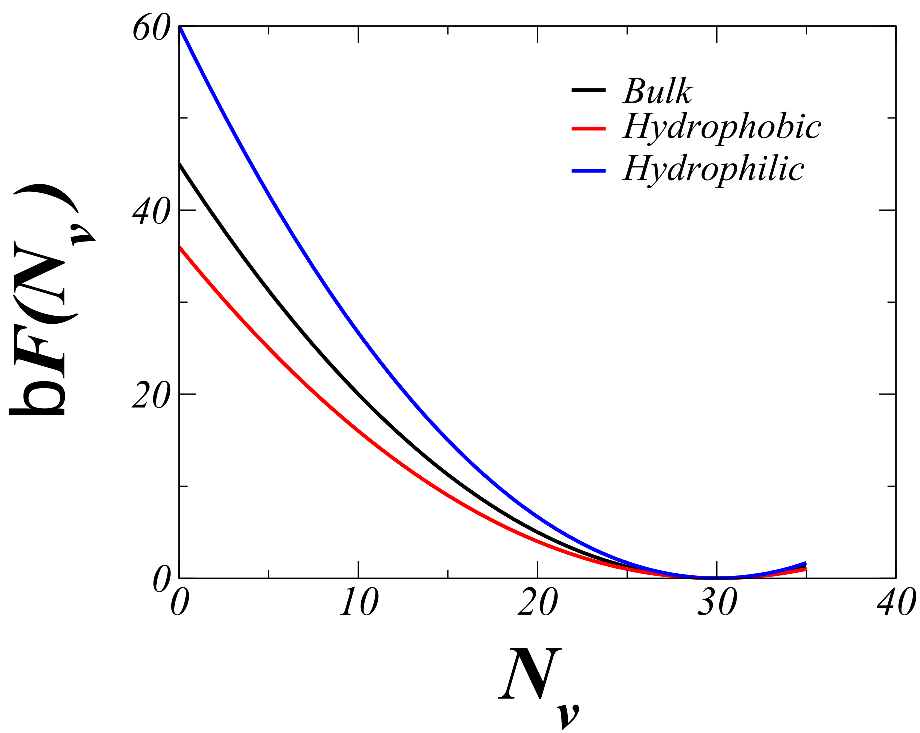


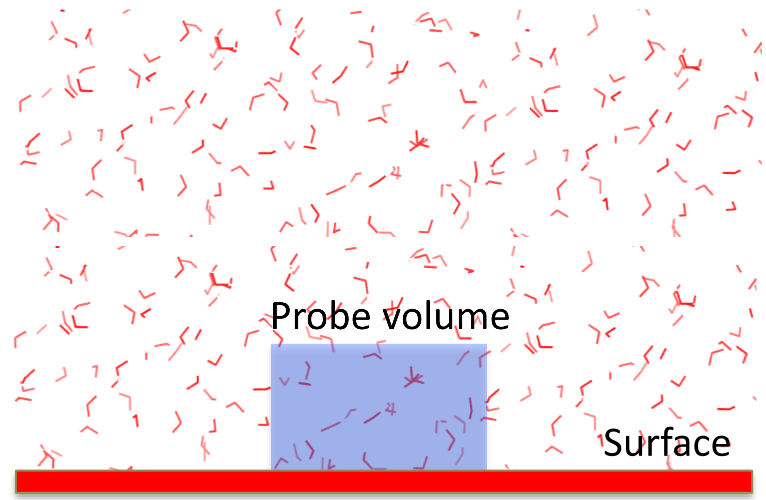


Free energy of dewetting the probe volume in vicinity of a surface

Excess chemical potential is an indication of hydrophilicity or hydrophobicity of the surface

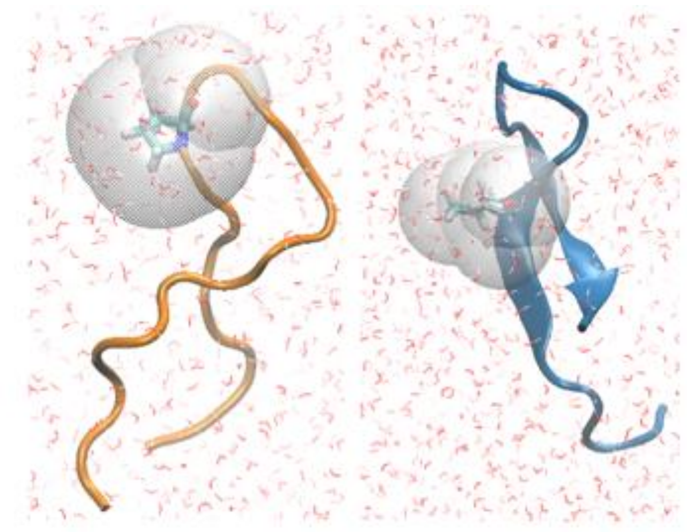
$$\mu_{\mathbf{ex}}^{\mathbf{phil}} > \mu_{\mathbf{ex}}^{\mathbf{bulk}} > \mu_{\mathbf{ex}}^{\mathbf{phob}}$$



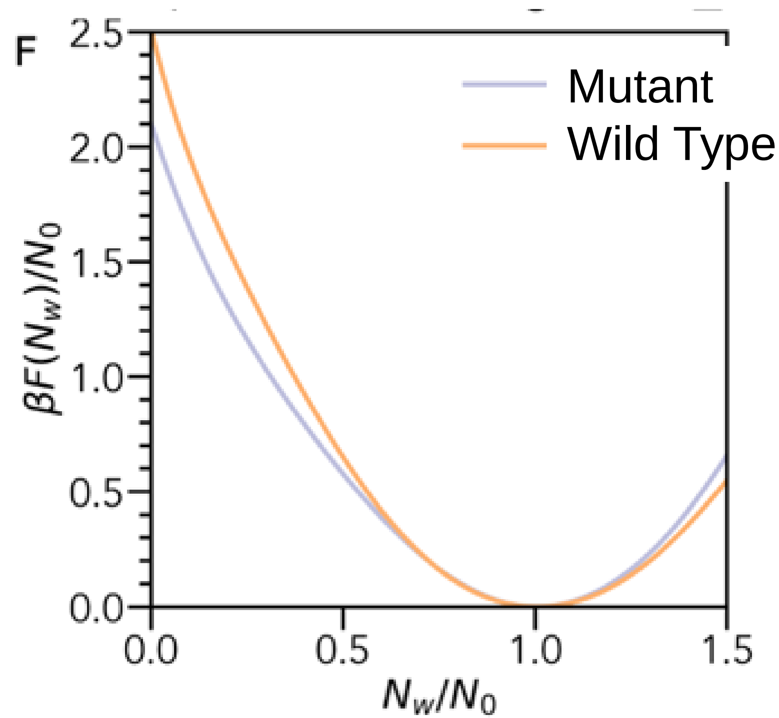


$$\mu_{\mathbf{ex}} = \mathbf{F(0)} = -\mathbf{lnP_v(0)}$$

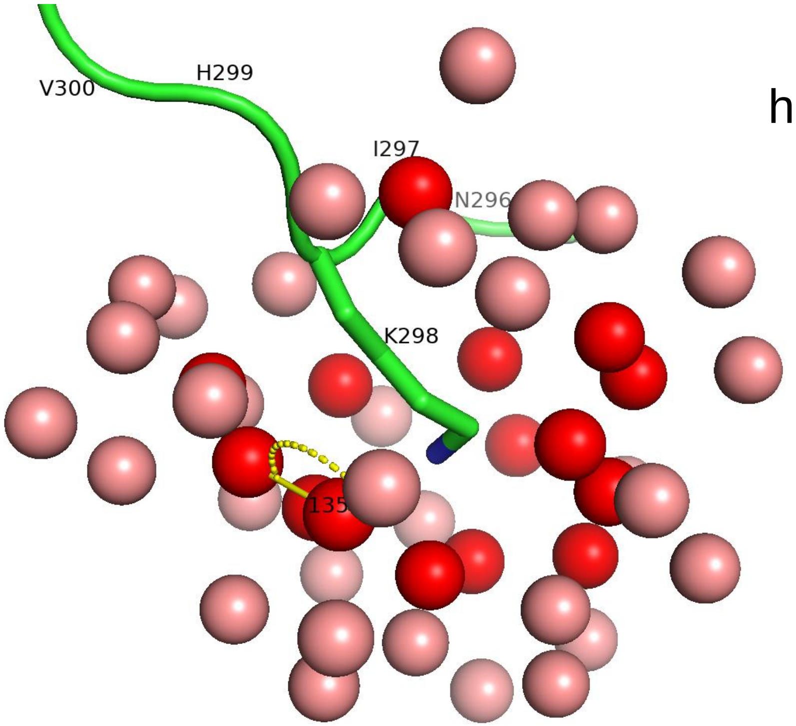
Free energy of dewetting lower for jR2R3-P301L: an additional factor favoring association of jR2R3-P301L



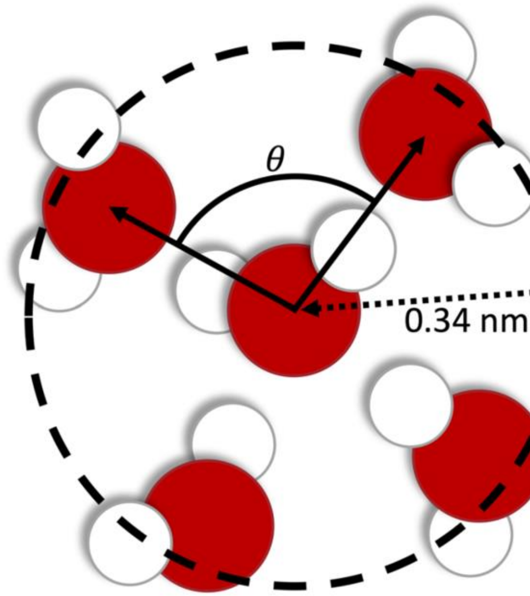
Wild Type Mutant



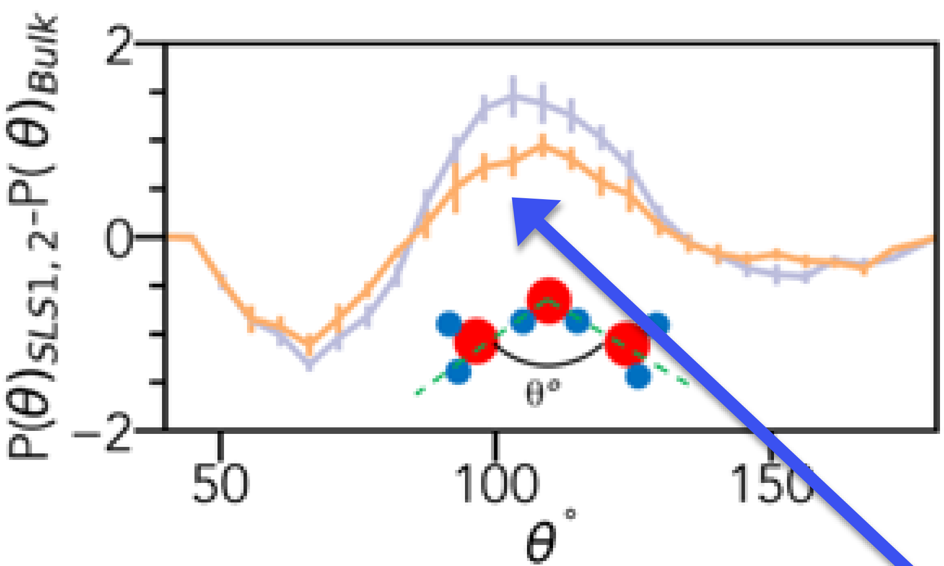
Quantifying Water Structure: *water triplet distribution*

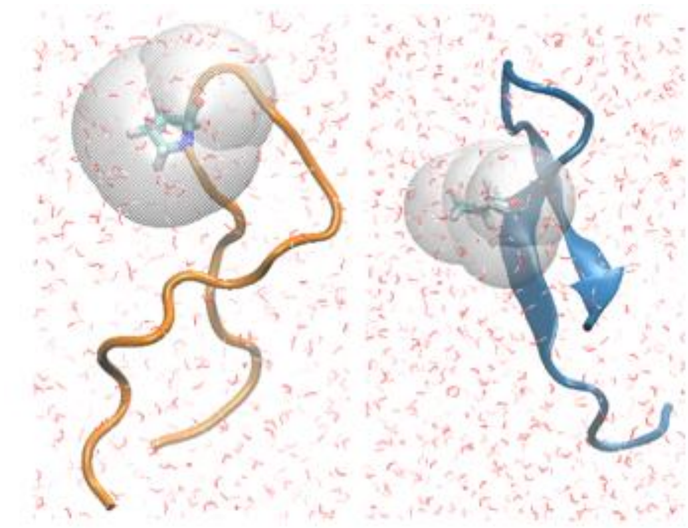


histogram
the
angles



Increased tetrahedral ordering of hydration waters near the L301 (mutant) compared to P301 (wild type)



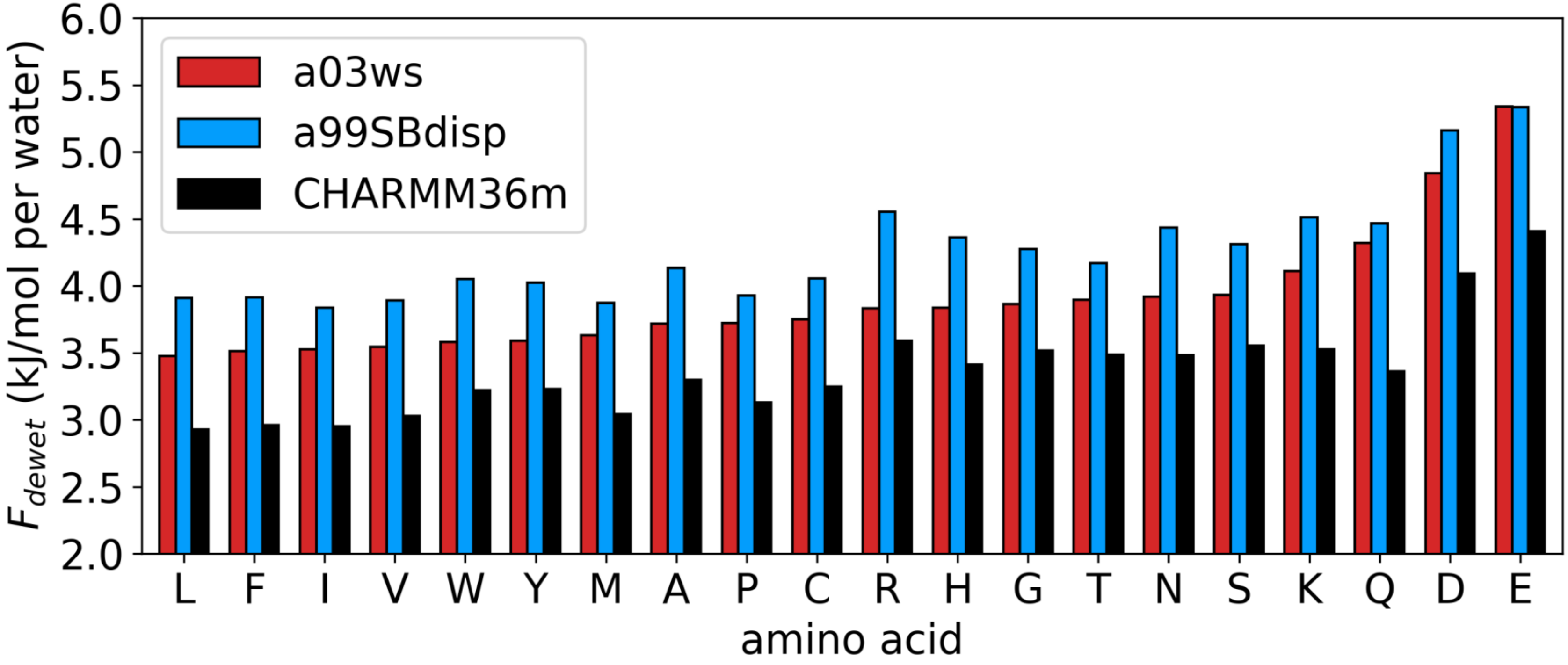


Wild Type

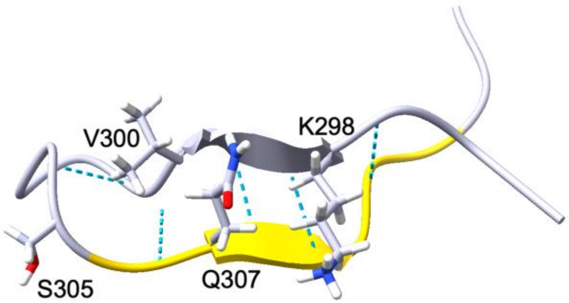
Mutant

TETRAHEDRAL
“hydrophobic”

Different Force Fields Show Different Hydrophobicity

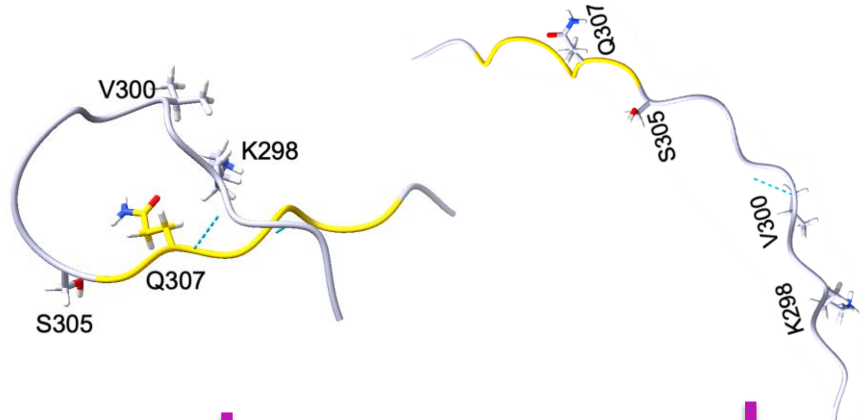


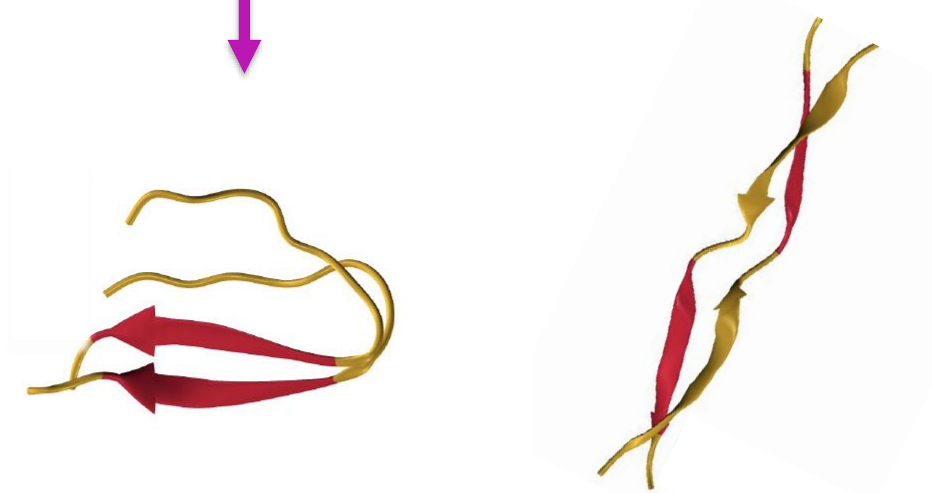
”fibril protective structure”



