

INTRINSIC STIFFNESS AND THETA SOLVENT REGIME OF MODEL AMINO ACID CHAINS: IMPLICATIONS FOR LIQUID LIQUID PHASE SEPARATION

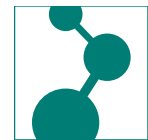
Kurt Kremer

Max Planck Institute for Polymer Research, Mainz

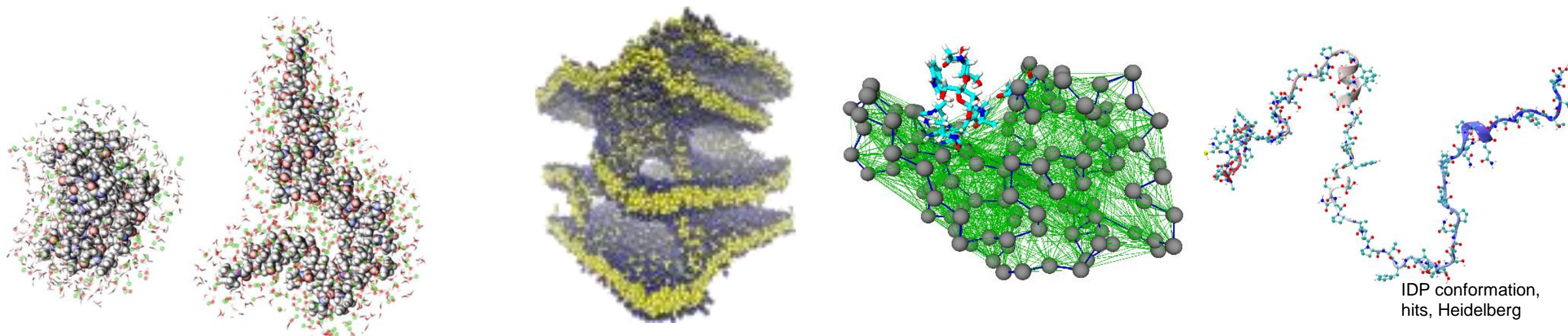
May 27, 2024 – Zaragoza, Spain

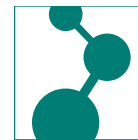
Y. Zhao, R. Cortes-Huerta, KK, J.F. Rudzinski J. Phys. Chem. B 2020, 124, 20, 4097–4113

L. Baidya, KK, G. Reddy, PNAS Nexus, 2025, 4, pgaf039



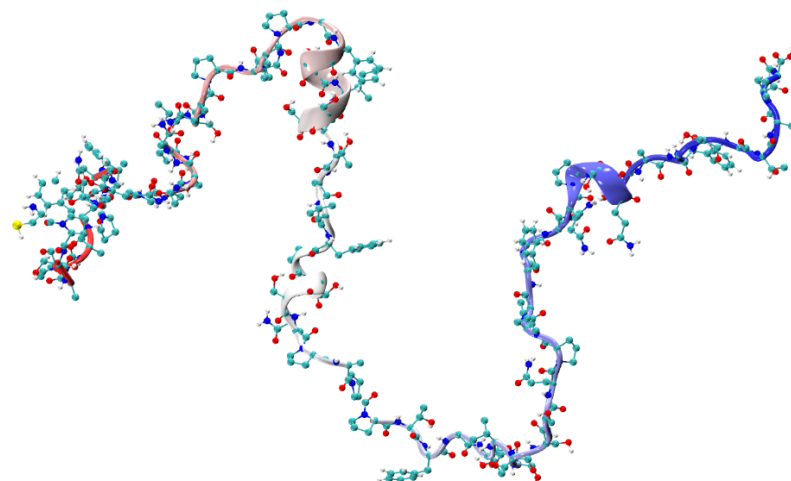
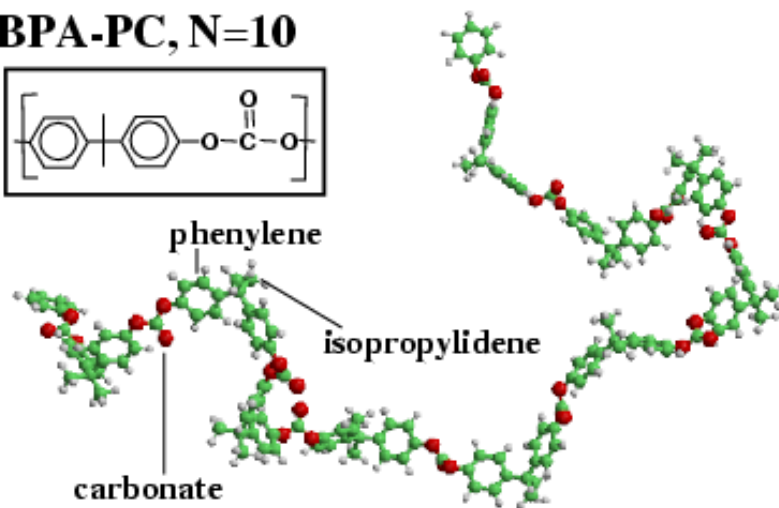
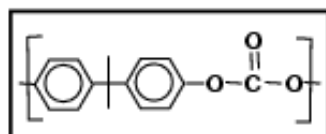
MULTISCALE STUDIES OF INTRINSICALLY DISORDERED PROTEINS (IDPS)





MULTISCALE STUDIES OF INTRINSICALLY DISORDERED PROTEINS (IDPS)

BPA-PC, N=10



IDP conformation, hits, Heidelberg

- Differences/similarities between IDP and 'regular' polymer
- Polymer physics analysis of model systems



INTRINSICALLY DISORDERED PROTEINS (IDPS)

- Long random block copolymers (polymer physics point of view)
- No ordered tertiary structure
- Strongly fluctuating conformation
- Temporarily well defined local secondary structure
- Experimentally difficult to characterize
- Force field issues compared to folded proteins
- AA simulations are prohibitively expensive (time scales, conformation landscape,...)

How far can standard polymer models be applied?

- **Differences/similarities between IDP and 'regular' polymer**
- **Polymer physics analysis of model systems**



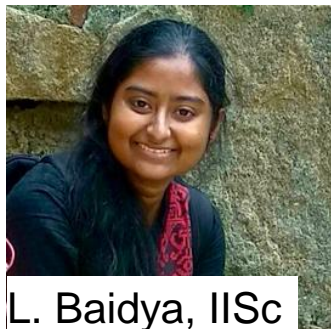
CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDP_s AND RELATION TO LLPS



G. Reddy, IISc

- **SINGLE CHAIN ANALYSIS**
- **Two homopolymers**
 - polyQ: polyglutamine in water
 - polyL: polyleucine in water
- **polyQ, polyL in mixed solvents**