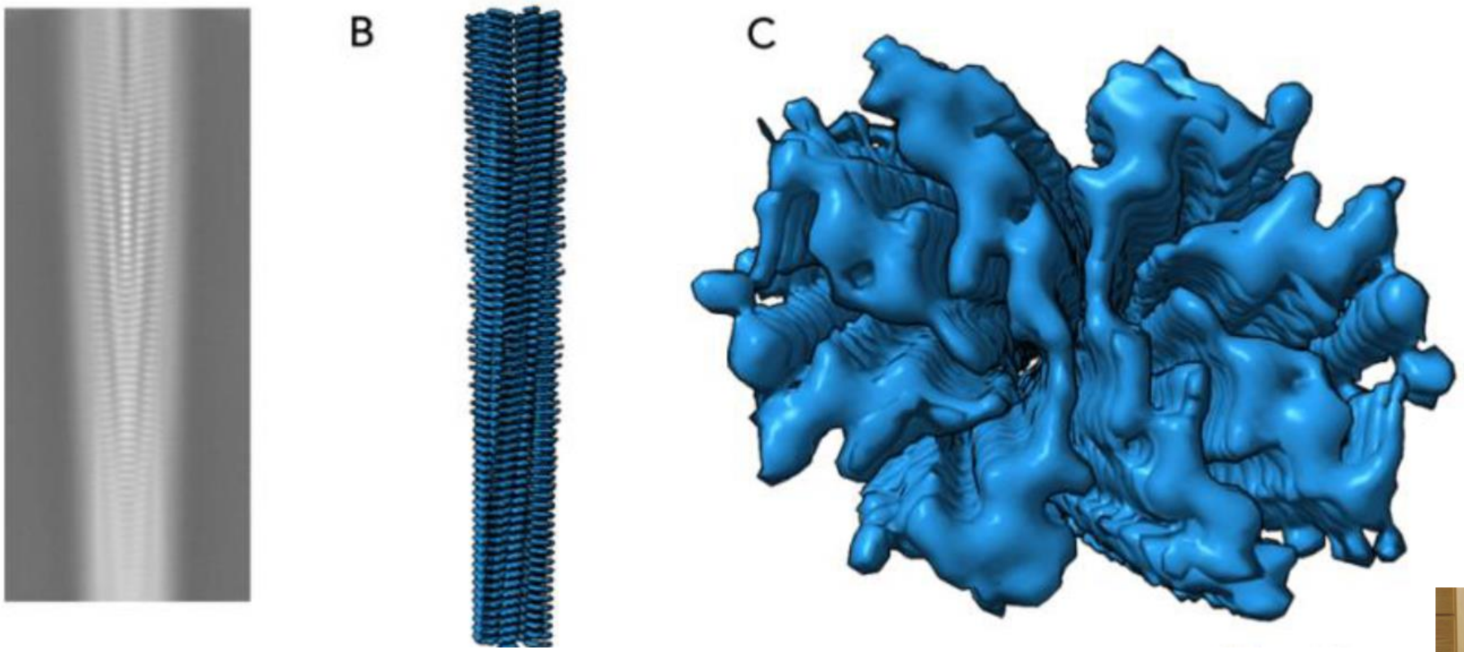
Dead End

”fibril inducing structure”



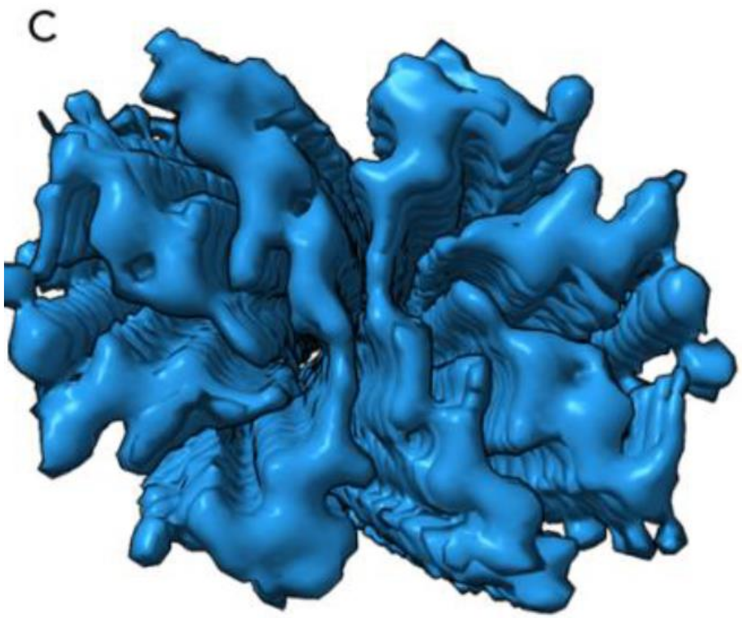


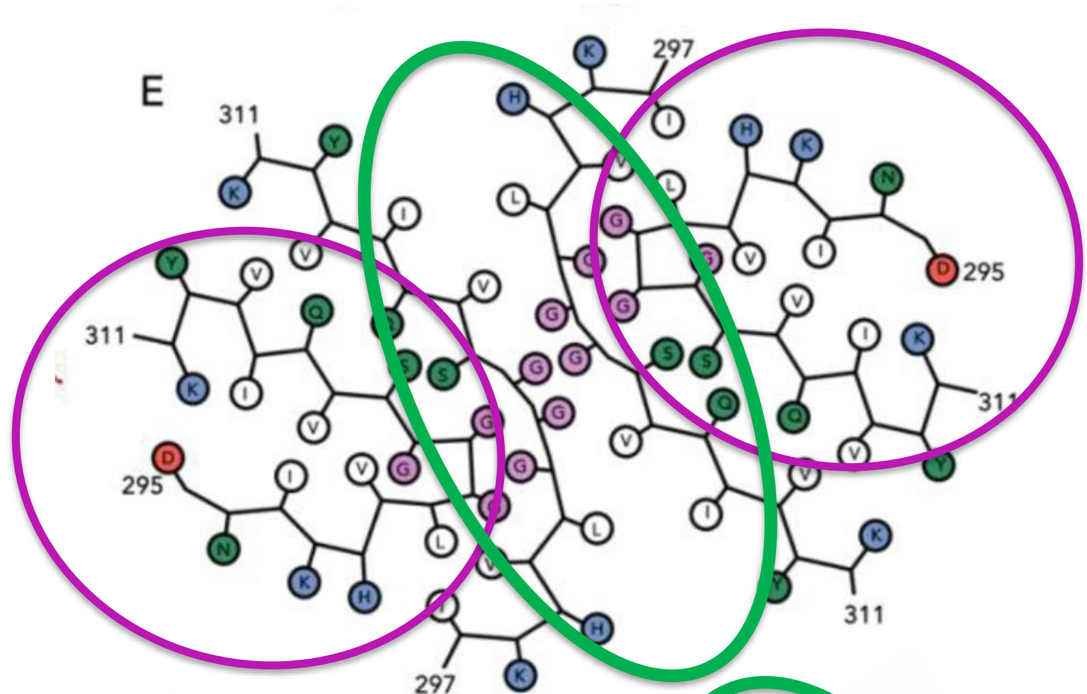
Recent CryoEM structure of jR2R3-P301L



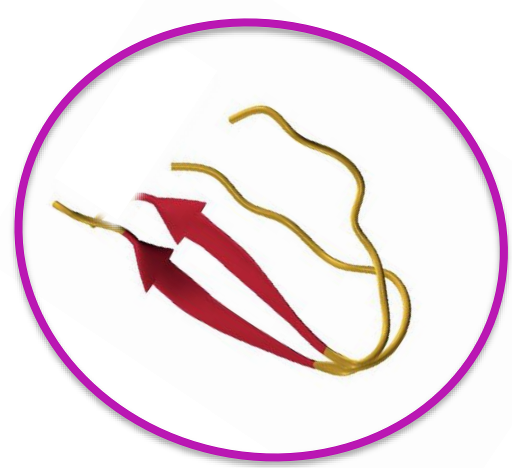
Professor Songi Han
UCSB/
Northwestern University

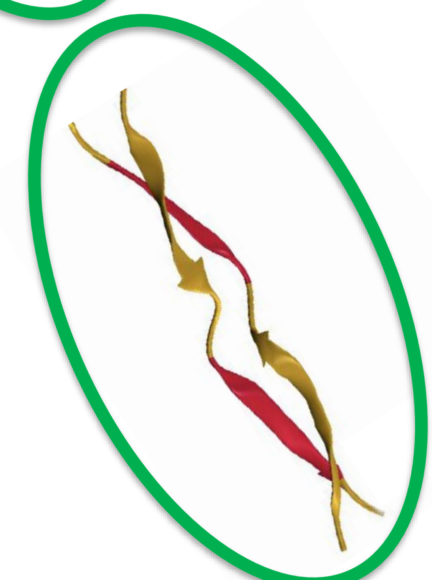
CryoEM structure of jR2R3-P301L

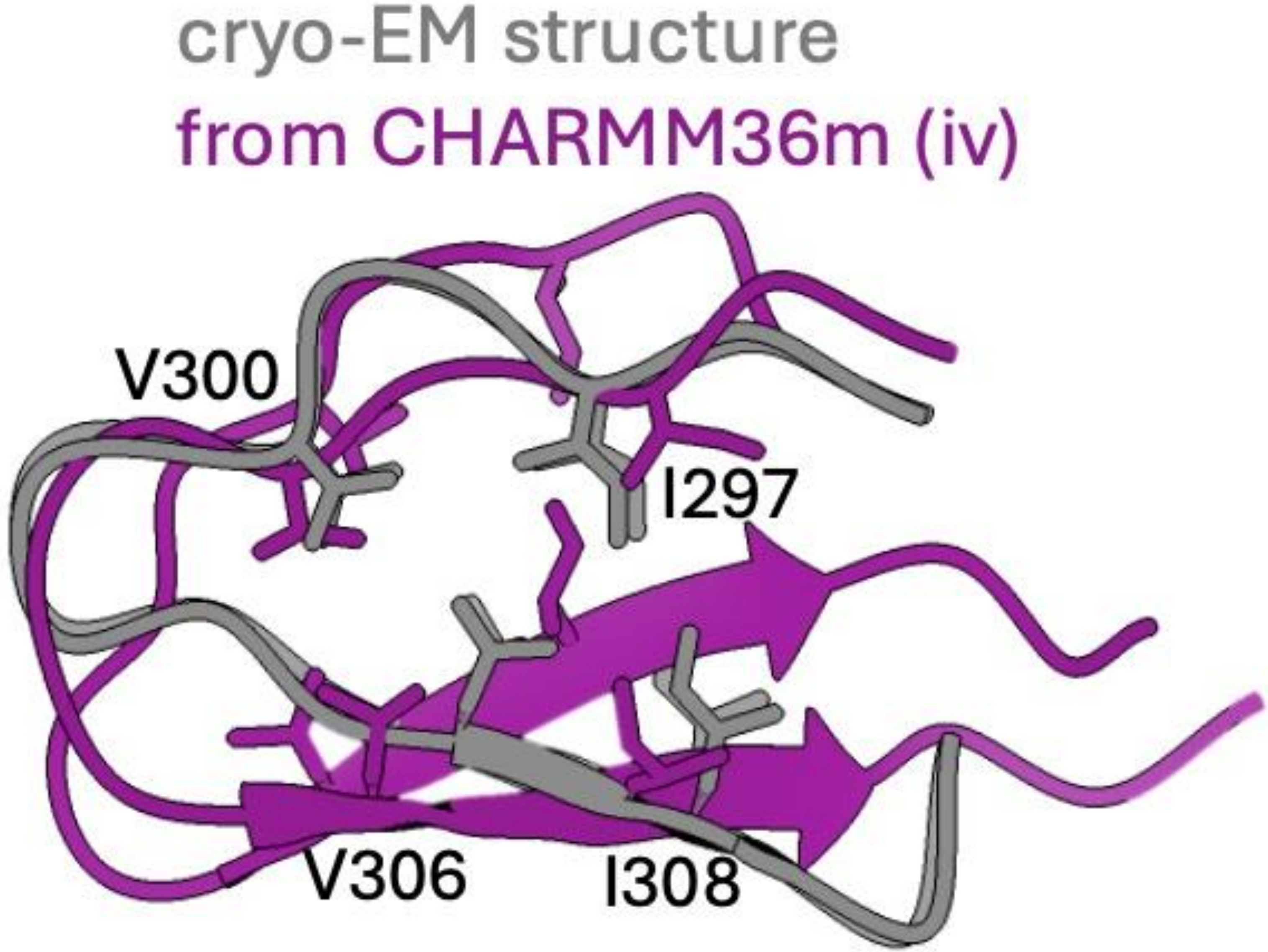




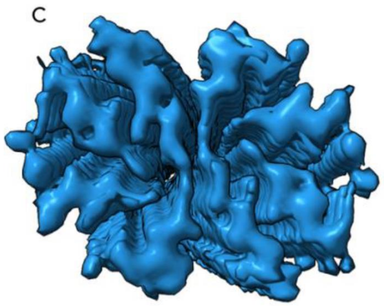
Simulation

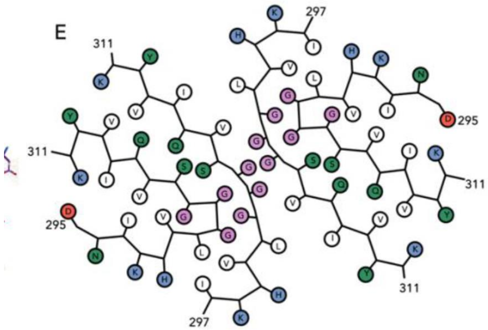


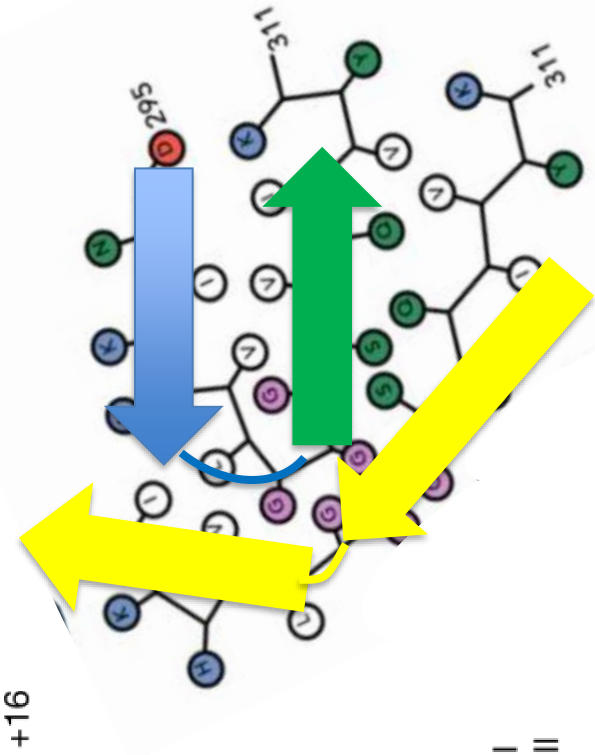



cryo-EM structure
from CHARMM36m (iv)
V300
I297
V306
I308

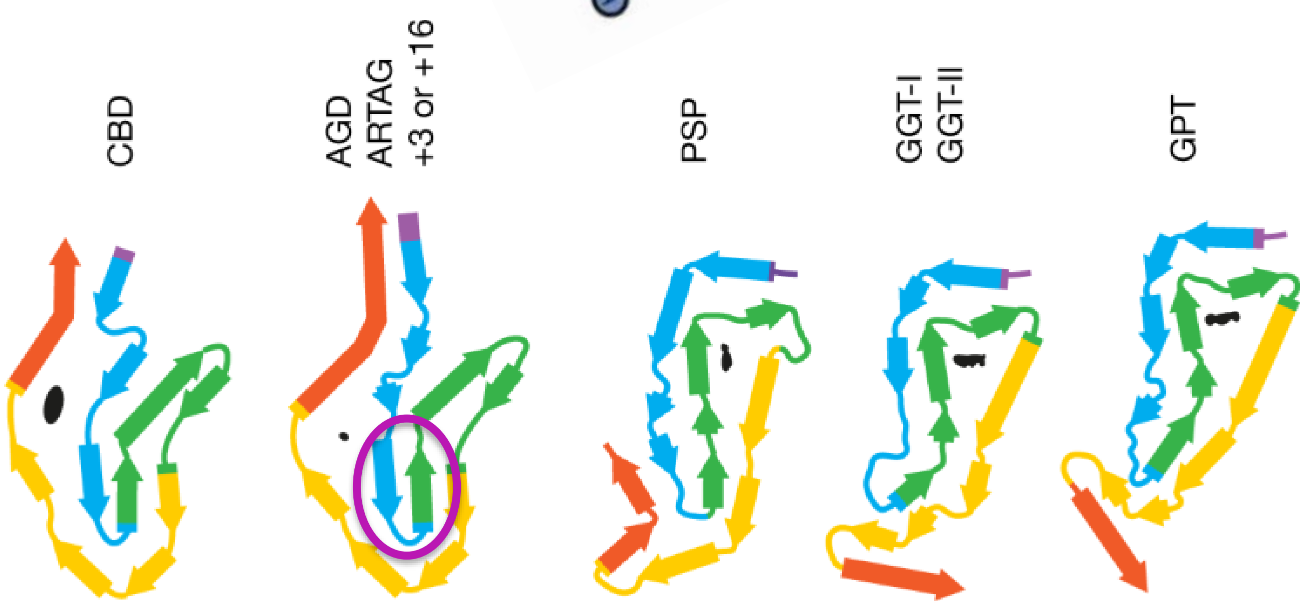
Importance of the Counter-Strand



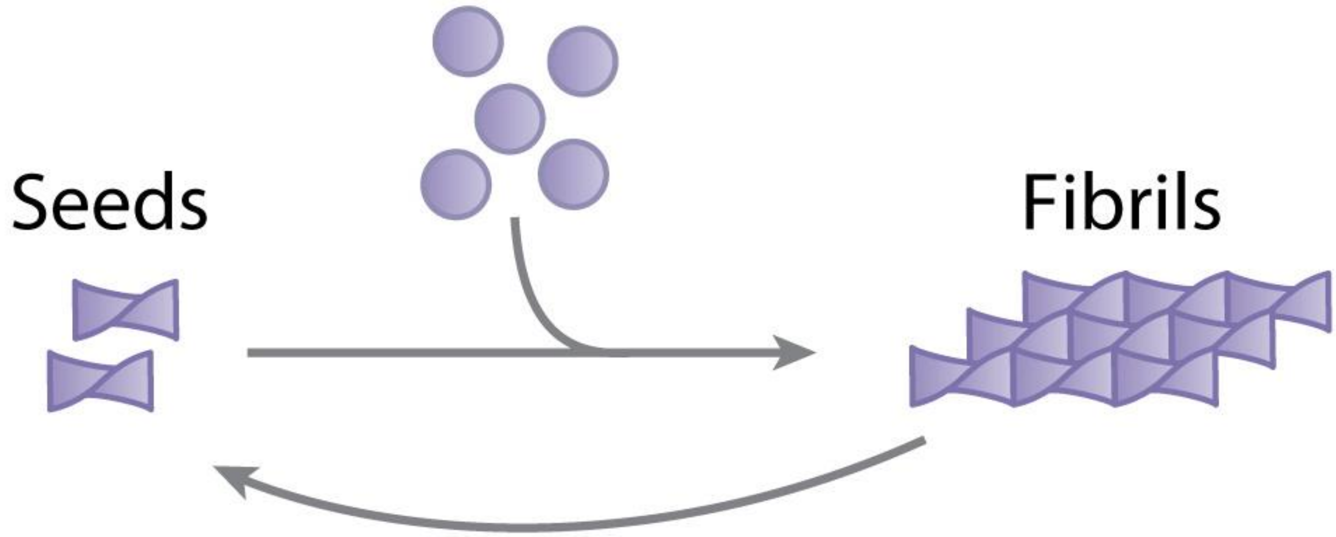




jR2R3-P301L

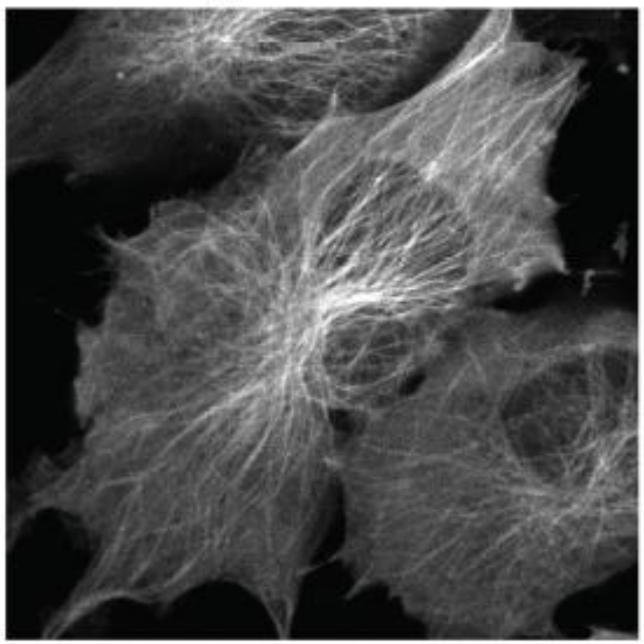


jR2R3-P301L can seed the fibrillization of full length Tau in Vivo

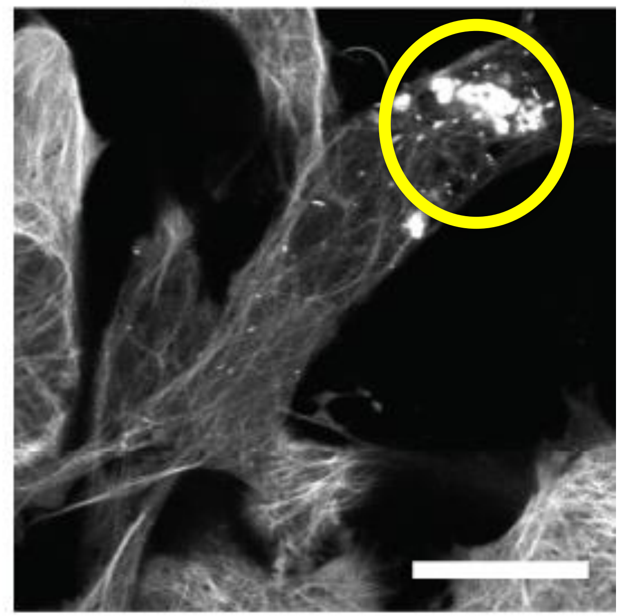


jR2R3-P301L can seed the fibrillization of full length Tau in Vivo

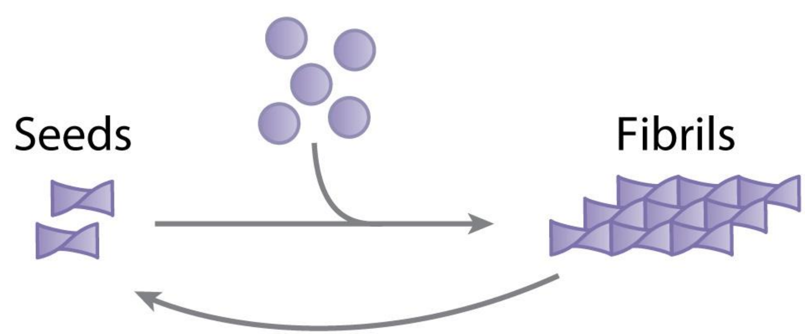
Before jR2R3 P301L



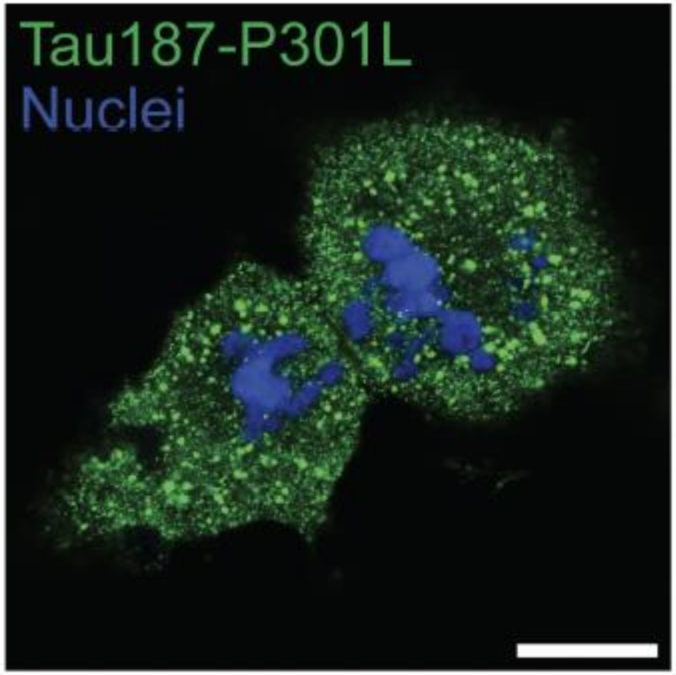
After jR2R3 P301L



Prof. Ken Kosik, UCSB

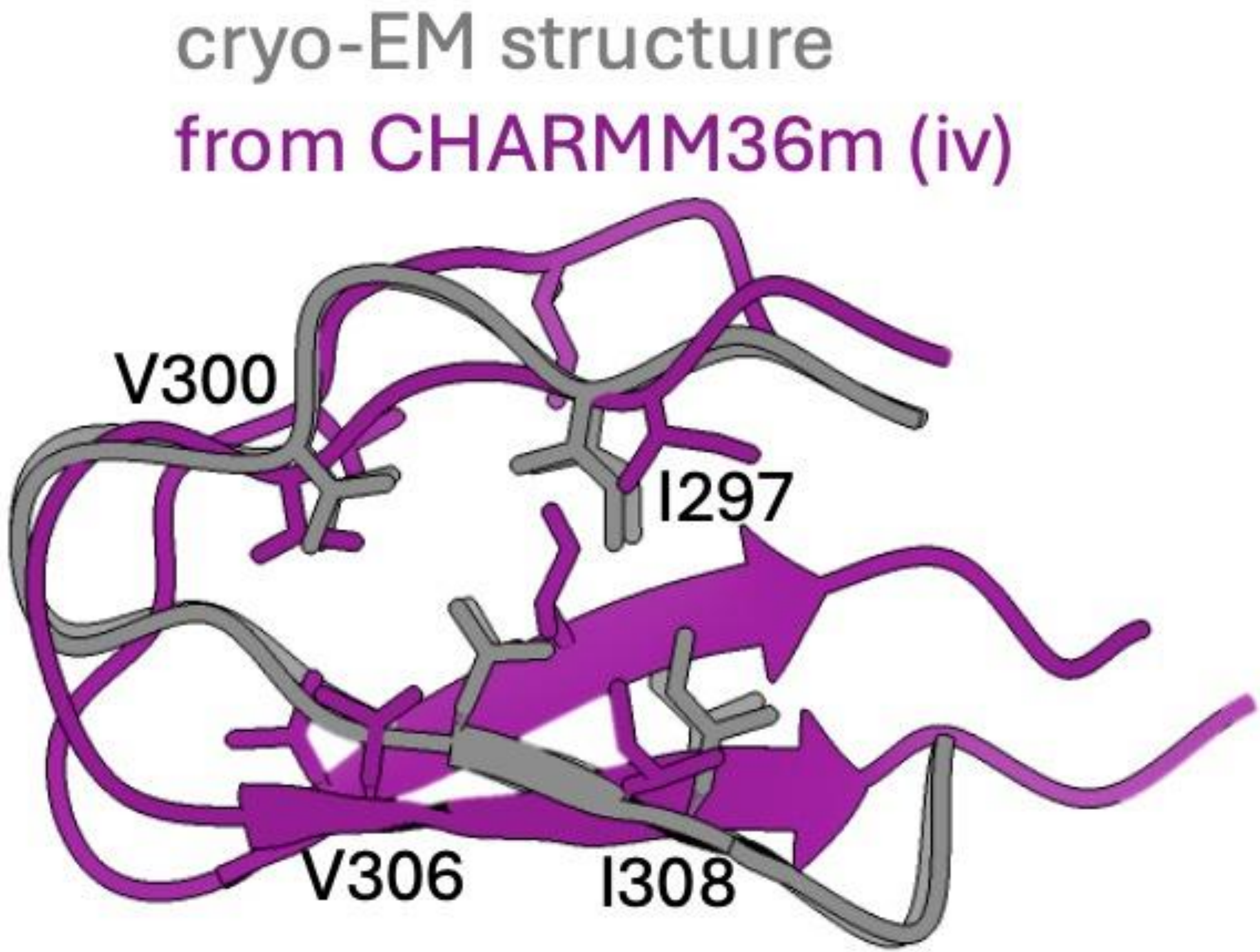


jR2R3-P301L acts as a prion: propagates the strain



PNAS (2024)
121 (15) e2320456121

Cells seeded with jR2R3-P301L fibrils undergo division and propagate aggregates to daughter cells

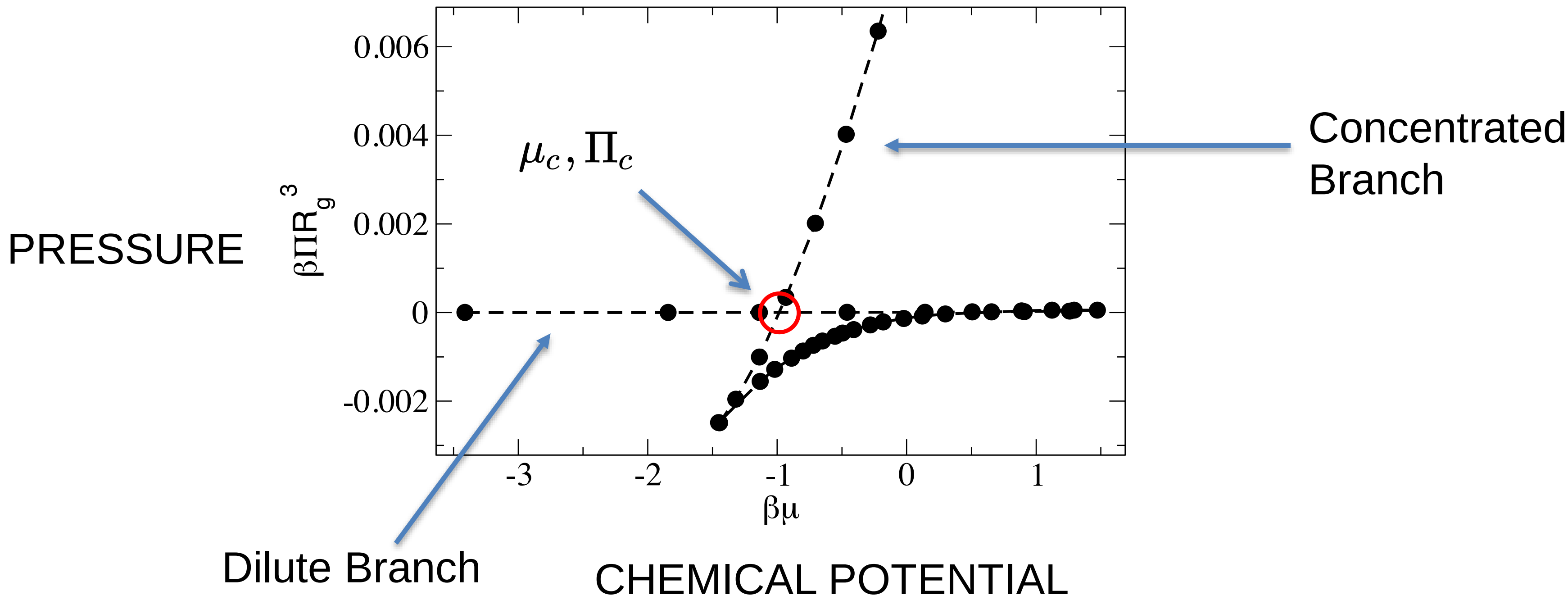

cryo-EM structure
from CHARMM36m (iv)
V300
I297
V306
I308

Back-up Slides

Determining Critical Points

diblock $E = 6.0$ $B = 0.1$

sv30 EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK

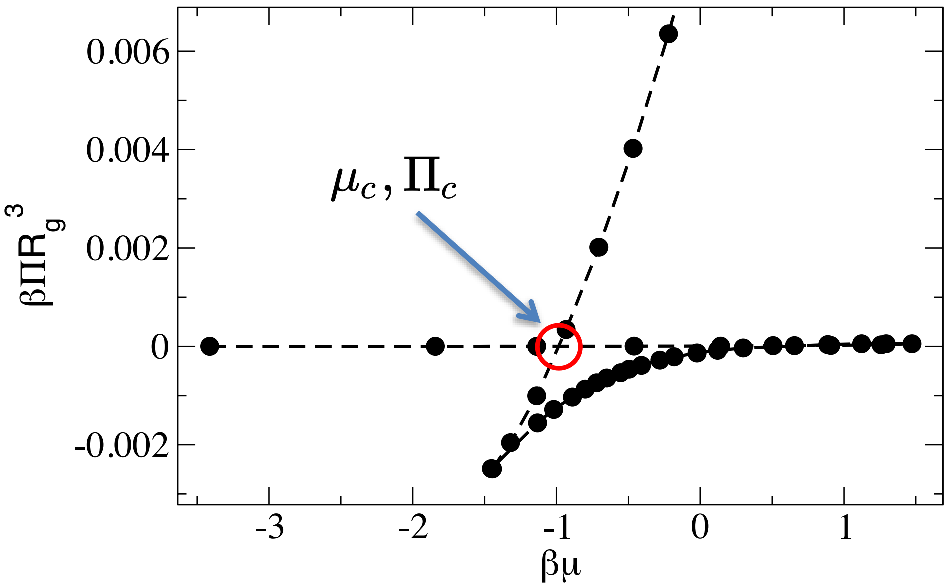


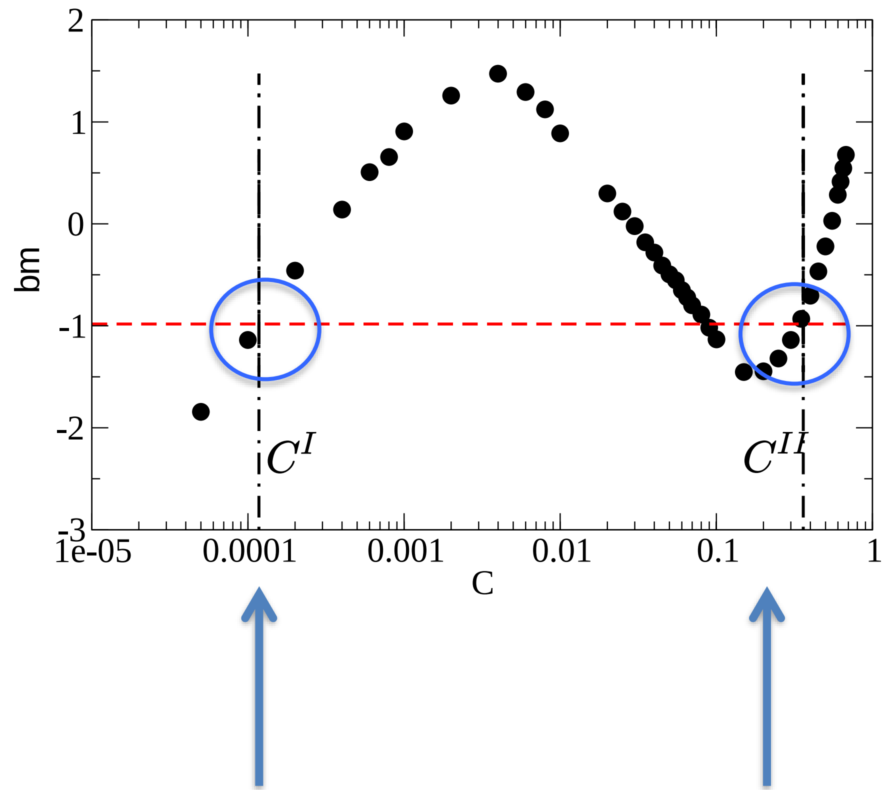
Mechanical (Pressure Π) and Chemical (Chemical Potential μ) Equilibrium