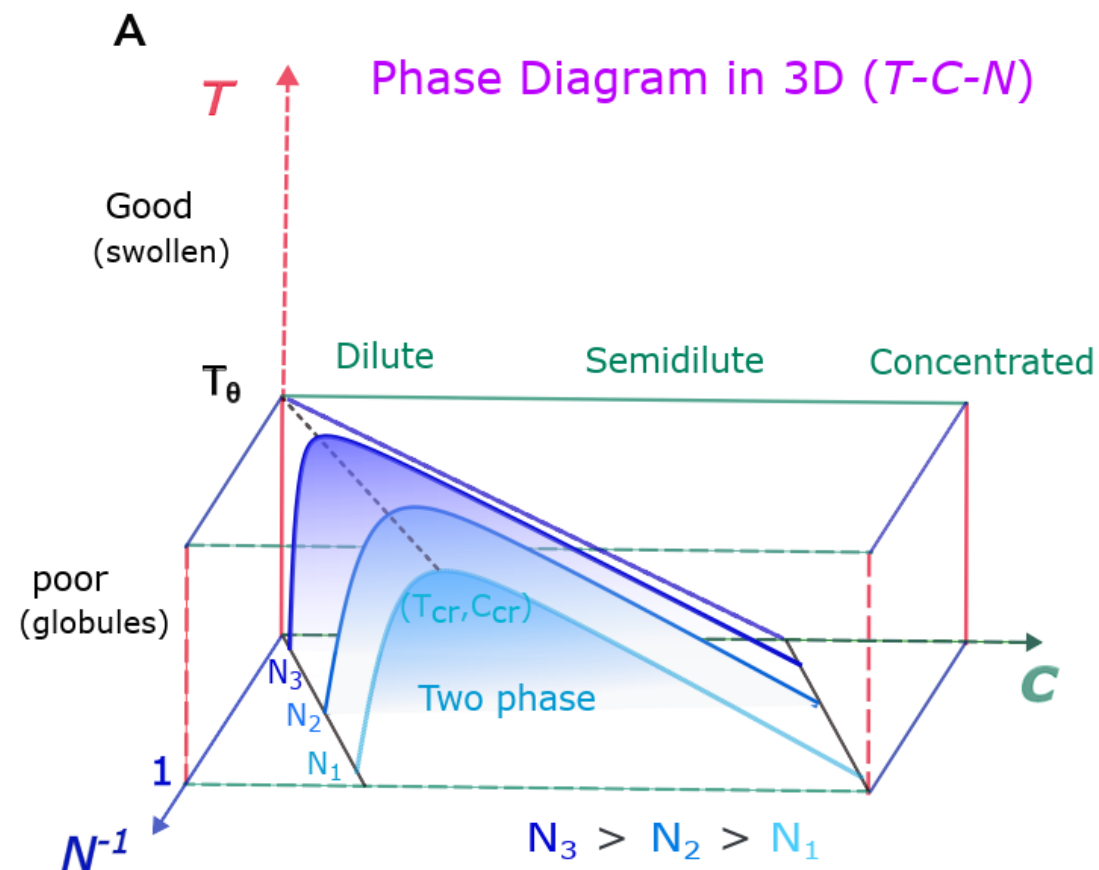
L. Baidya, IISc



CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND θ REGIME OF MODEL IDP_s AND RELATION TO LLPS

- Experimental situation
 - Single chain analysis used to infer solvation behaviour
 - Good solvent \Rightarrow no contribution to LLPS
 - Poor solvent \Rightarrow tendency to trigger LLPS
- Two homopolymers as example
 - polyQ: hydrophilic polyglutamine in water
 - polyL: hydrophobic polyleucine in water
 - polyQ, polyL in mixed solvents

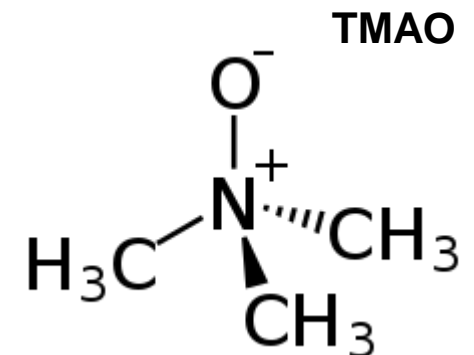
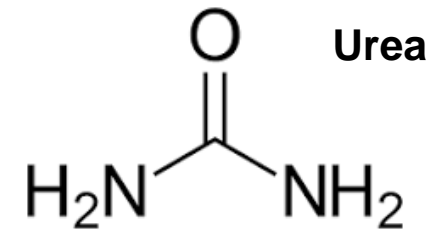




CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDPS AND RELATION TO LLPS

- Two homopolymers as example
 - polyQ: hydrophilic polyglutamine in water
 - polyL: hydrophobic polyleucine in water
 - polyQ, polyL in water + cosolvent
- Cosolvents:
 - Urea – denaturant, destabilizes compact states **“good solvent”**
 - Trimethylamine N-oxide (TMAO) -
protective osmolyte, stabilizes compact states
“poor solvent”





CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDP_s AND RELATION TO LLPS

- **Experimental situation**

Attempts to use single chain properties
to study propensity for LLPS

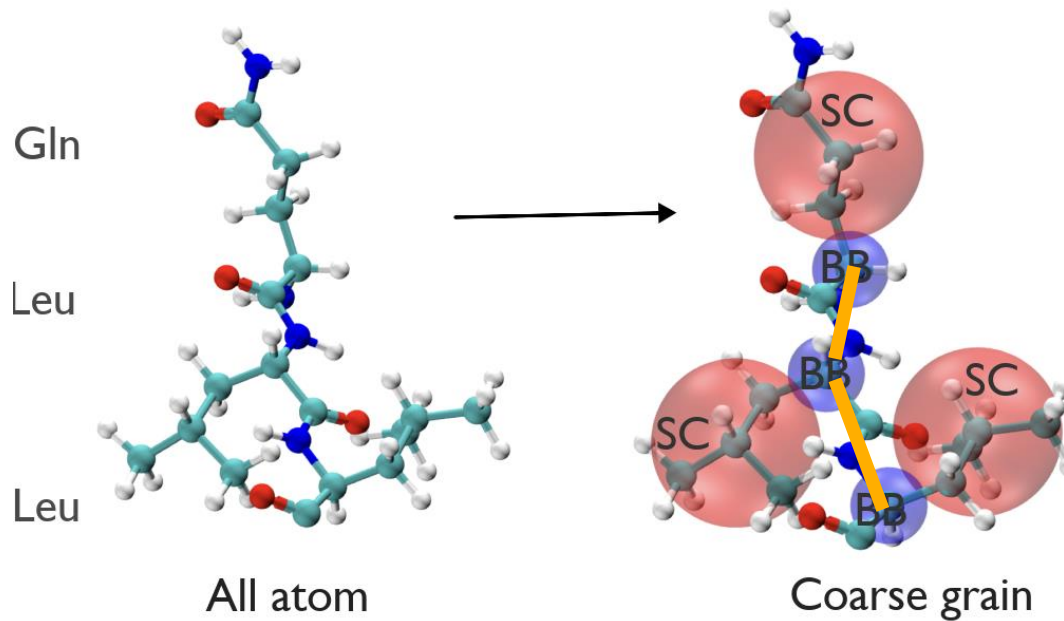
**Experimental results are
contradictory**

- **Experiments:**

- X-ray scattering (SAXS)
=> R_g , internal distance distributions
- single molecular fluorescence resonance energy transfer (smFRET)
=> close contacts
Walters & Murphy J. Mol. Bio. 2009
- fluorescence correlation spectroscopy (FCS)
=> R_h
Pappu et al, PNAS 2006.
- Experiments typically on short chains



CLAISSICAL POLYMER THEORY AND INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDP_s

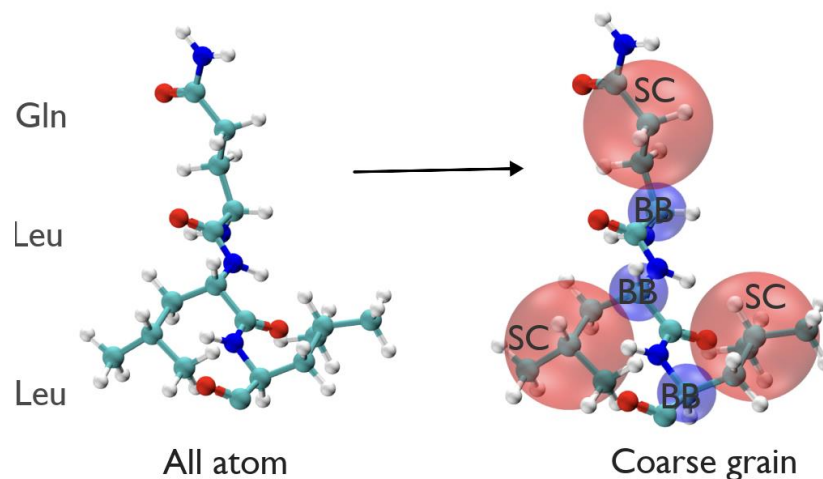


- **Self-Organized Polymer Model for Intrinsically Disordered Proteins (SOP-IDP) (D. Thirumalai et al 2019)**
- **blue: backbone α carbon**
red: side chains
- **Implicit solvent model**



CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDPs



$$E_{CG}(\{\mathbf{r}\}, 0) = E_B + E_{NB}^L + E_{NB}^{NL}$$

$$E_B = - \sum_{i=1}^{N_B} \frac{k}{2} R_0^2 \log \left(1 - \frac{(r_i - r_i^0)^2}{R_0^2} \right),$$

$$E_{NB}^L = \sum_{i=1}^{N_i} \epsilon_l \left(\frac{\sigma_i}{r_i} \right)^6,$$

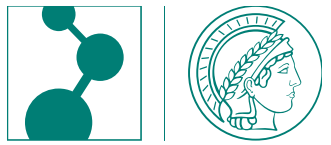
$$E_{NB}^{NL} = \sum_{i=1}^{N_{bb}} \epsilon_{bb} \left[\left(\frac{\sigma_i^{bb}}{r_i} \right)^{12} - 2 \left(\frac{\sigma_i^{bb}}{r_i} \right)^6 \right] + \sum_{i=1}^{N_{bs}} \epsilon_{bs} \left[\left(\frac{\sigma_i^{bs}}{r_i} \right)^{12} - 2 \left(\frac{\sigma_i^{bs}}{r_i} \right)^6 \right] + \sum_{i=1}^{N_{ss}} \epsilon_{ss} \left| 0.7 - \epsilon_i \right| \left[\left(\frac{\sigma_i^{ss}}{r_i} \right)^{12} - 2 \left(\frac{\sigma_i^{ss}}{r_i} \right)^6 \right].$$

Table S1: Parameters used in SOP-IDP Model

Parameter	Value
k	20.0 kcal/(mol.Å ²)
R_0	2.0 Å
ϵ_l	1.0 kcal/mol
ϵ_{bb}	0.12 kcal/mol
ϵ_{bs}	0.24 kcal/mol
ϵ_{ss}	0.18 kcal/mol

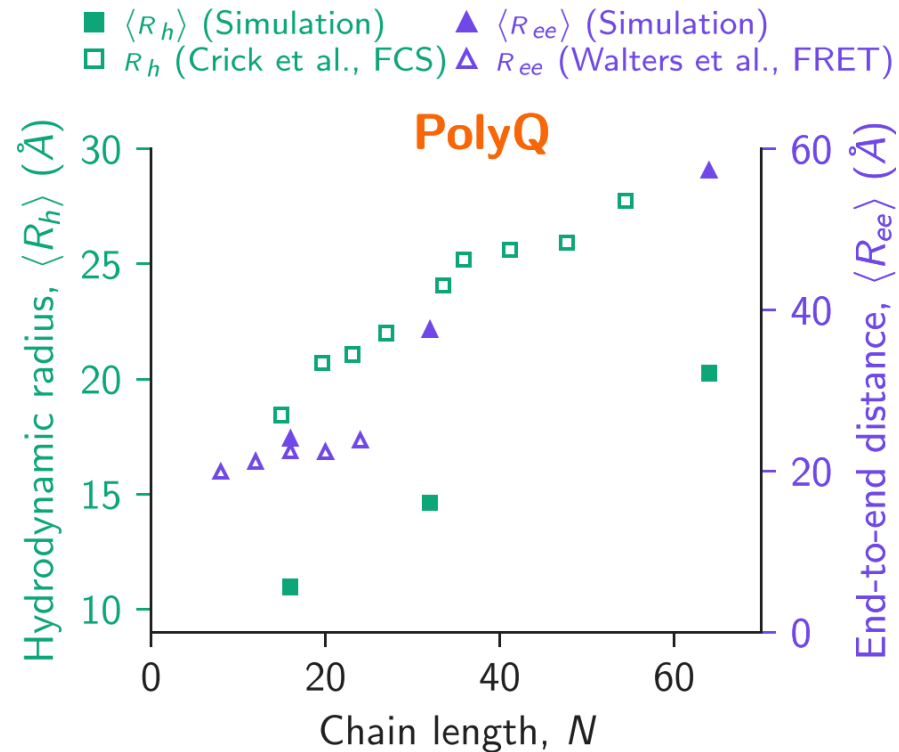
Table S2: Parameters of bead radius

Bead	vdW radius (Å)
C_α	1.90
Gln (Q)	3.01
Leu (L)	3.09



CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDPs

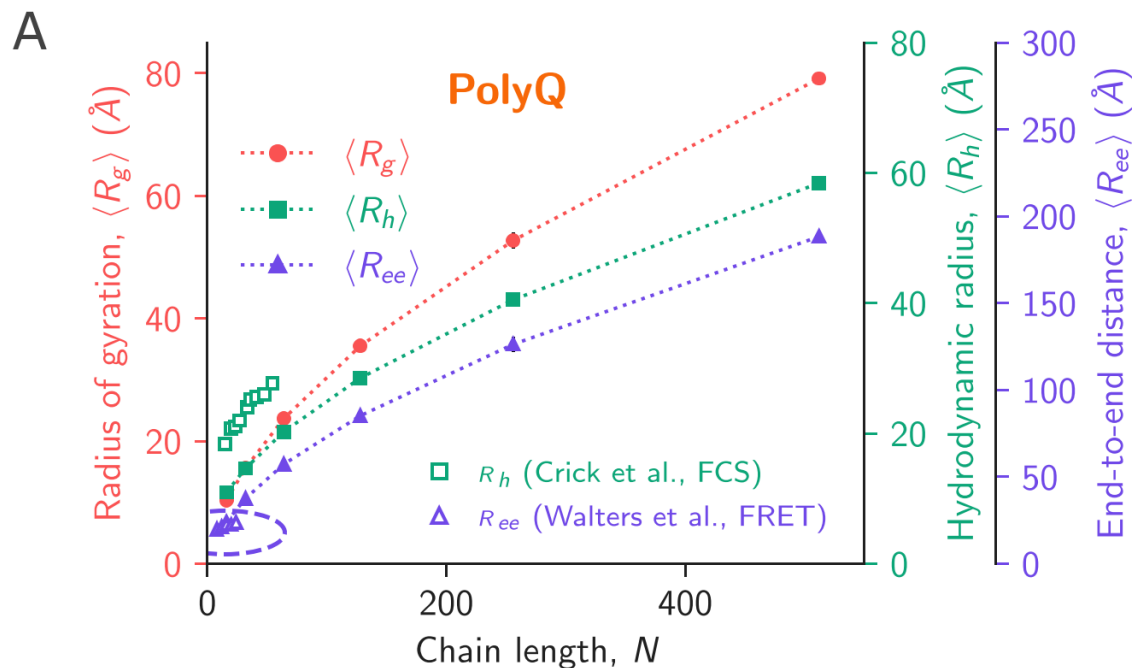
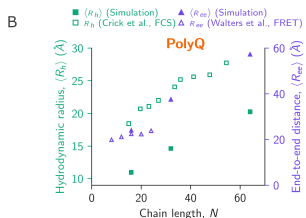


- polyQ: **hydrophilic** polyglutamine in water
- Comparison to experiment
- Semiquantitative agreement, different conclusions