Determining Critical Points

diblock $E = 6.0$ $B = 0.1$

sv30 EEEEEEEEEEEEEEEEEEEEEEEEEKKKKKKKKKKKKKKKKKKKKKKKKK

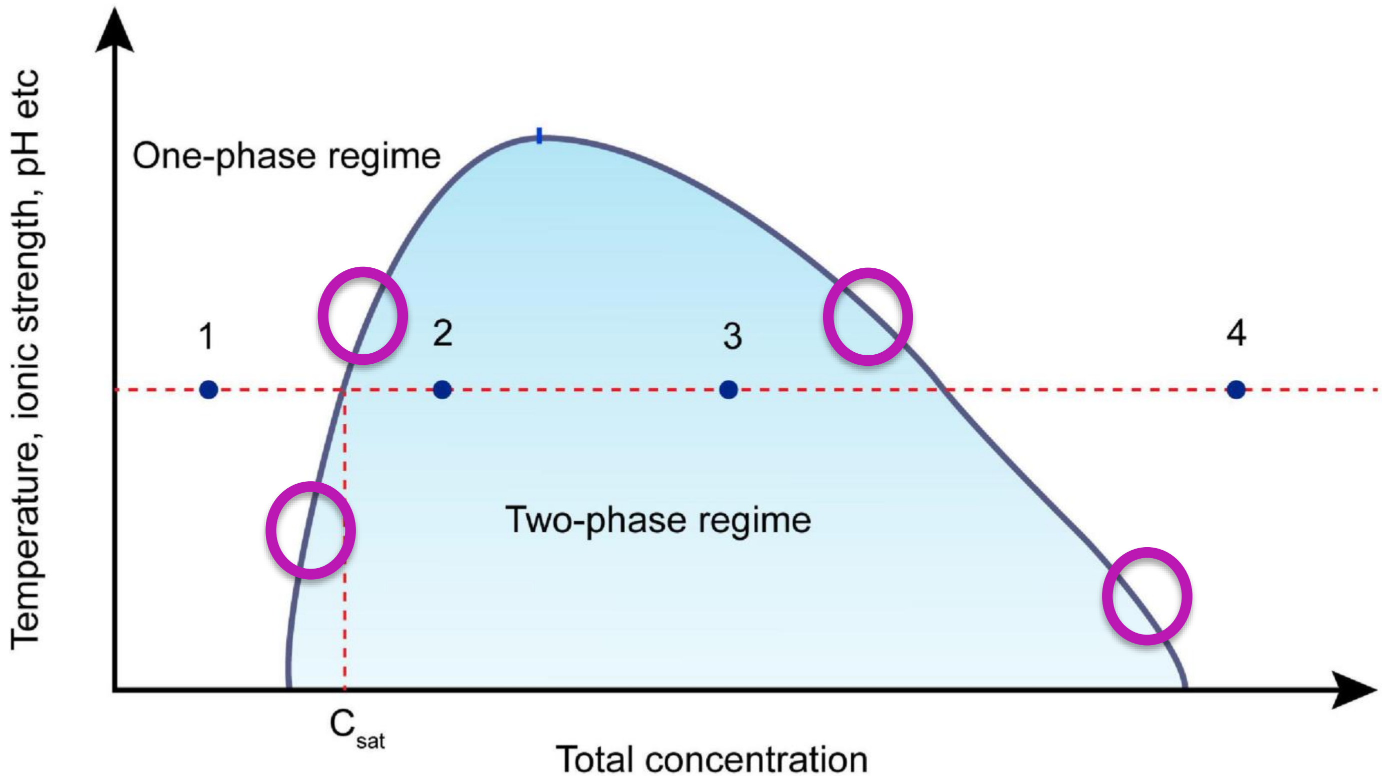


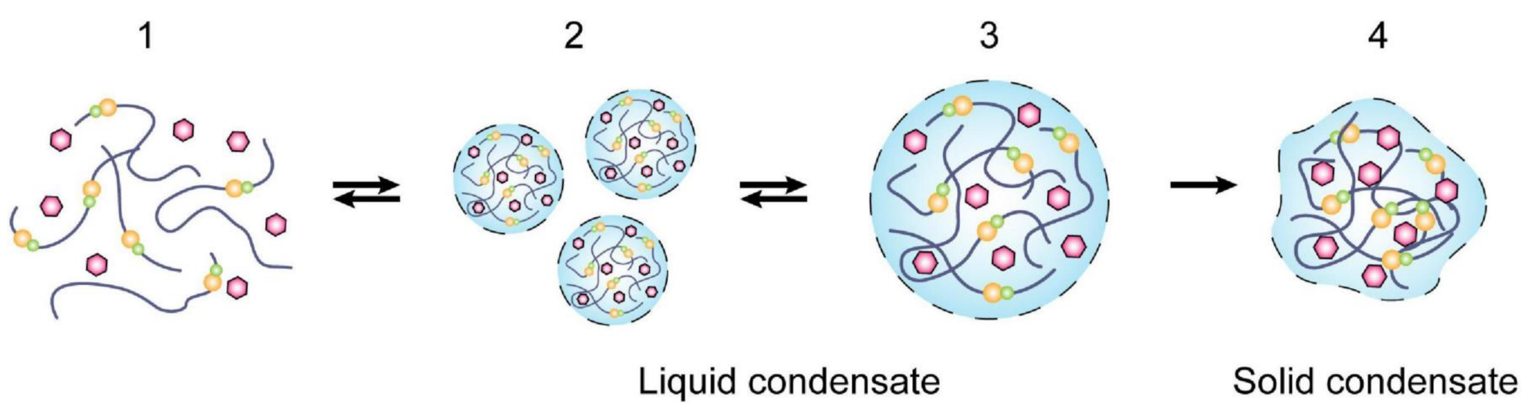


Protein depleted

Protein rich (coacervate)

We map the boundaries of the phase diagram





Field Theoretic Model

Partition function for a system of charged Gaussian chains and salt in implicit solvent

$$Z = Z_0 \int Dw \int D\psi \, e^{-H[w,\psi]}$$

Excluded volume interactions

Electrostatic interactions

$$H[w,\psi] = \frac{1}{2B}\int d\mathbf{r}\; w(\mathbf{r})^2 + \frac{1}{2E}\int d\mathbf{r}\; |\nabla\psi|^2 - \sum_l \frac{CV\bar{\phi}_l}{N_l}\ln Q_l$$

Fluctuating chemical potential field

Fluctuating electrostatic potential field

Single chain partition function for polymers and salt ions (complex valued)

B: dimensionless excluded volume parameter

E: dimensionless Bjerrum length

C: dimensionless monomer density

Field Theoretic Simulations

Complex Langevin equations of motion

$$\frac{\partial w(r,t)}{\partial t} = -\lambda_w \frac{\delta H[w,\psi]}{\delta w(r,t)} + \eta_w(r,t)$$

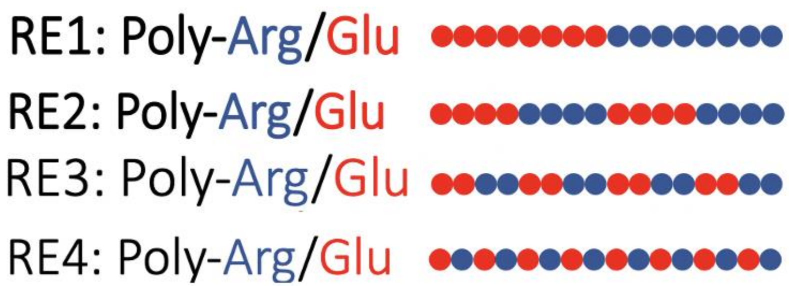
$$\frac{\partial \psi(r,t)}{\partial t} = -\lambda_\psi \frac{\delta H[w,\psi]}{\delta \psi(r,t)} + \eta_\psi(r,t)$$

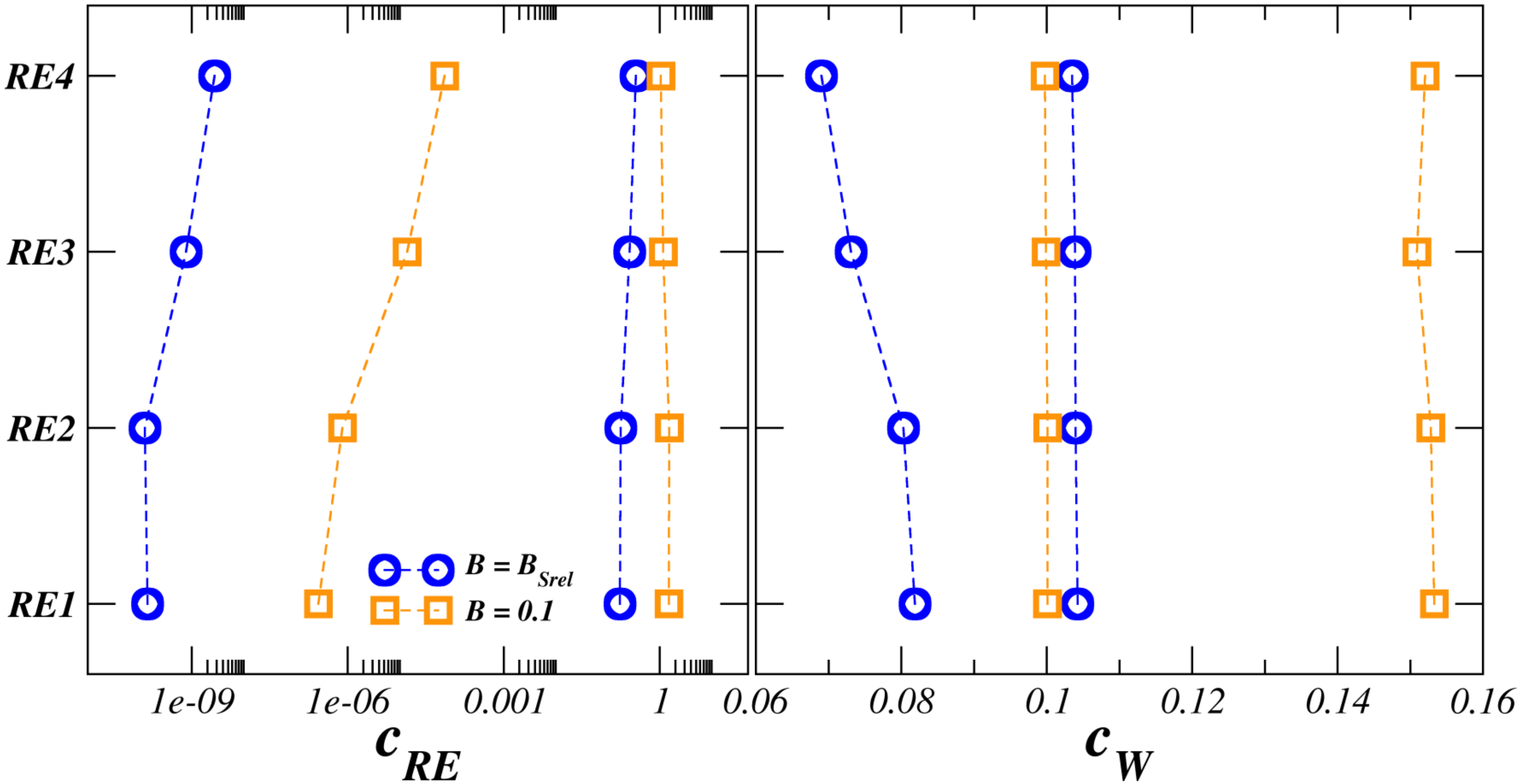
real Gaussian
white noise

Ensemble averages over the field configurations

$$\langle O \rangle = \frac{\int Dw \int D\psi \; O e^{-H[w,\psi]}}{\int Dw \int D\psi \; e^{-H[w,\psi]}}$$

RE peptides phase behavior





Discrete Gaussian chain polyelectrolyte model

dimensionless monomer density

$$\boxed{C} \sim \rho b^3$$

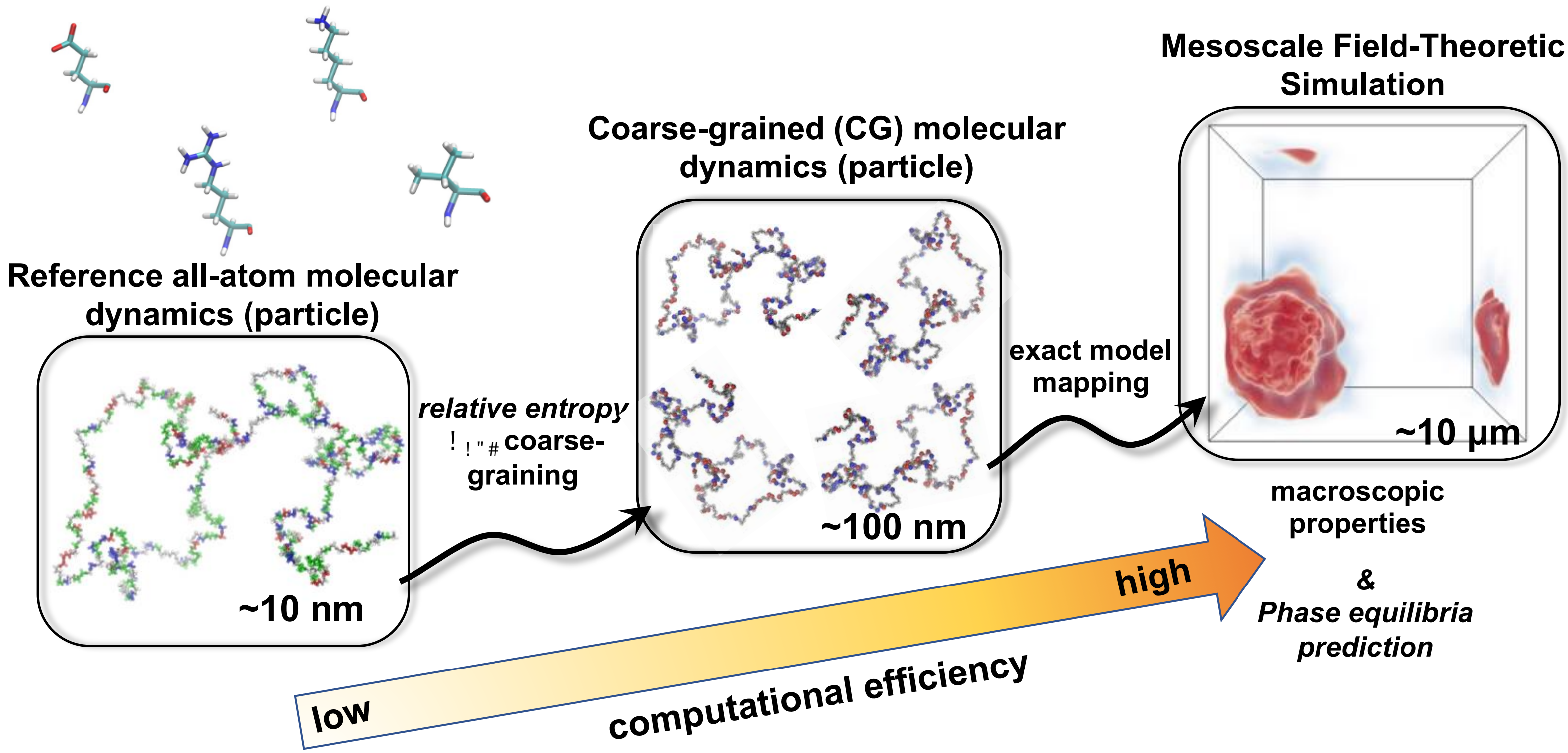
dimensionless excluded volume parameter

$$\beta U = \frac{v}{2} \int d\mathbf{r} \bar{\rho}^2(\mathbf{r}) \qquad \boxed{B} \sim \frac{v}{b^3}$$

dimensionless Bjerrum length

$$\beta U = \frac{l_B}{2} \int d\mathbf{r} \int d\mathbf{r}' \frac{\bar{\rho}_e(\mathbf{r})\bar{\rho}_e(\mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|} \qquad \boxed{E} \sim \frac{l_B}{b}$$

Coarse graining though minimizing S_{rel} (Information Loss)



Chemical Detail & transferability

Minimizing the relative entropy (information-loss):

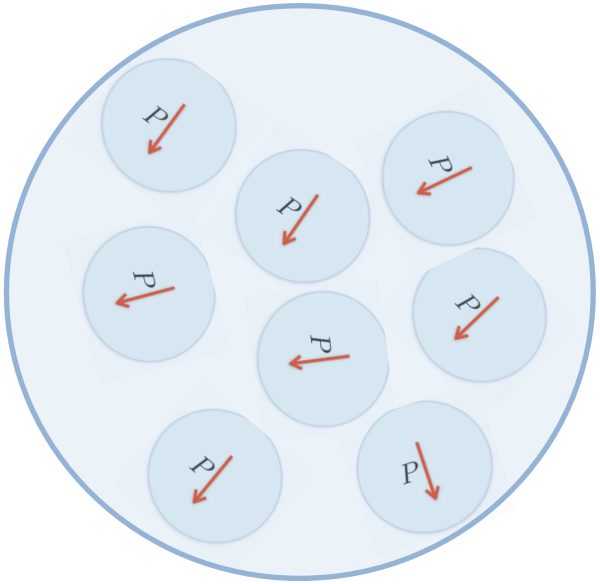
$$S_{rel} = \int\int \wp_{AA}(\boldsymbol{r}) \ln\left(\frac{\wp_{AA}(\boldsymbol{r})}{\wp_{CG}(\boldsymbol{R})}\right) \delta(\boldsymbol{M}(\boldsymbol{r}) - \boldsymbol{R}) d\boldsymbol{r}\, d\boldsymbol{R}$$

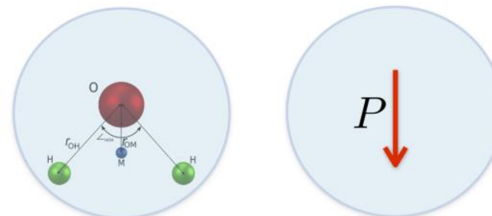
Shell Adv. Chem. Phys. 2016

Field theoretic simulation water model

FTS simulation in NVT ensemble

8 AA waters → 1 CG water



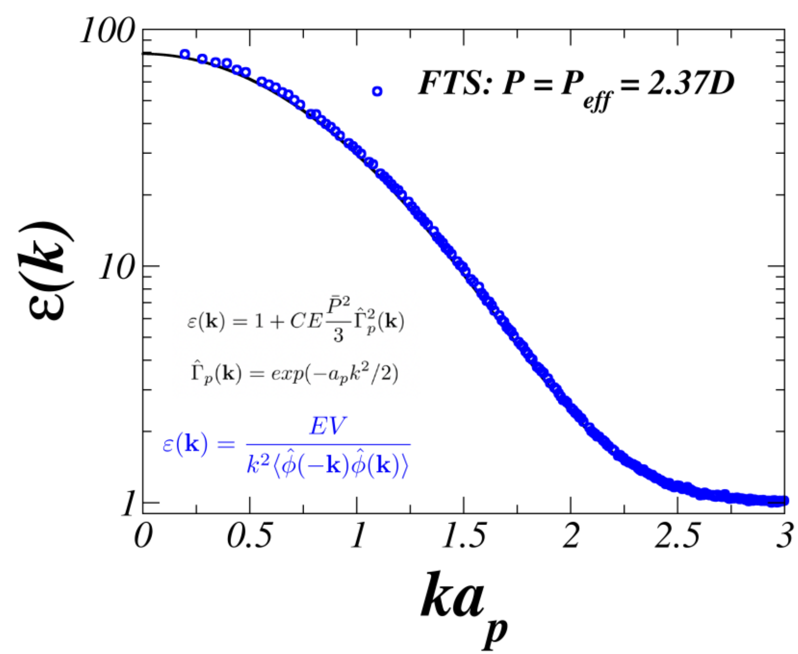


Atomistic model Dimer model

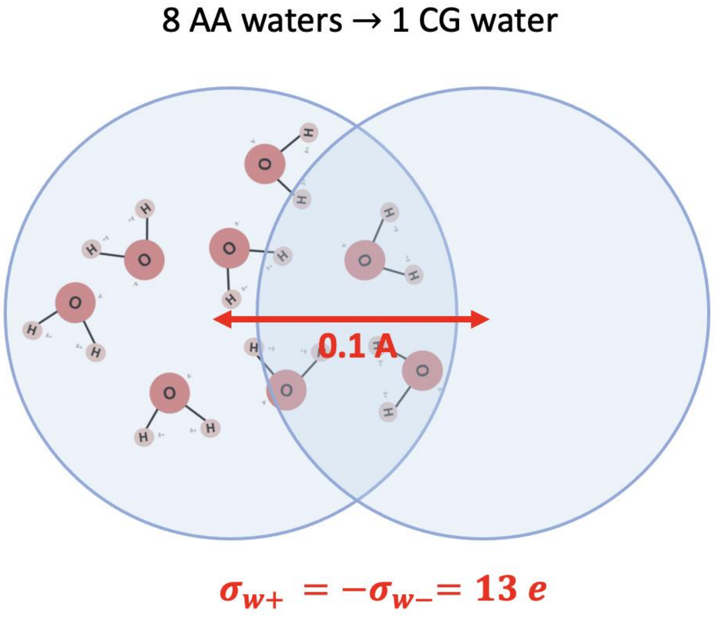
$$\beta \bar{U}[\mathbf{r}] = \frac{v}{2}\int d\mathbf{r}\, \bar{\rho}^2(\mathbf{r}) + \frac{l_B}{2}\iint d\mathbf{r}\, d\mathbf{r}' \frac{\bar{\rho}_e(\mathbf{r})\bar{\rho}_e(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|}$$

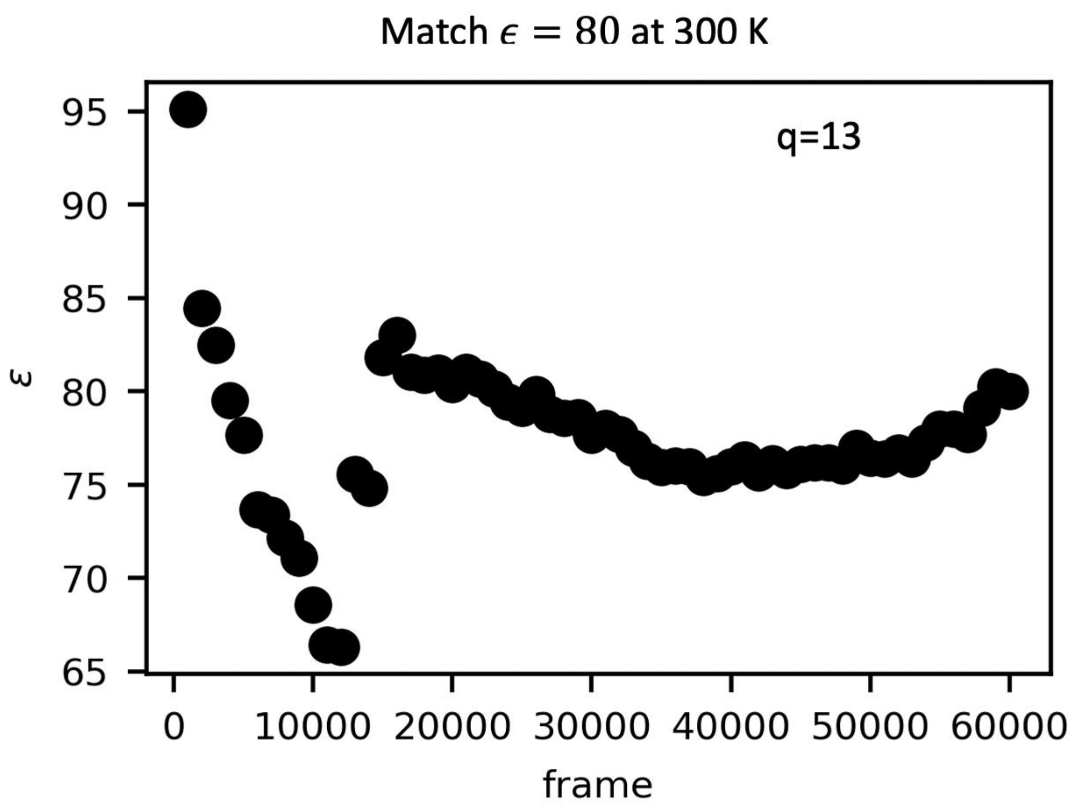
$$\bar{\rho}(\mathbf{r}) = \int d\mathbf{r}'\, \Gamma(|\mathbf{r}-\mathbf{r}'|)\hat{\rho}(\mathbf{r}')$$

$$\Gamma(r) = \left(\frac{1}{2\pi a^2}\right)^{3/2} \exp(-r^2/2a^2)$$



MD coarse-grained water model

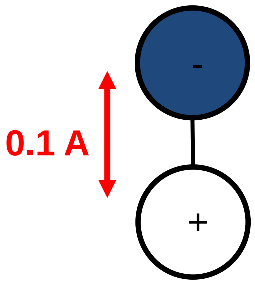




Relative entropy parametrization of peptide and water interaction

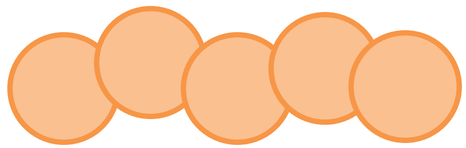
RE1: Poly-Arg/Glu

water



$\sigma_{w+} = -\sigma_{w-} = 13e$

peptide



$\sigma_i = +/-1$

$\sigma = a_i = R_w = 0.158\, nm$

- $\beta U_{bond,water}$: stiff harmonic with $b = 0.01\, nm$
- $\beta U_{bond,residue}$: harmonic with $b = \sqrt{6}\,\sigma$
- Electrostatics: $\beta U_{el,W-,W+}, \beta U_{el,W+,W+}, \beta U_{el,W-,W-}$

- Excluded volumes:
  - $u_{W-,W-} = u_{Lys,Lys} = u_{Cl,Cl} = u_{W-,Cl} = u_{Lys,Cl} = 0.1\ kT\ \sigma^3$
  - $u_{W-,residue} = \left[B^{Srel}(4\pi\sigma^2)^{\frac{3}{2}}\sigma^3\right] N_{ref}^2 \sigma^{-3} = B_{W-,residue}^{Srel}(4\pi)^{3/2}\sigma^3$

Coarse-grain interactions

$$U_{CG} = \sum_{bonds} U_{bond} + \sum_{i}^{N_T - 1} \sum_{j=i+1}^{N_T} U_{ev}(r_{ij}) + U_{el}(r_{ij})$$

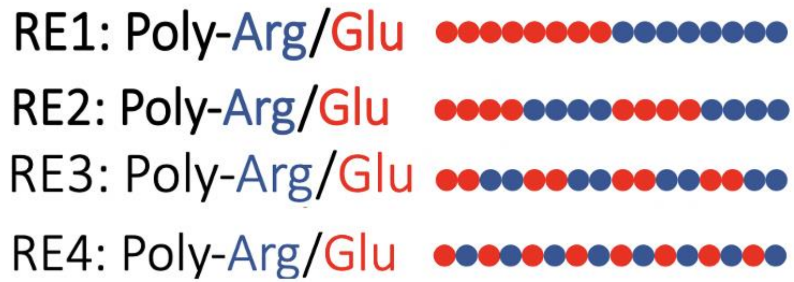
$$\beta U_{bond}(r_{ij}) = \frac{3}{2b^2}(r_{ij} - r_0)^2$$

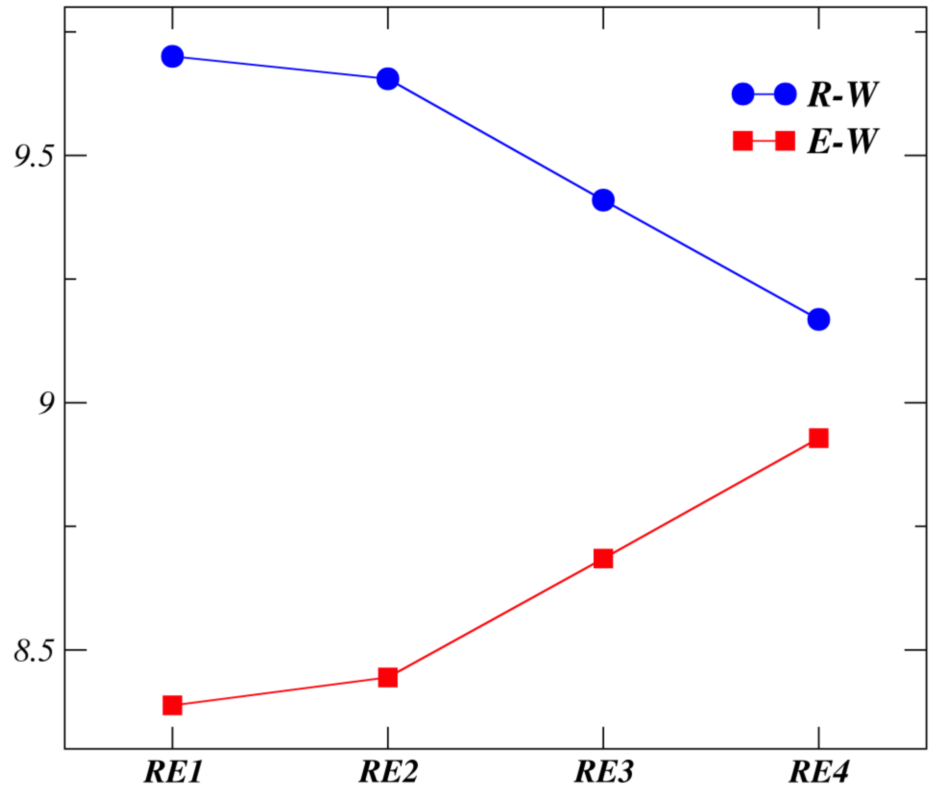
$$\beta U_{ev}(r_{ij}) = \frac{u_{ij}}{(2\pi(a_i^2 + a_j^2))^{3/2}} e^{-r_{ij}^2/2(a_i^2 + a_j^2)}$$

$$\beta U_{el}(r_{ij}) = \frac{l_B \sigma_i \sigma_j}{r_{ij}} erf\left(\frac{r_{ij}}{2\sqrt{a_i^2/2 + a_j^2/2}}\right)$$

$a_i = 0.316$ nm: bead radius
b: statistical segment length
$r_0 = 0$: equilibrium bond distance
u_{ij}: excluded volume
σ_i: charge
$l_B = 561.6\ nm$: Bjerrum length

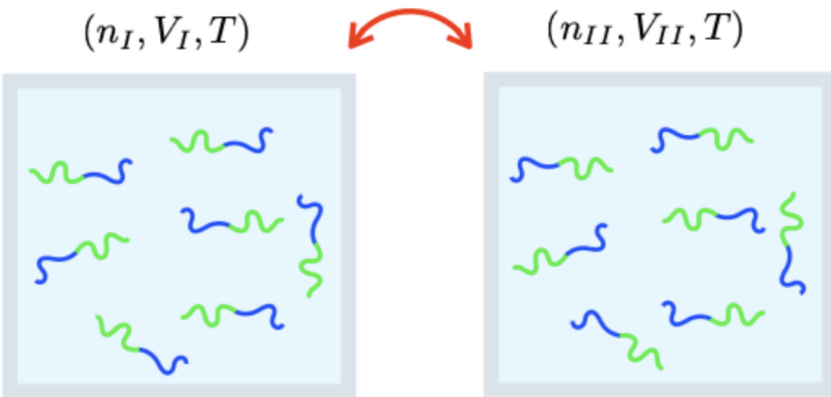
Sequence Dependent Water-Residue Effective Interaction





Excluded volume interaction with water

Gibbs Ensemble Simulations

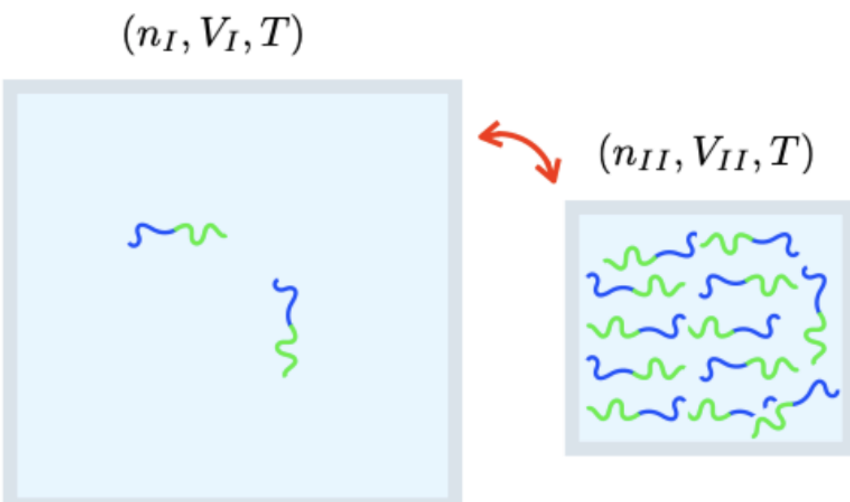


$$V_T = V_I + V_{II} \qquad n_T = n_I + n_{II}$$

$$F(n, V_T, T) = F_I(n_I, V_I, T) + F_{II}(n_{II}, V_{II}, T)$$

$$\frac{\partial F}{\partial V_I} = -(\Pi_I - \Pi_{II})$$

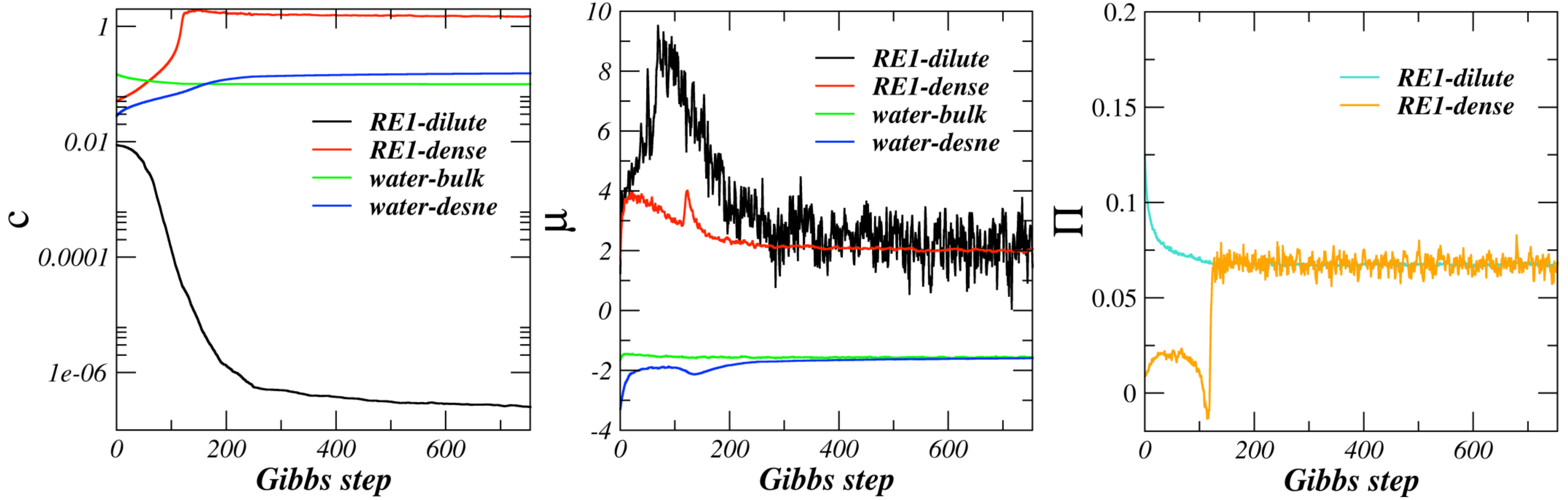
$$\frac{\partial F}{\partial n_I} = (\mu_I - \mu_{II})$$



$$\frac{\partial F}{\partial V_I} = -(\Pi_I - \Pi_{II}) = 0$$

$$\frac{\partial F}{\partial n_I} = (\mu_I - \mu_{II}) = 0$$

Gibbs Ensemble Field Theory Simulation (FTS) convergence



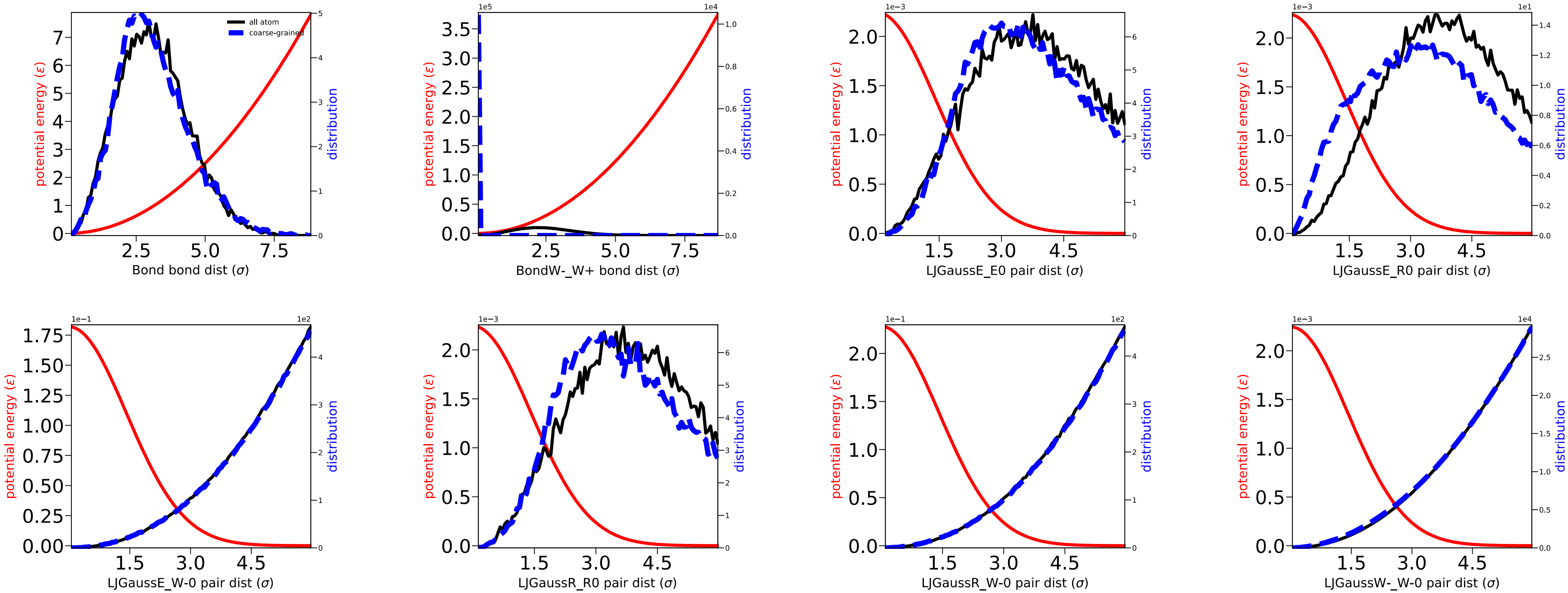
CG parametrization of peptide-water interaction using relative-entropy

Optimized parameters:

Blurring sigma 0.35

E-W B=0. 182

R-W B=0.228



- The implicit solvent model cannot capture the heterogeneous dielectricity that may occur within the peptide-dense phase. In contrast, the explicit solvent model accounts for environment-dependent dielectricity, unlike the uniform dielectricity assumed in the implicit solvent model.