CLAISSICAL POLYMER THEORY AND

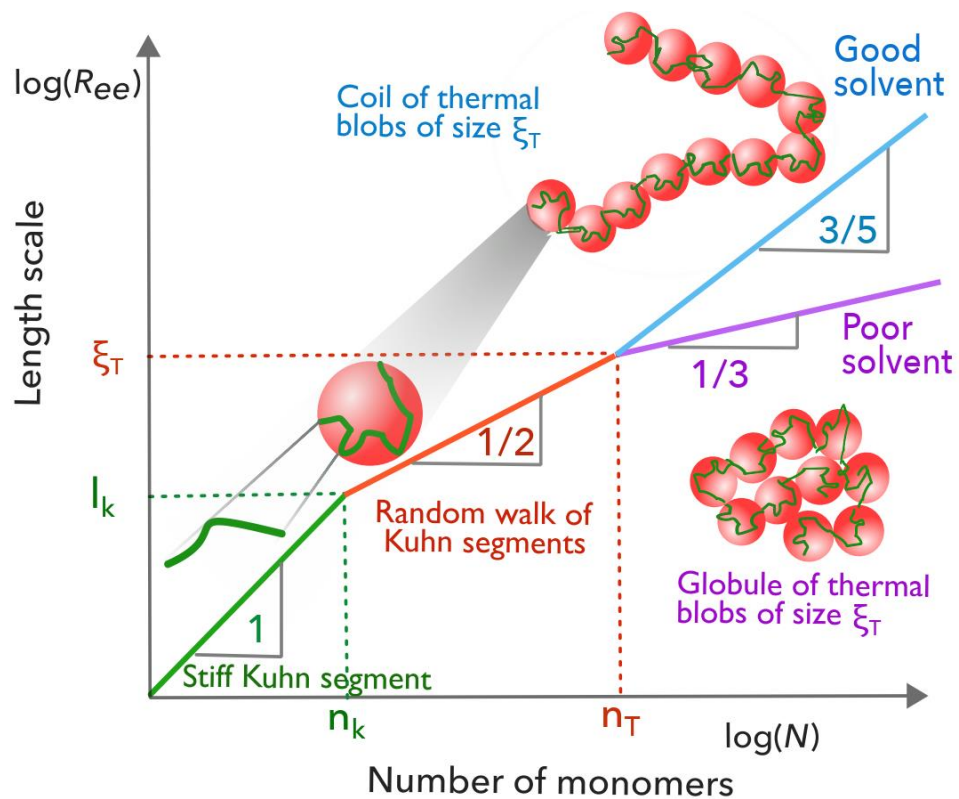
INTRINSIC STIFFNESS AND Θ REGIME OF MODEL IDPs



- polyQ: **hydrophilic** polyglutamine in water
- Comparison to experiment
- Semiquantitative agreement, different conclusions
- **To infer solvent quality long chains are needed**

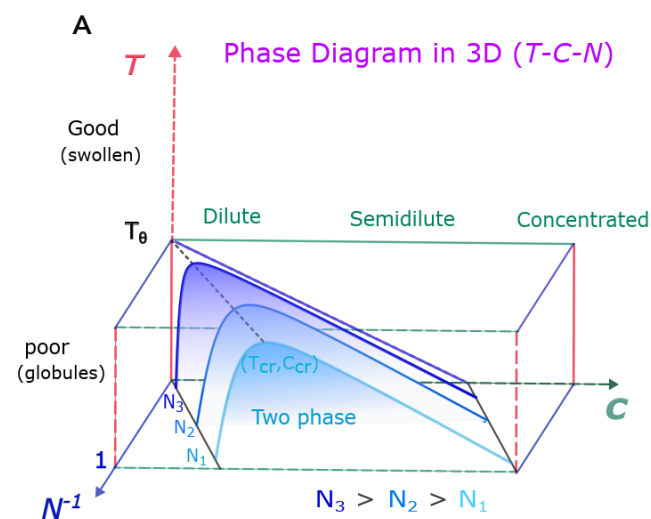


BLOB MODEL TO ANALYSE DATA OF MODEL IDP_s



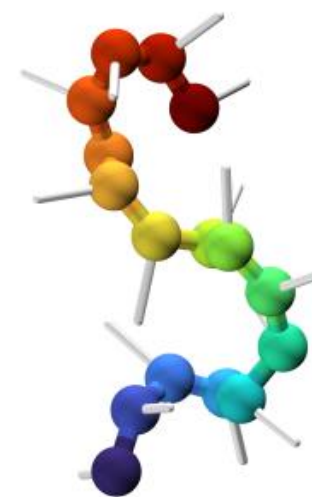
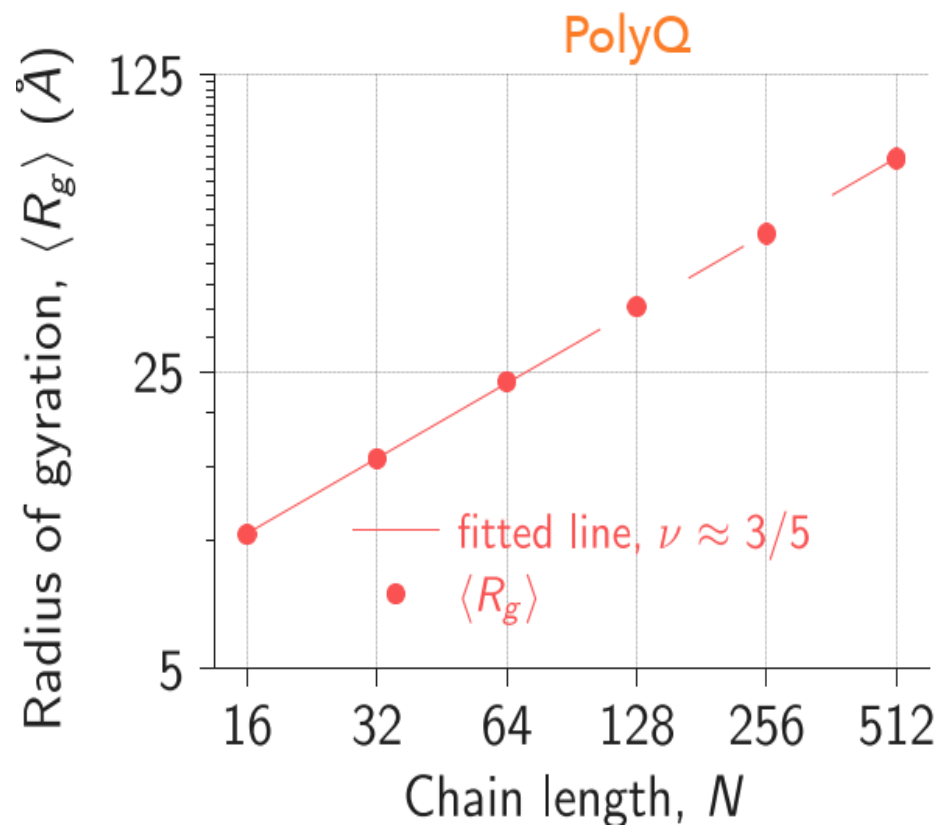
- Comparison to experiment
- Semiquantitative agreement, different conclusion

$$\langle R_{ee}^2(N) \rangle^{1/2} \sim N^{1/2} f_{\pm}(N\tau^{1/\phi})$$

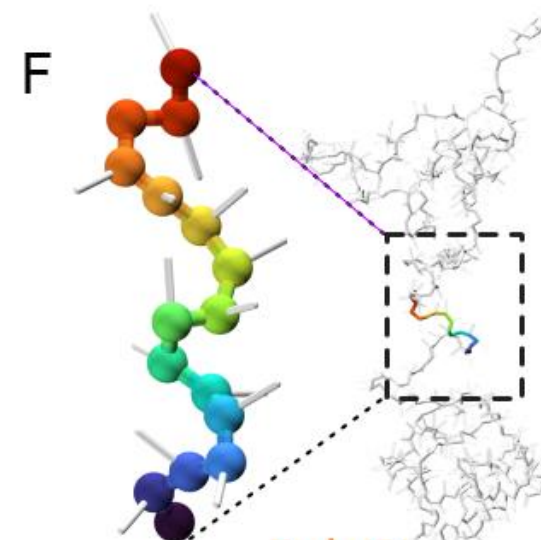




SCALING PROPERTIES OF MODEL IDP_s



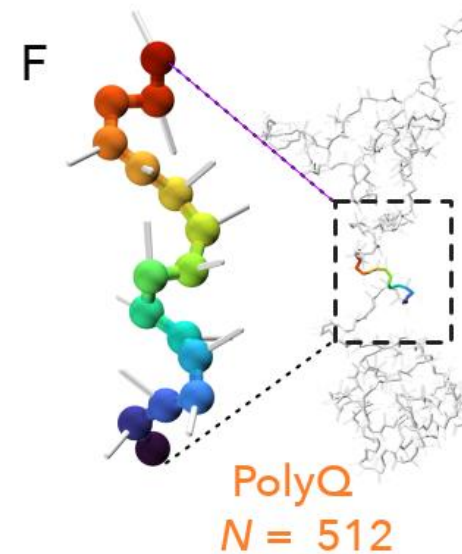
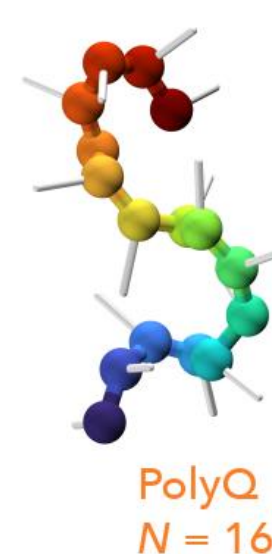
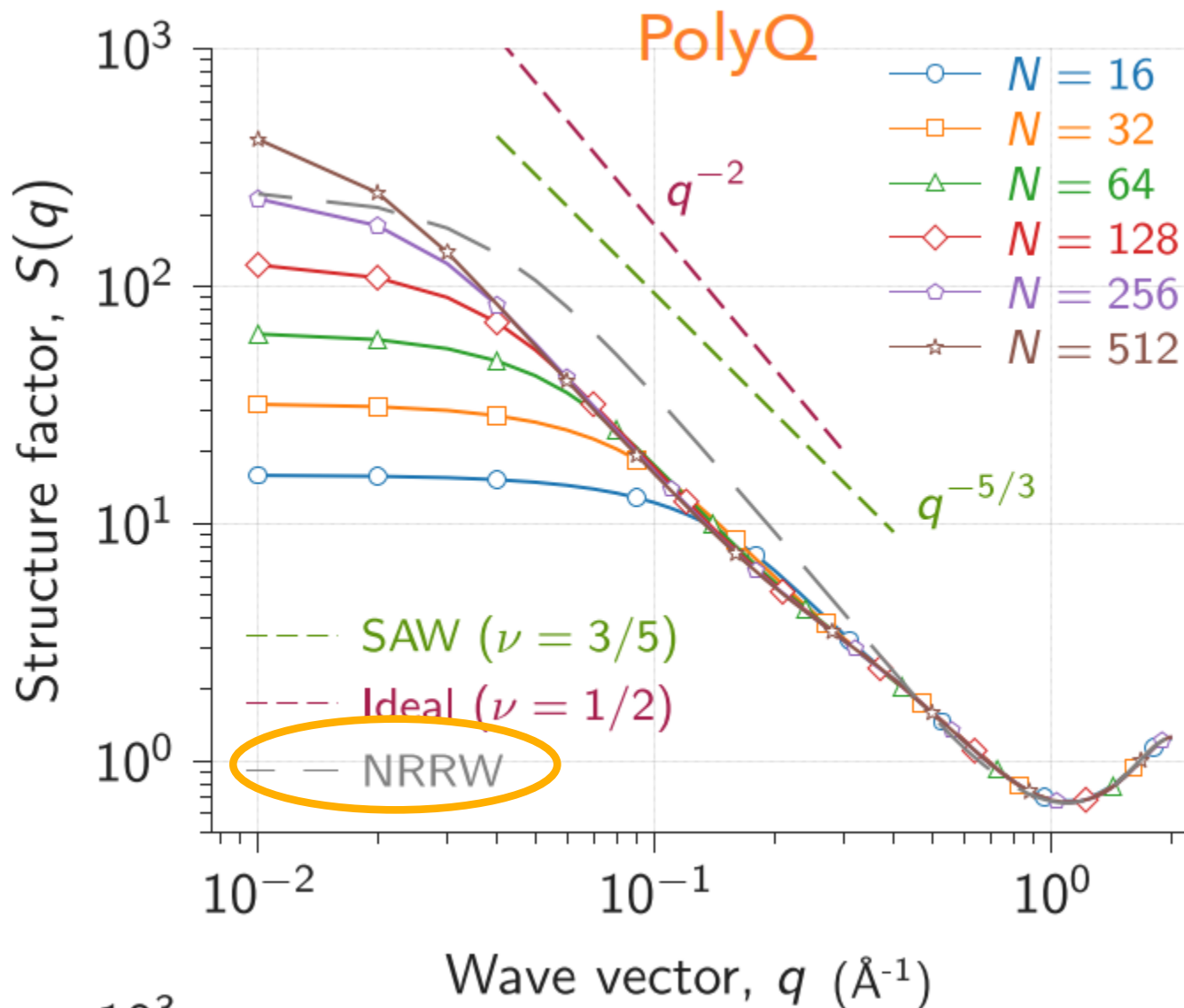
PolyQ
 $N = 16$



PolyQ
 $N = 512$



SCALING PROPERTIES OF MODEL IDP_s



NRRW: Nonreversal Random Walk, RW with short range EV along the backbone: 1:3,1:4...1:6

Intrinsic stiffness relevant up to $q \approx 0.35-0.4 \text{ \AA}^{-1}$

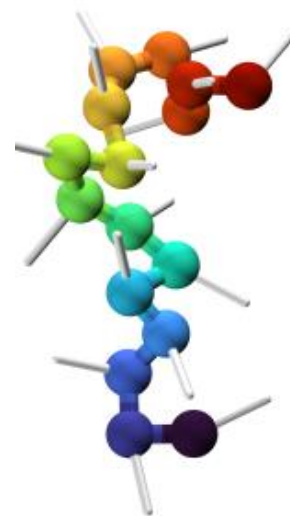
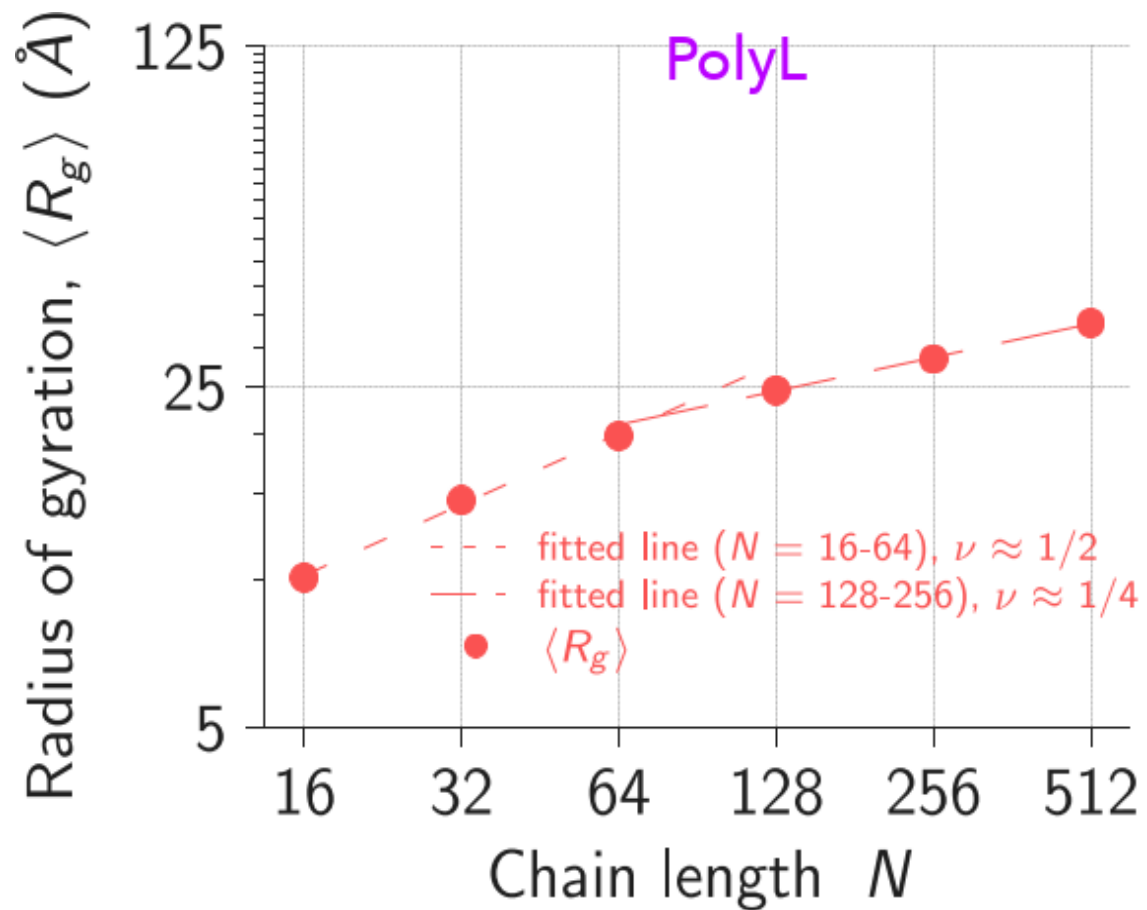
\Rightarrow Up to about 15 – 18 \AA