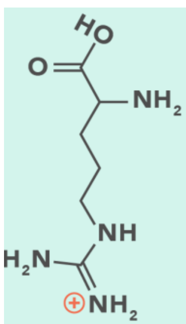
- This explicit solvent model can be parameterized to incorporate the effect of the peptide sequence on the effective interaction between residues and water into the coarse-graining (CG) process. Previous CG methods totally ignore this.

- As a result, the phase diagram derived from accurately transferring chemical details to the CG system is expected to capture any mesoscopic phase transitions and morphological complexities.

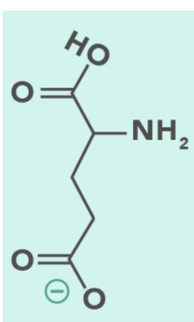
-

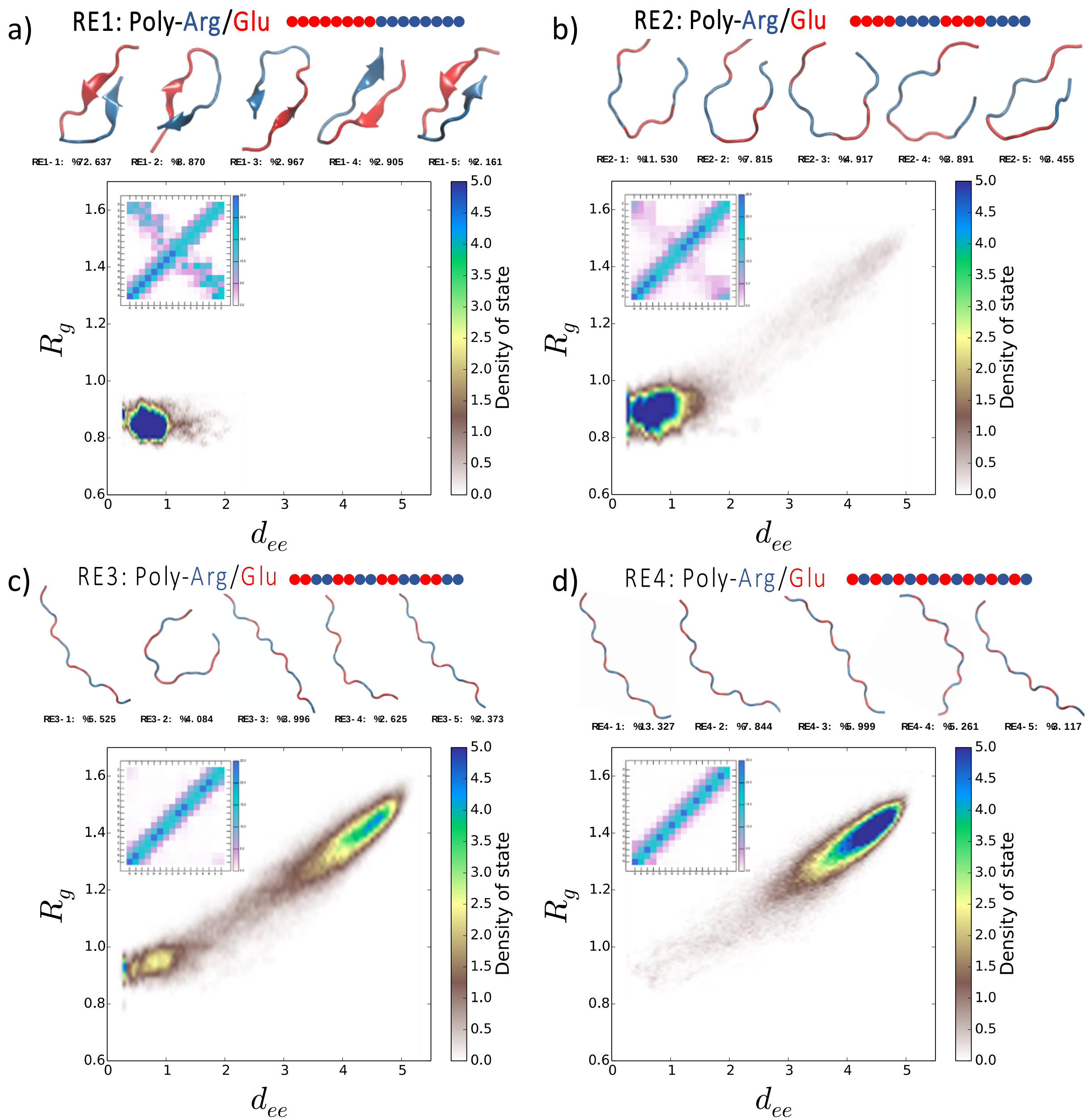
All atom reference simulations for coarse graining

Arg



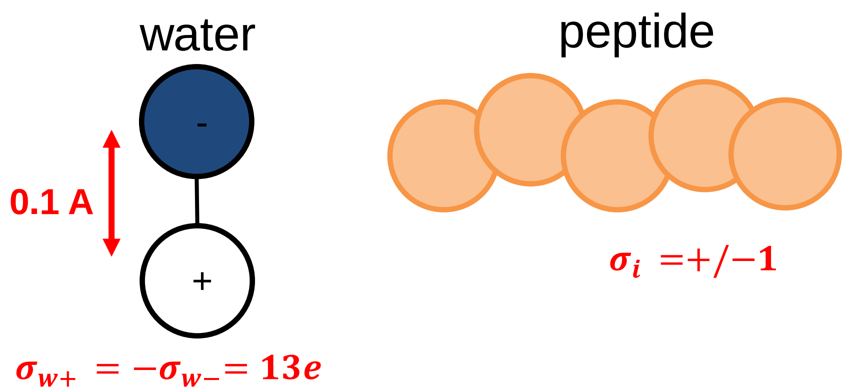
Glu





Relative entropy parametrization of peptide and water interaction

RE1: Poly-Arg/Glu



$$\sigma = a_i = R_w = 0.158\, nm$$

- $\beta U_{bond,water}$: stiff harmonic with $b = 0.01\ nm$
- $\beta U_{bond,residue}$: harmonic with $b = \sqrt{6}\ \sigma$
- Electrostatics: $\beta U_{el,W-,W+}, \beta U_{el,W+,W+}, \beta U_{el,W-,W-}$

- Excluded volumes:
  - $u_{W-,W-} = u_{Lys,Lys} = u_{Cl,Cl} = u_{W-,Cl} = u_{Lys,Cl} = 0.1\ kT\ \sigma^3$
  - $u_{W-,residue} = \left[B^{Srel}(4\pi\sigma^2)^{\frac{3}{2}}\sigma^3\right]N_{ref}^2\sigma^{-3} = B^{Srel}_{W-,residue}(4\pi)^{3/2}\sigma^3$

Coarse-grain interactions

$$U_{CG} = \sum_{bonds} U_{bond} + \sum_{i}^{N_T-1}\sum_{j=i+1}^{N_T} U_{ev}(r_{ij}) + U_{el}(r_{ij})$$

$$\beta U_{bond}(r_{ij}) = \frac{3}{2b^2}(r_{ij} - r_0)^2$$

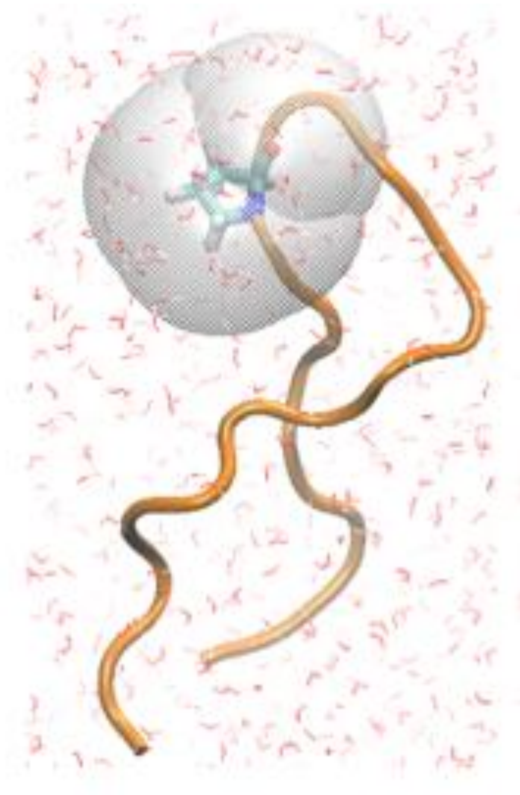
$$\beta U_{ev}(r_{ij}) = \frac{u_{ij}}{(2\pi(a_i^2 + a_j^2))^{3/2}} e^{-r_{ij}^2/2(a_i^2+a_j^2)}$$

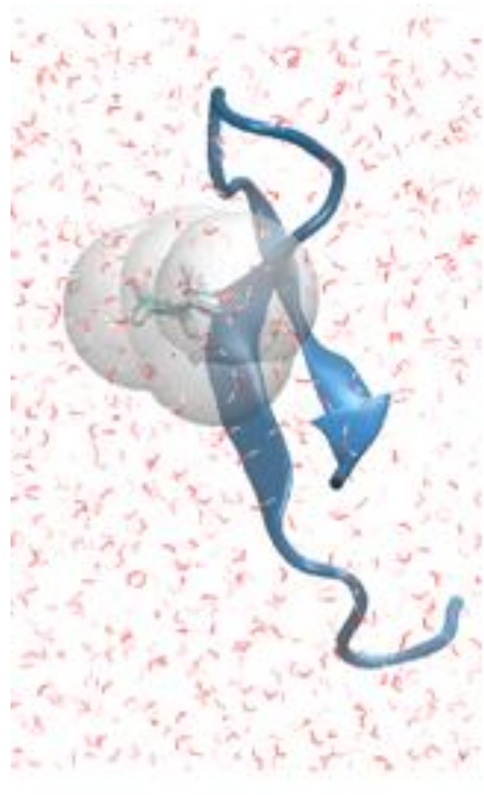
$$\beta U_{el}(r_{ij}) = \frac{l_B \sigma_i \sigma_j}{r_{ij}} erf\left(\frac{r_{ij}}{2\sqrt{a_i^2/2 + a_j^2/2}}\right)$$

$a_i = 0.316$ nm: bead radius
b: statistical segment length
$r_0 = 0$: equilibrium bond distance
u_{ij}: excluded volume
σ_i: charge
$l_B = 561.6\ nm$: Bjerrum length

Tau and Traumatic Brain Injury

Overhauser Dynamic Nuclear Polarization (ODNP) experiments show a reduction in hydration water dynamics around 301 site for the P→L mutant (jR2R3-P301L)





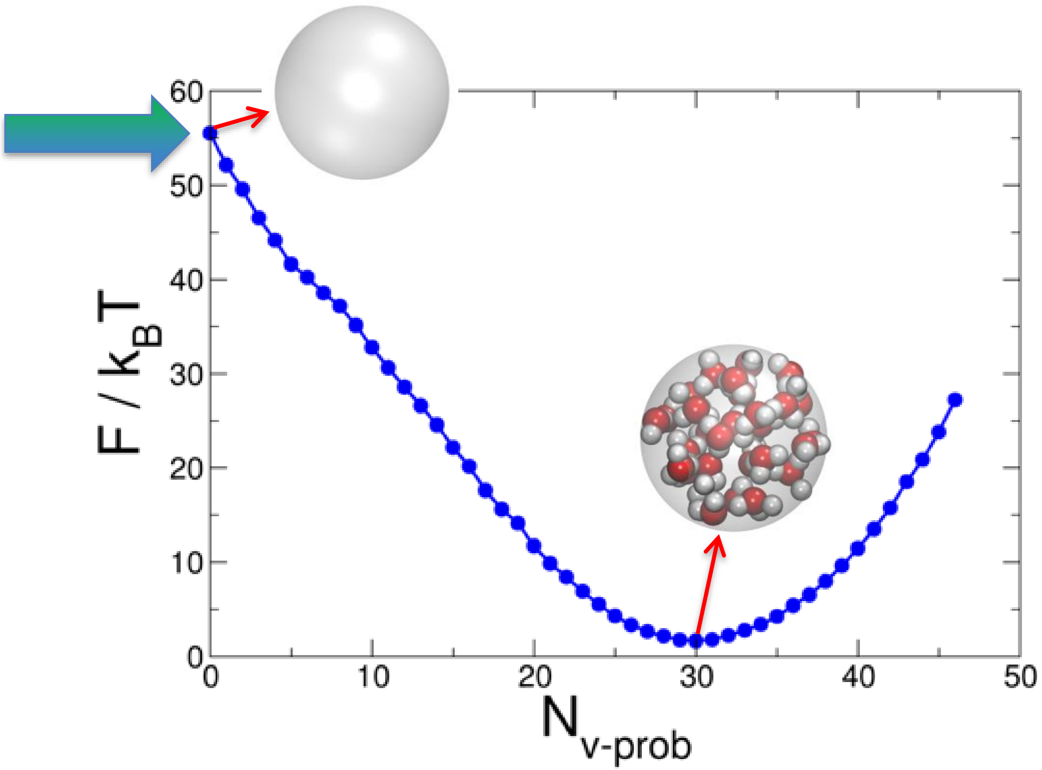
Increased ordering of water around mutation site

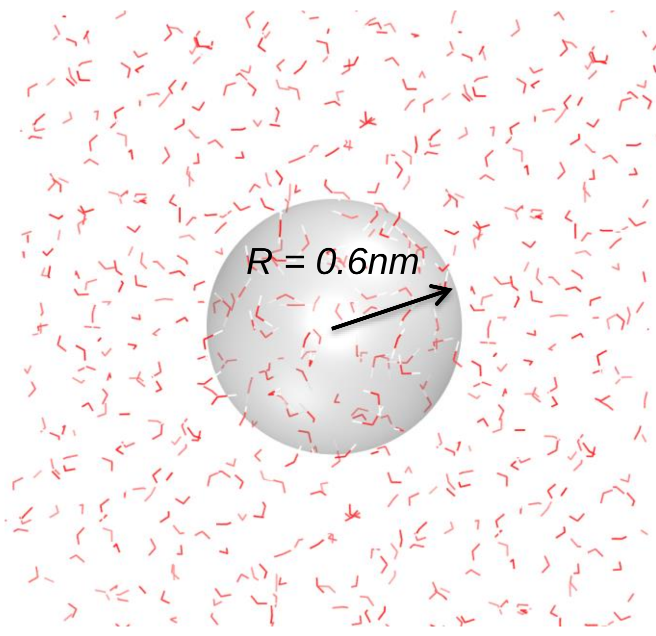
→ locally more hydrophobic

Probing Hydrophobicity Computationally through Umbrella Sampling (INDUS)

Free energy of dewetting a spherical volume in bulk

$$\mu_{ex} = \mathbf{F(0) = -lnP_v(0)}$$

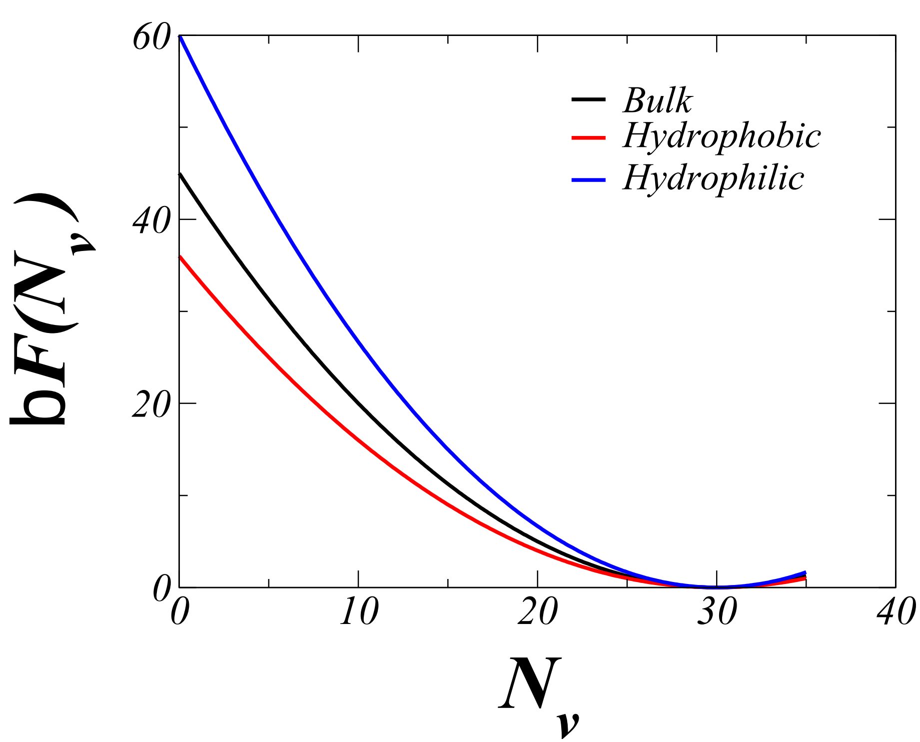


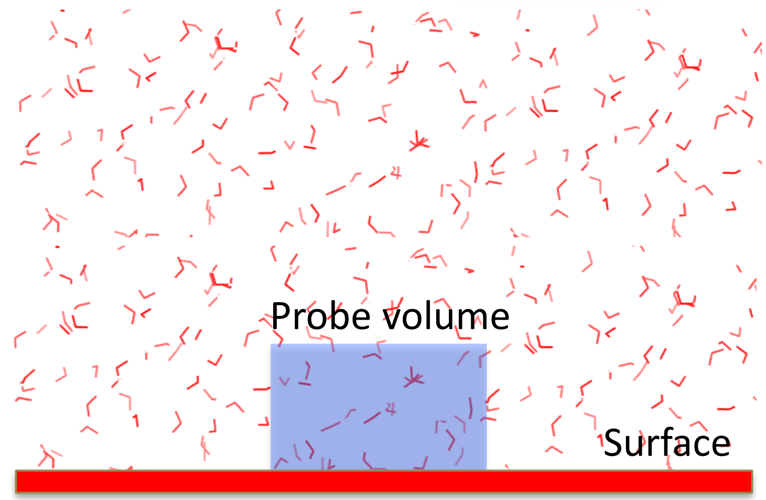


Free energy of dewetting the probe volume in vicinity of a surface

Excess chemical potential is an indication of hydrophilicity or hydrophobicity of the surface

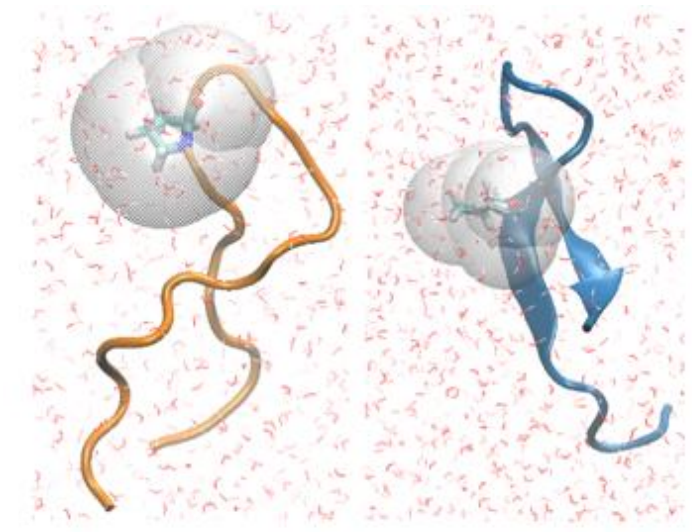
$$\mu_{\mathbf{ex}}^{\mathbf{phil}} > \mu_{\mathbf{ex}}^{\mathbf{bulk}} > \mu_{\mathbf{ex}}^{\mathbf{phob}}$$



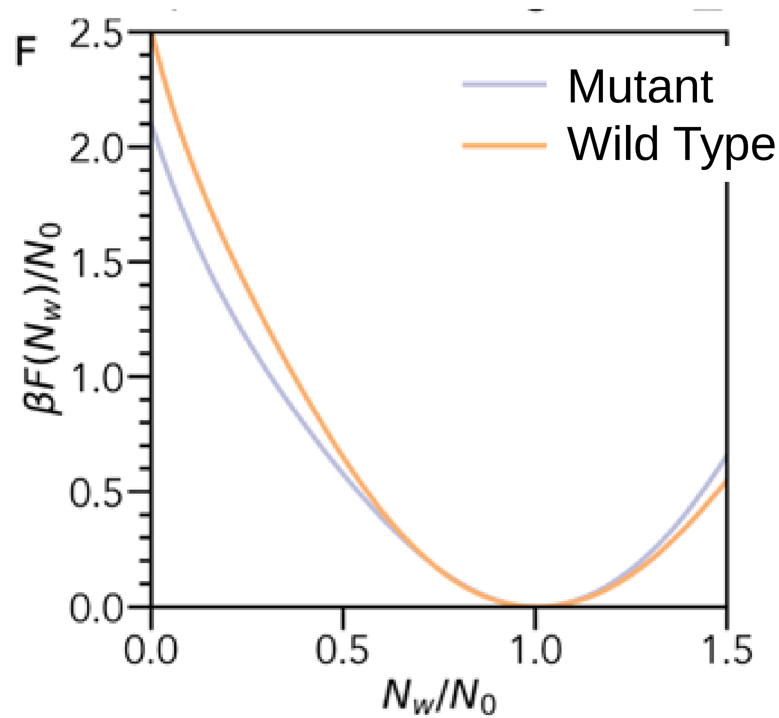


$$\mu_{\mathbf{ex}} = \mathbf{F(0)} = -\mathbf{lnP_v(0)}$$

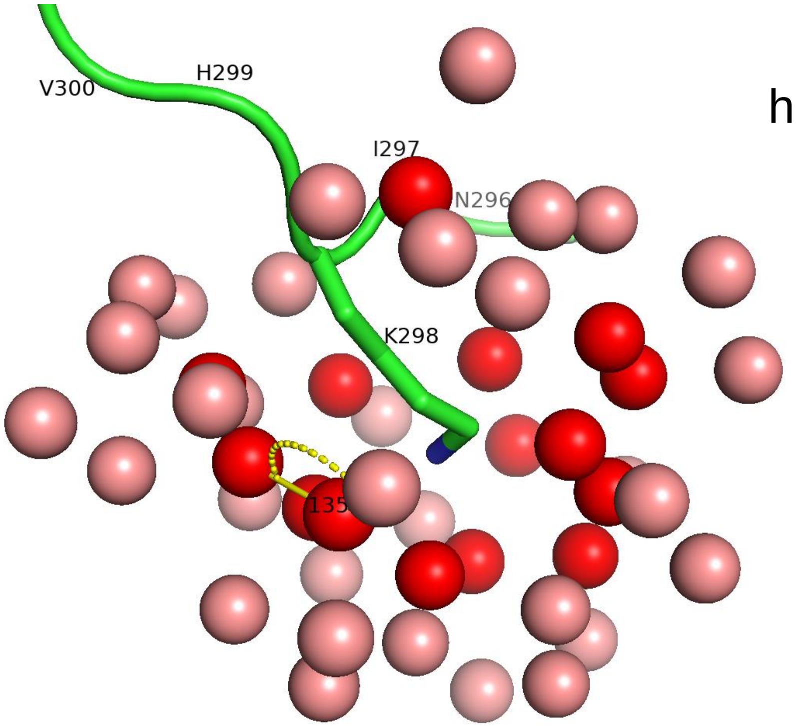
Free energy of dewetting lower for jR2R3-P301L: an additional factor favoring association of jR2R3-P301L



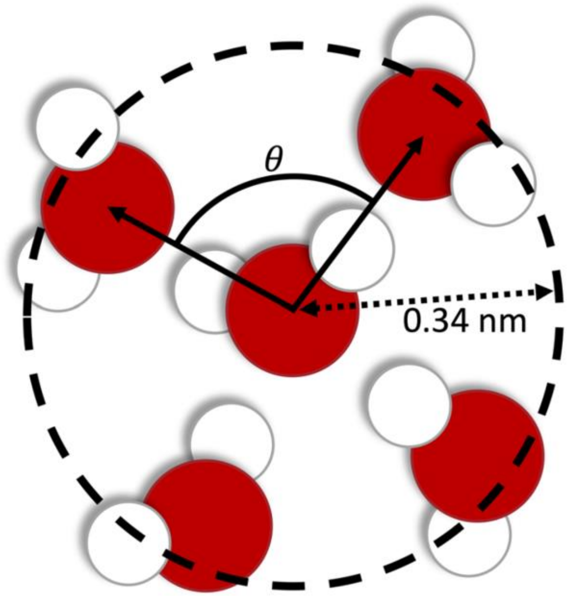
Wild Type Mutant



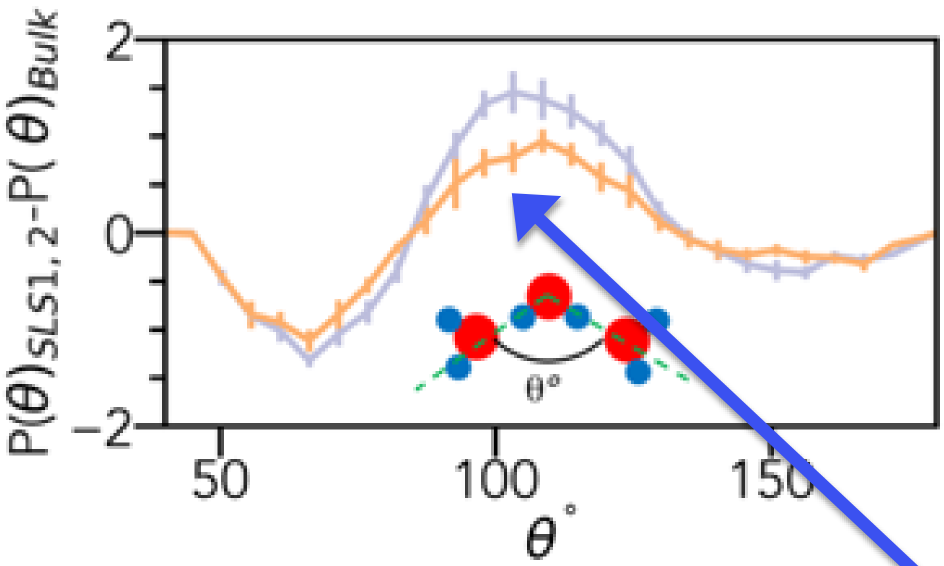
Quantifying Water Structure: *water triplet distribution*

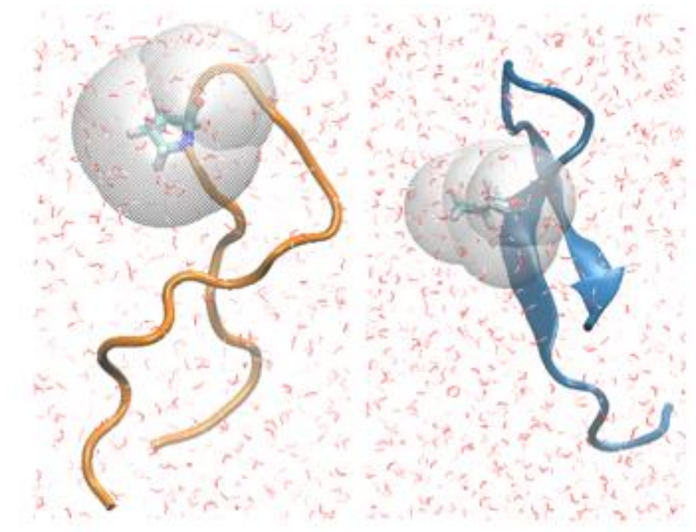


histogram
the
angles



Increased tetrahedral ordering of hydration waters near the L301 (mutant) compared to P301 (wild type)



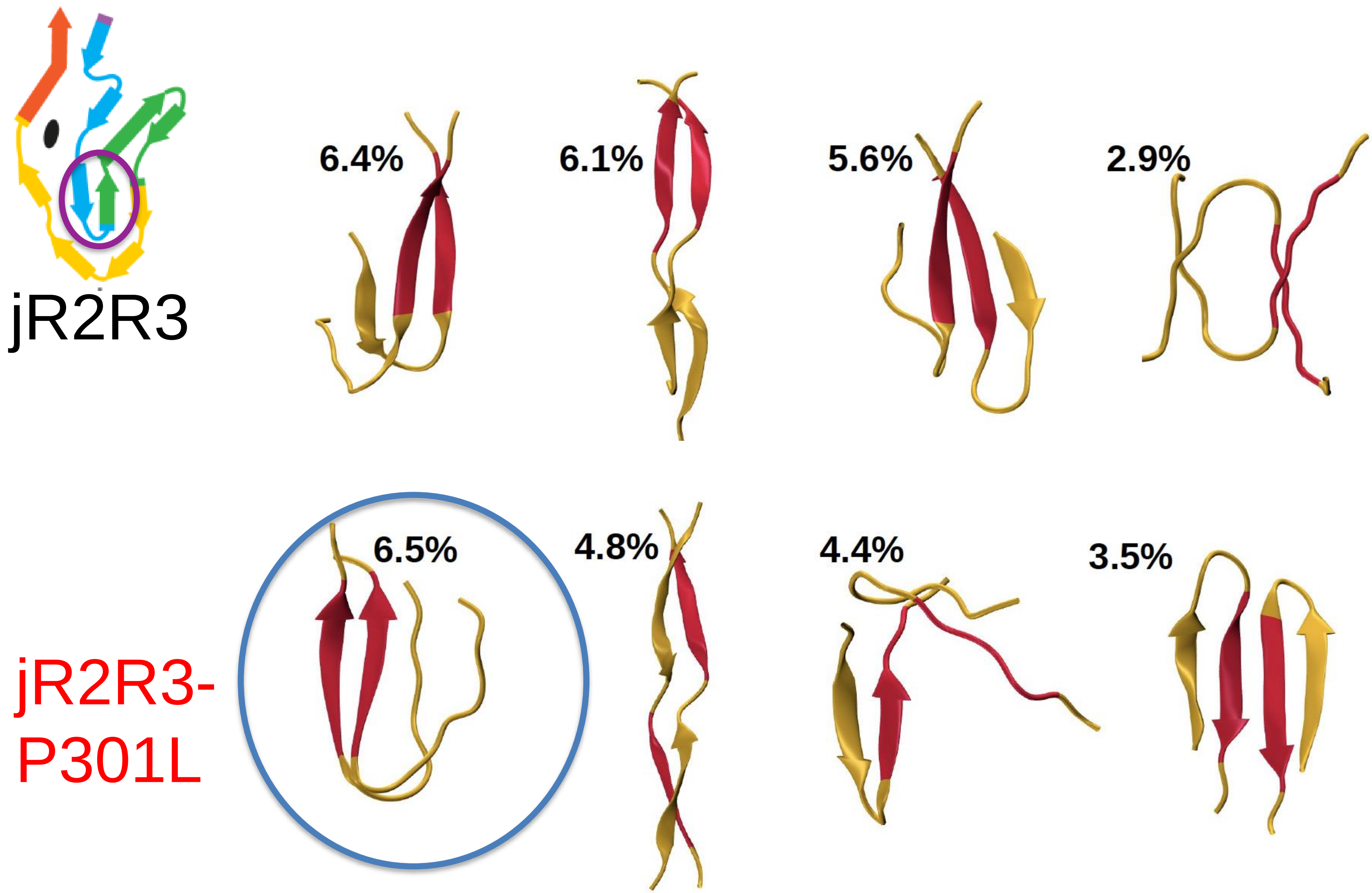


Wild Type

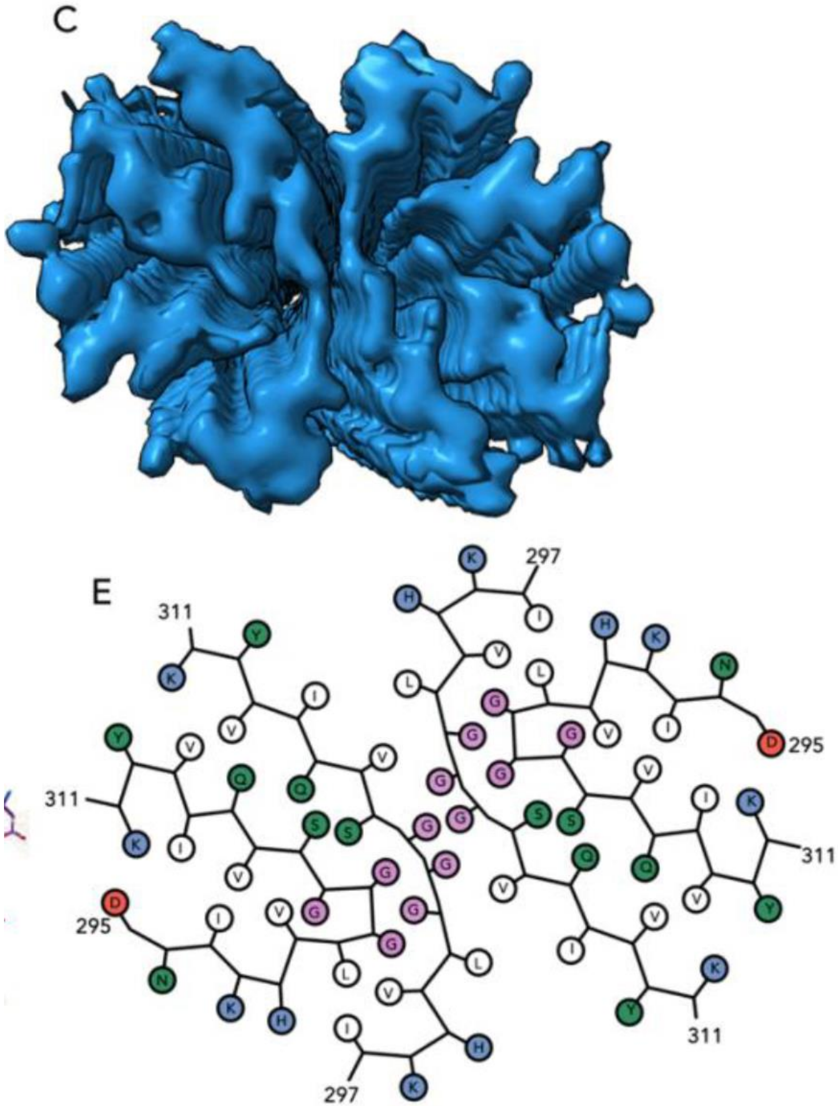
Mutant

TETRAHEDRAL
“hydrophobic”

Dimer Simulations of jR2R3 and JR2R3-P301L



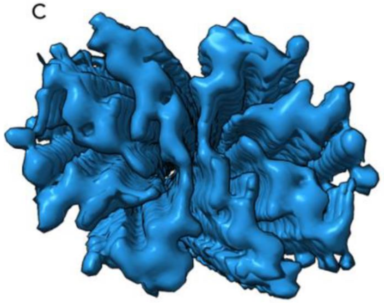
CryoEM structure



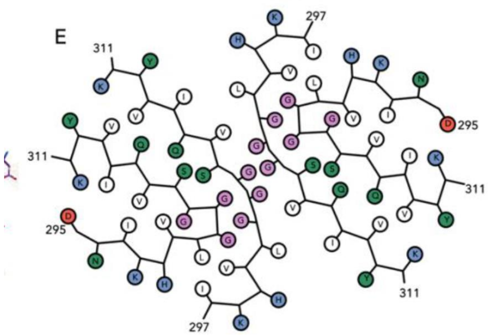
Prof. Songi Han
(Northwestern)