(corrected misprint, original talk: nm)



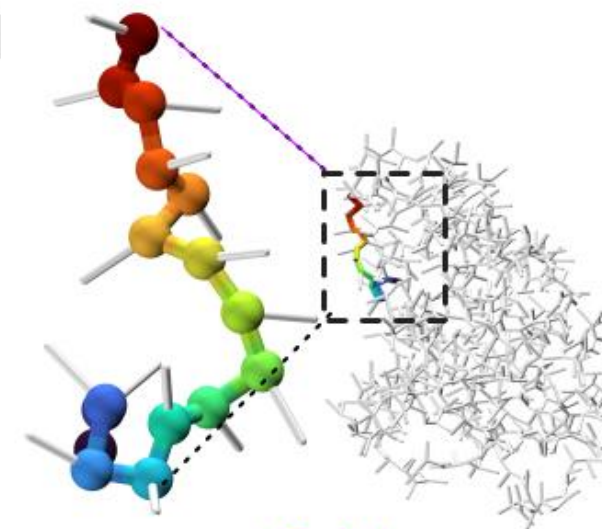
SCALING PROPERTIES OF MODEL IDP_s

POLYL: HYDROPHOBIC POLYLEUCINE IN WATER



PolyL
 $N = 16$

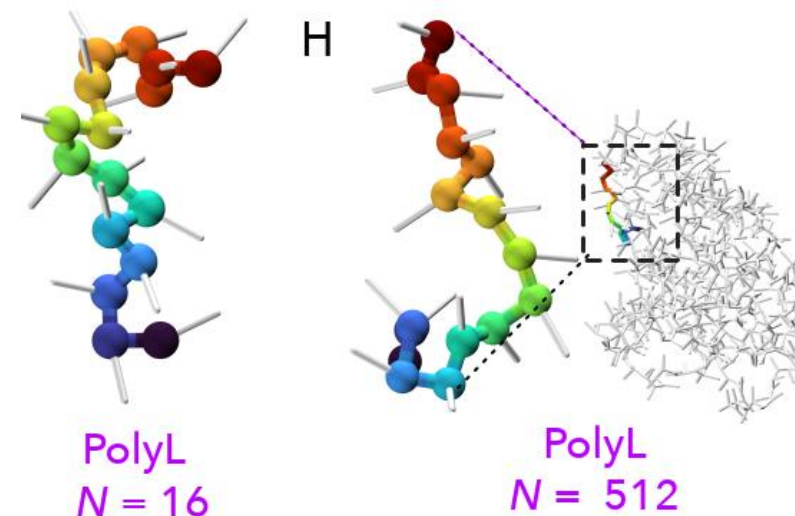
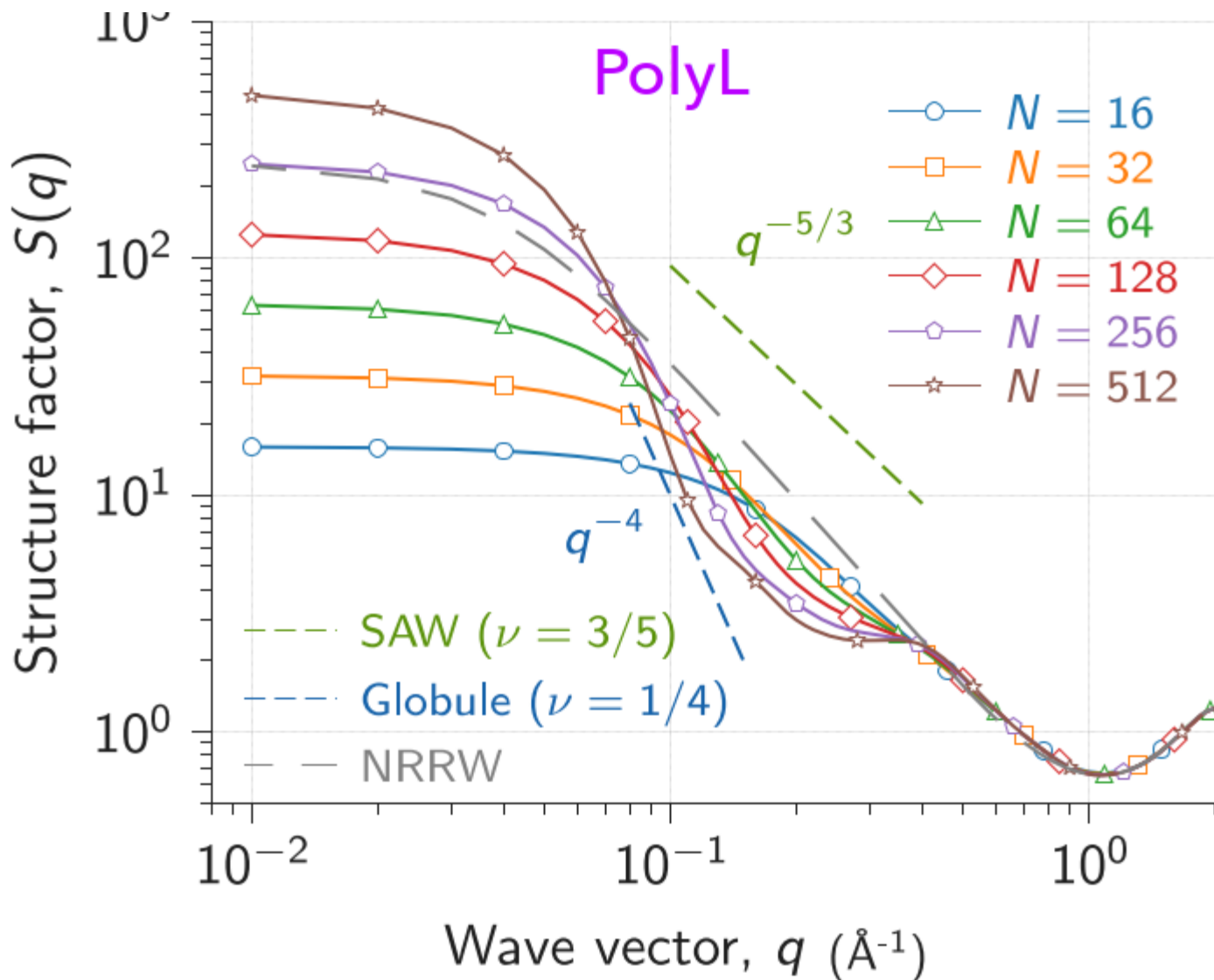
H