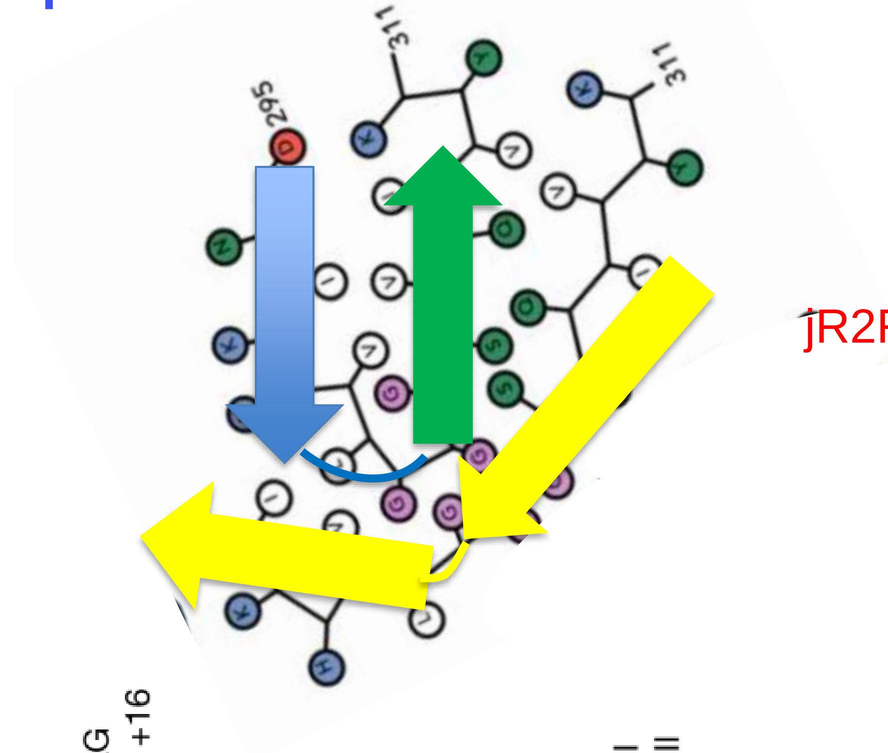
Importance of the Counter-Strand

C

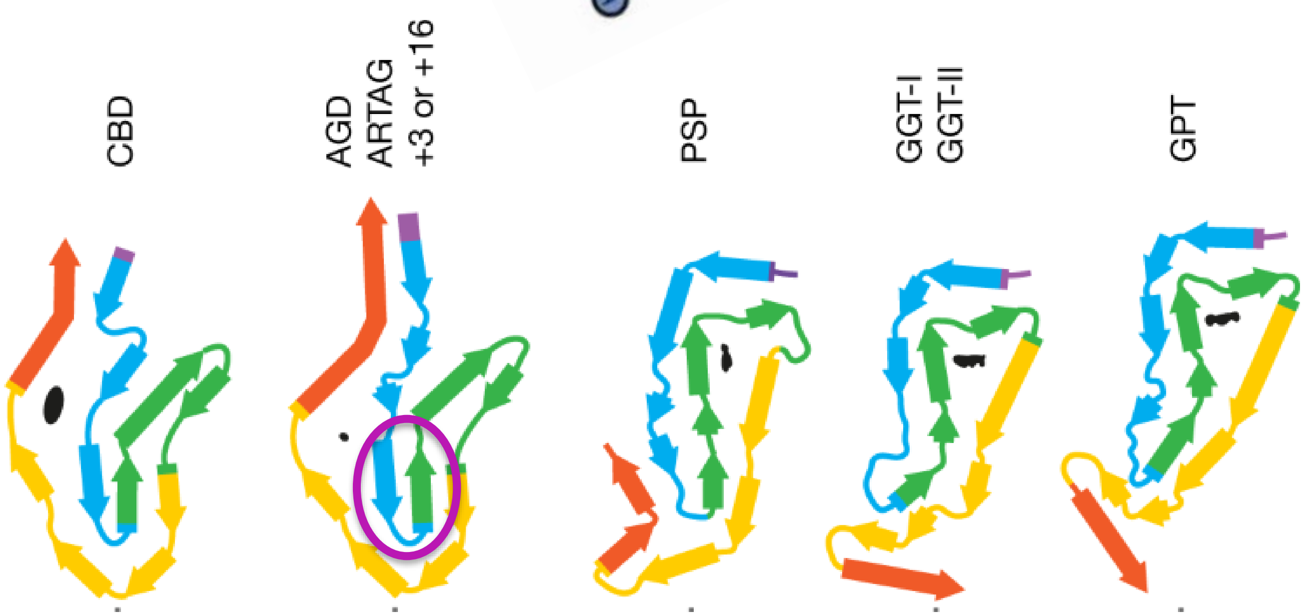


E

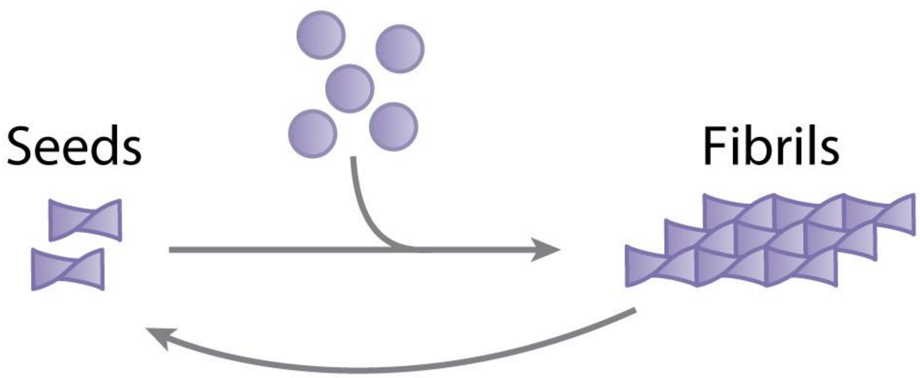


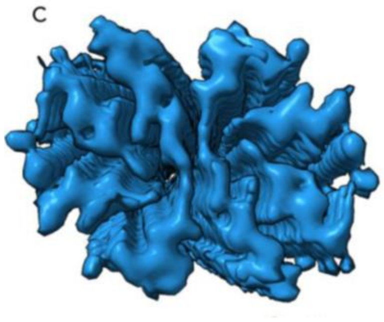


jR2R3-P301L



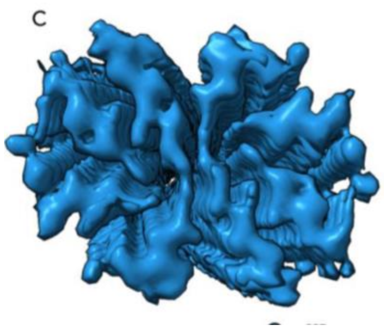
Seeding





Can jR2R3-P301L can seed the fibrillization of full length Tau?

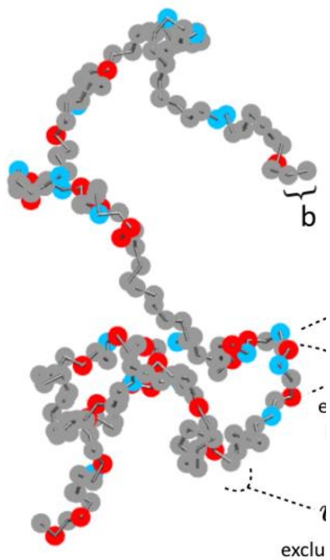
jR2R3-P301L can seed the fibrillization of full length Tau in Vitro



Fibril of Tau Fragment jR2R3-P301L

DNIKHVLGGS
VQIVYK

+

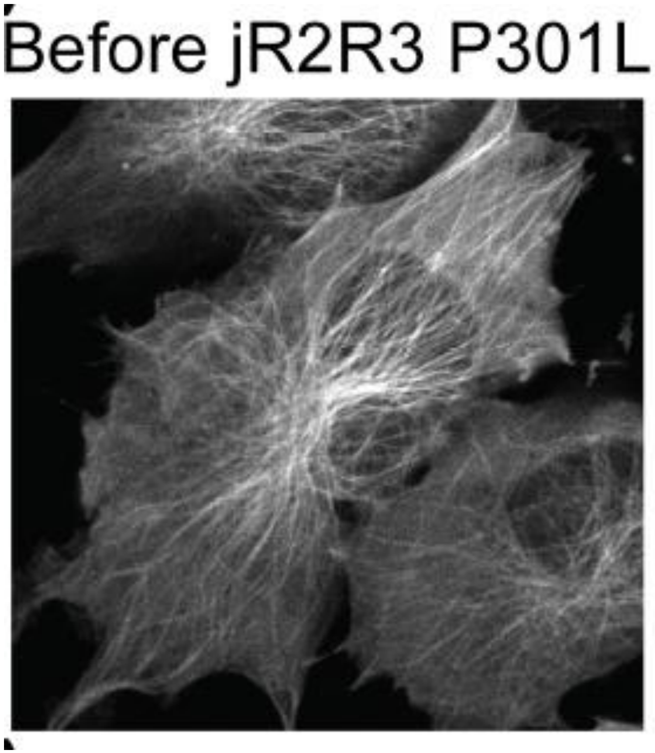


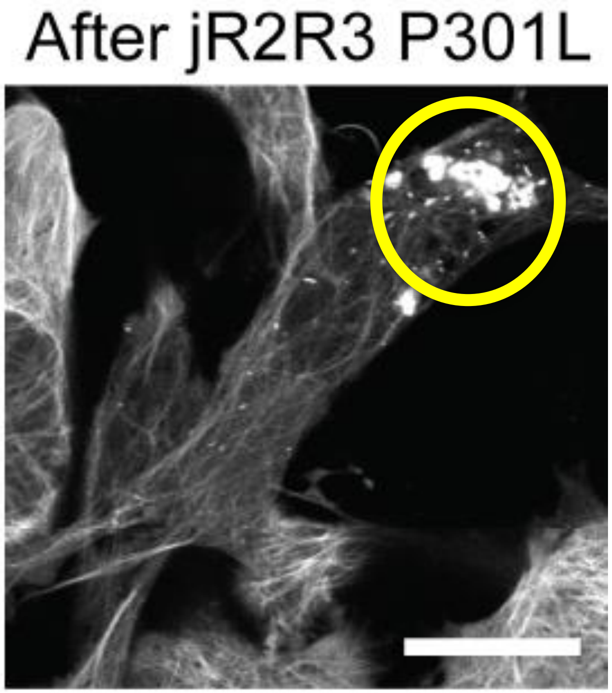
Full length Tau

272	GGK	274	R1
275	VQIINKKLDLS-NVQSKCGSKDNIKHVPGGGS	305	R2
306	VQIVYKPVDLS-KVTSKCGSLGNIHHKPGGGQ	336	R3
337	VEVKSEKLDFKDRVQSKIGSLDNITHVPGGGN	368	R4
369	KKIETHKLTFRENAKAKTD	387	C

Full Length Tau Fibrils

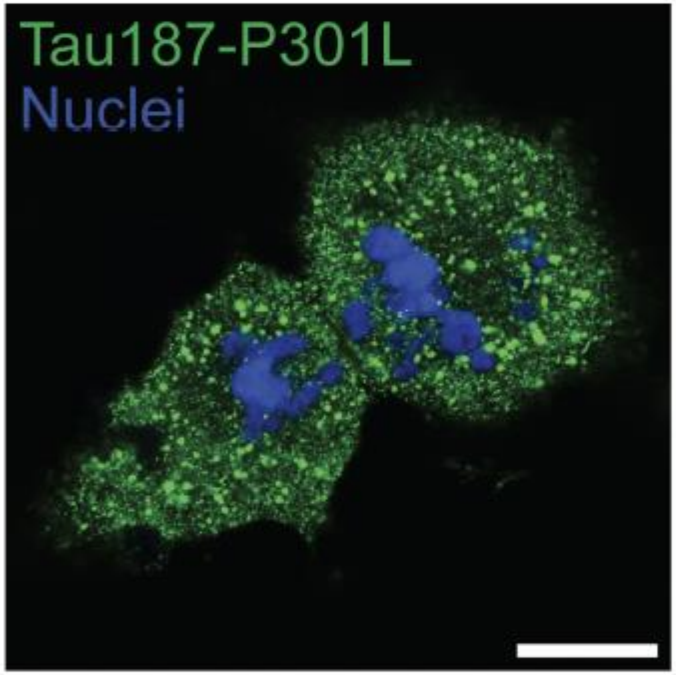
jR2R3-P301L can seed the fibrillization of full length Tau in Vivo





Cells expressing mclover3-Tau187-P301L seeded with jR2R3-P301L fibrils

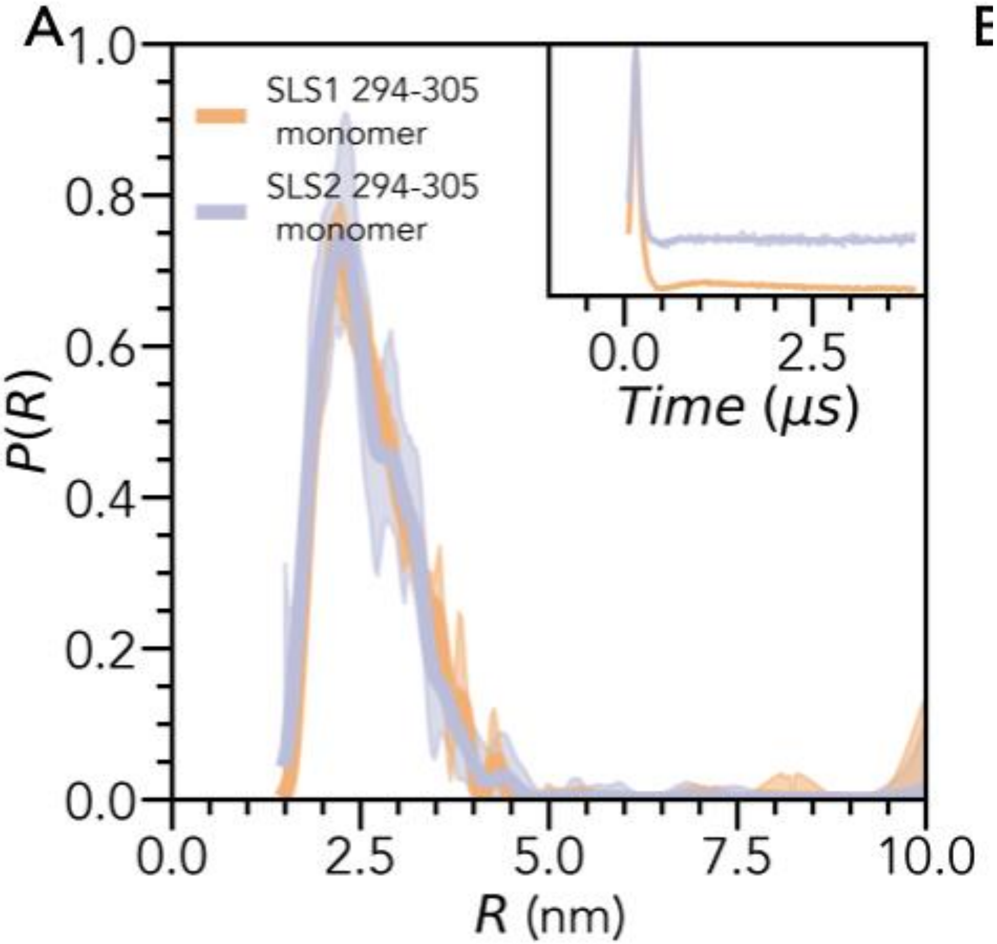
jR2R3-P301L acts as a prion: propagates the strain



Prof. Ken Kosik, UCSB

Cells seeded with jR2R3-P301L fibrils undergo division and propagate aggregates to daughter cells

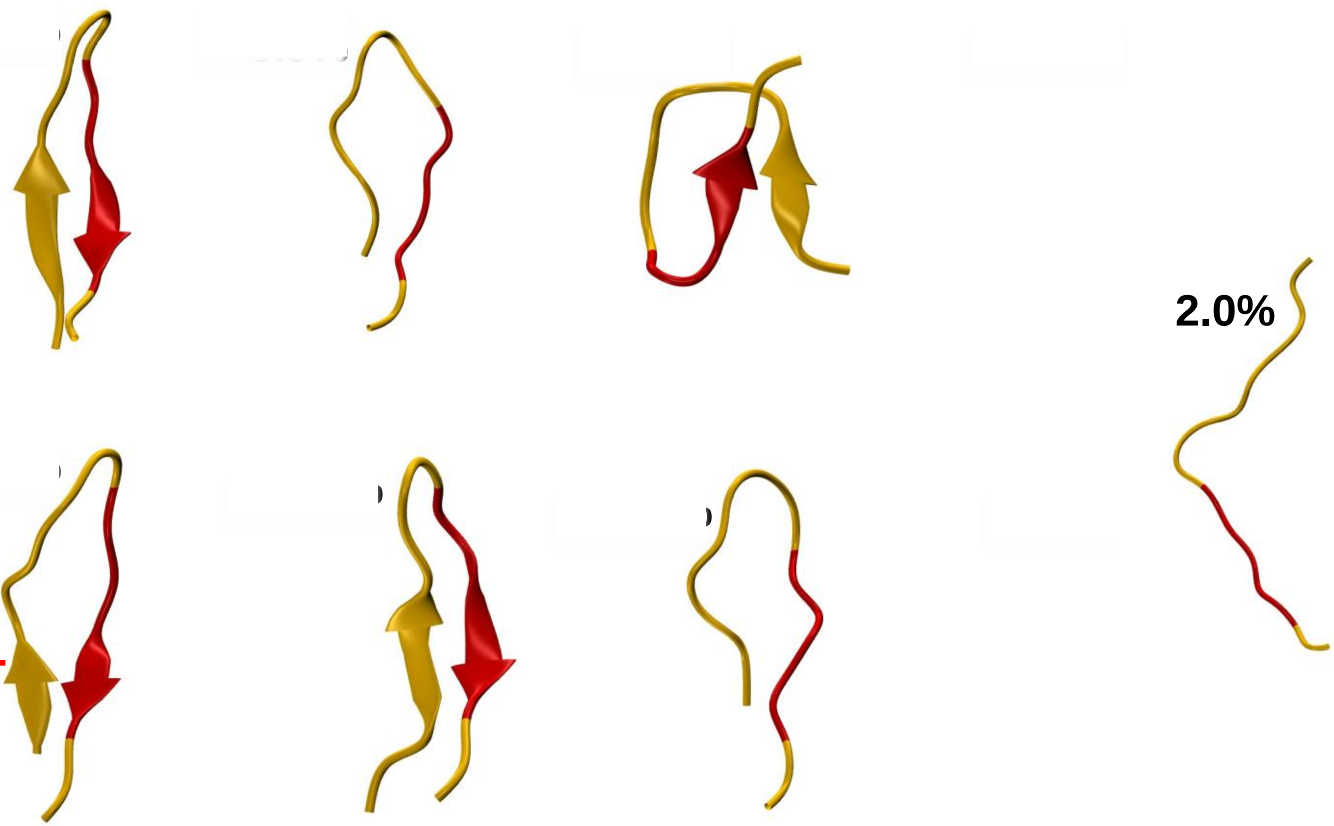
Double Electron-Electron Resonance (DEER) Spectroscopy cannot distinguish between wild type and mutant



Simulations of jR2R3 and JR2R3-P301L (Replica Exchange; Charmm36m)

jR2R3

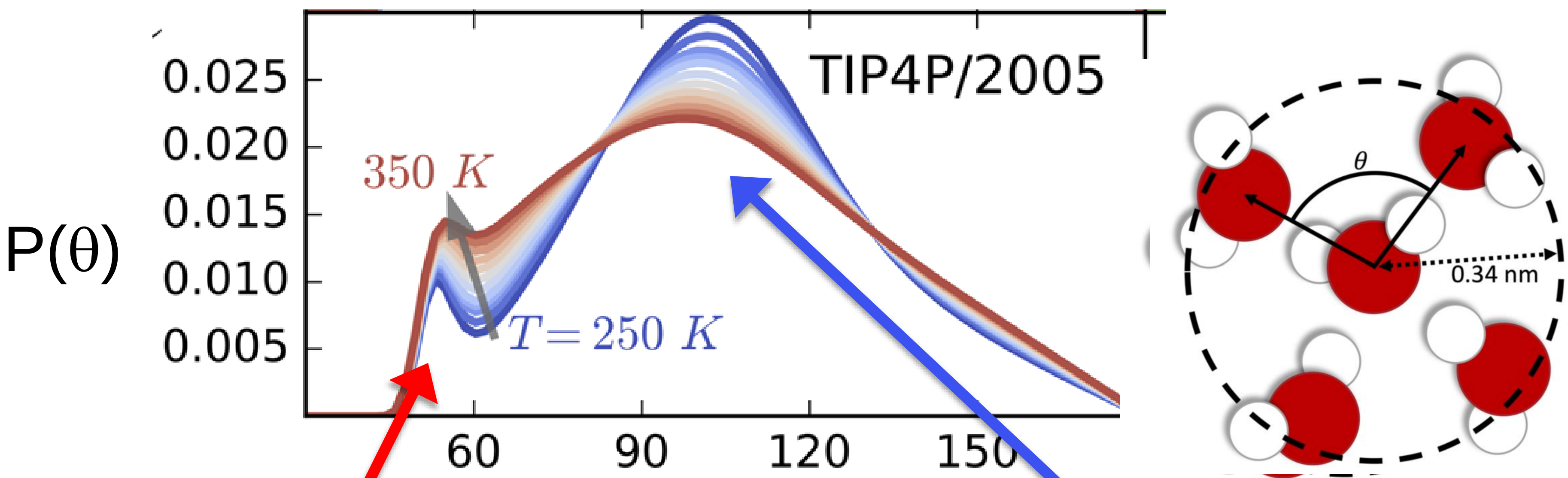
jR2R3-P301L



Pritam Ganguly

LITTLE DIFFERENCE

Water Triplet Distribution



3-body angle θ

"hydrophilic"

TETRAHEDRAL
"hydrophobic"