PolyL
 $N = 512$



SCALING PROPERTIES OF MODEL IDP_s



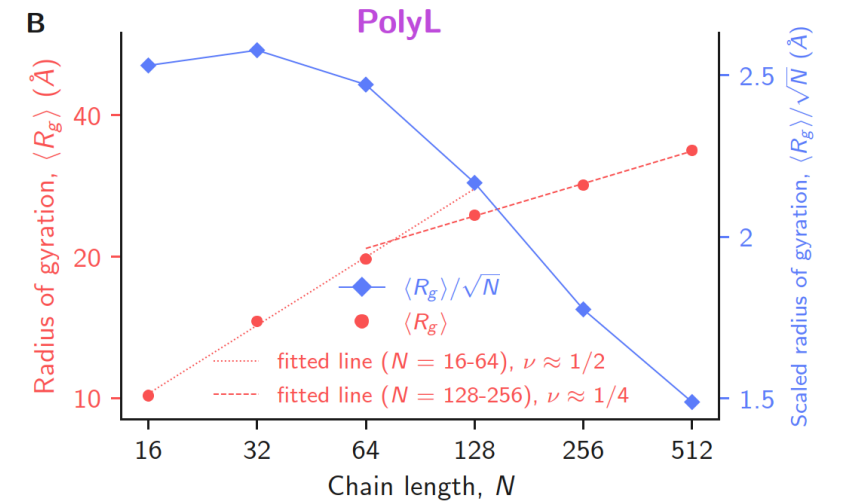
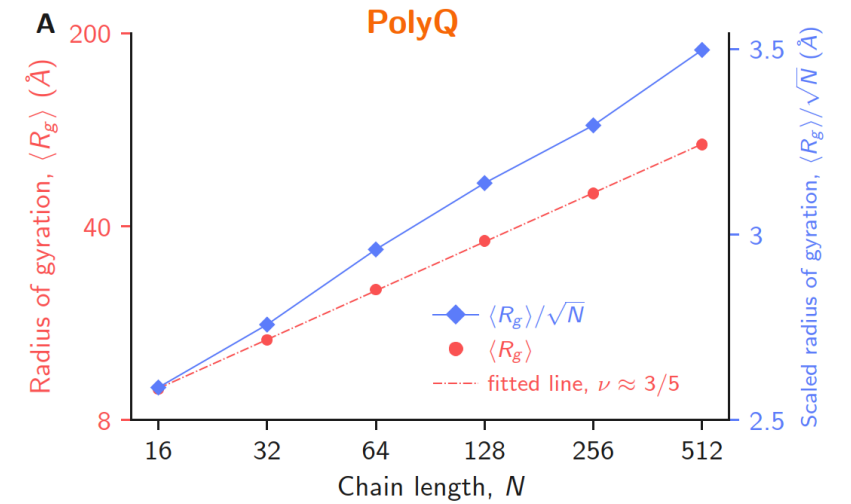
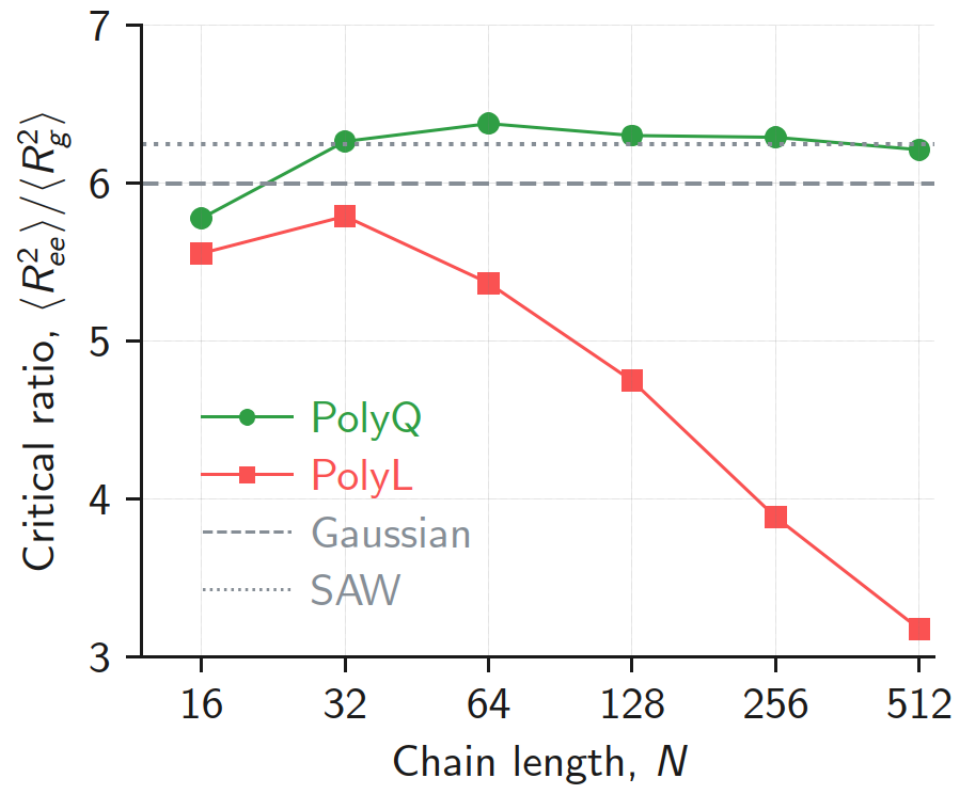
NRRW: Nonreversal Random Walk, RW with short range EV along the backbone: 1:3,1:4...1:6

Intrinsic stiffness relevant up to $q \approx 0.4 \text{ \AA}^{-1}$

\Rightarrow Up to about 15 \AA

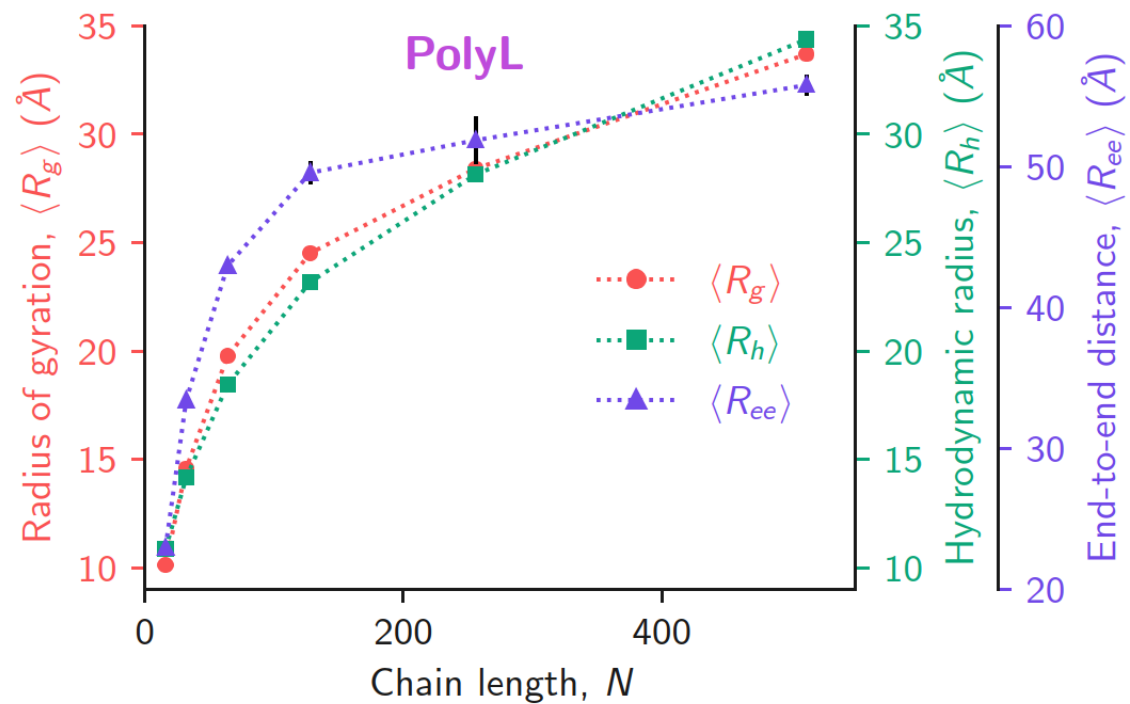
(corrected misprint, original talk: nm)

SCALING PROPERTIES OF MODEL IDP_s





FINITE SIZE CORRECTIONS SCALING PROPERTIES OF MODEL IDP_s



$$\langle R_g^2 \rangle = D_g N^{2\nu} (1 + a_g N^{-\Delta_1} + \dots)$$

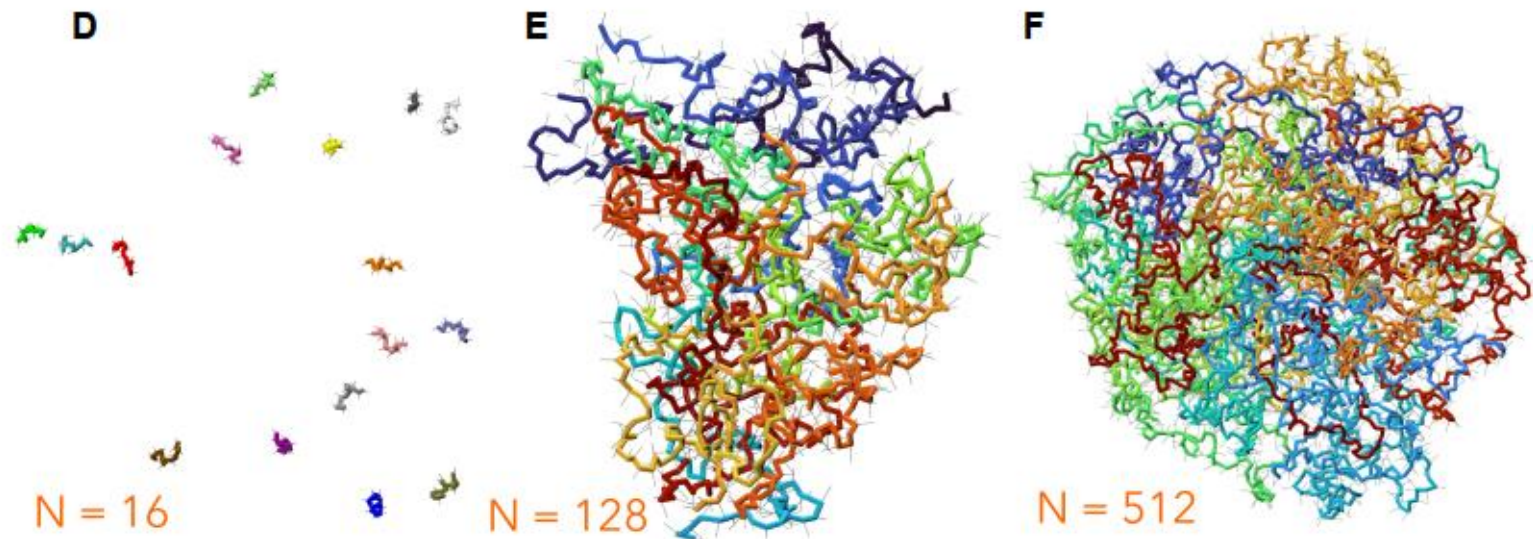
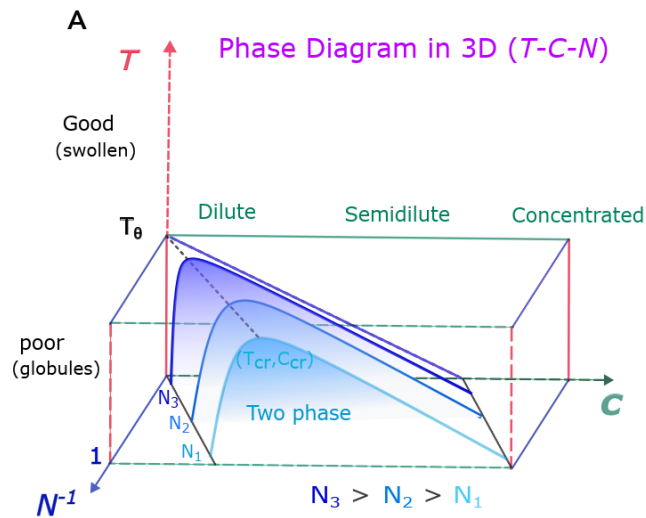
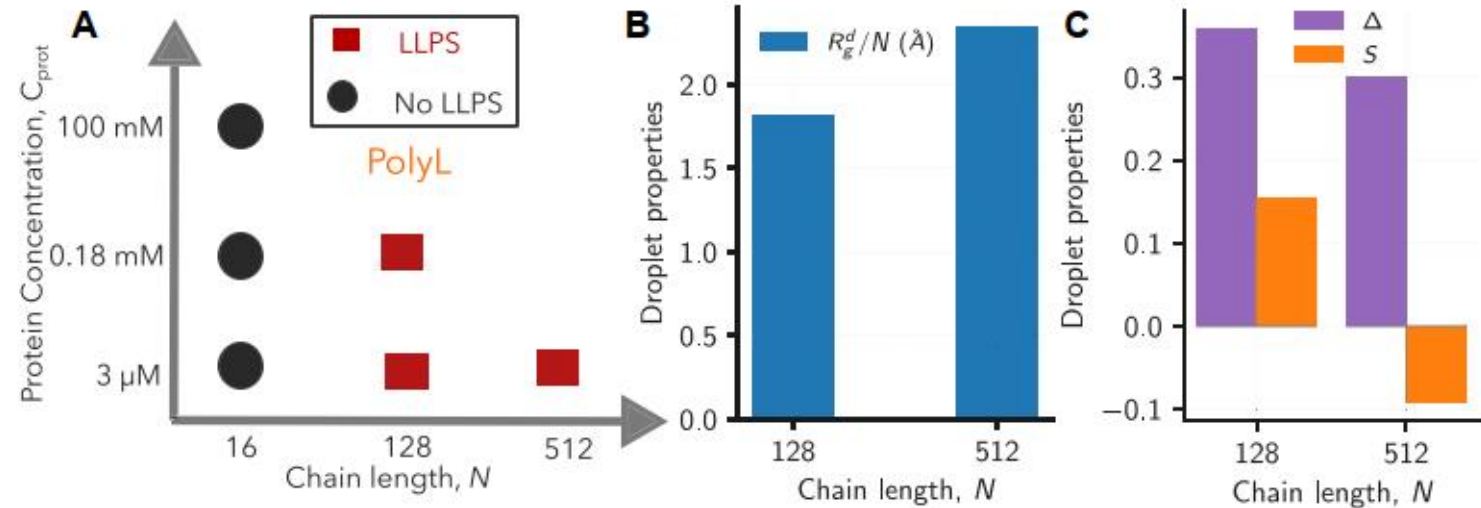
$$\langle R_{ee}^2 \rangle = D_e N^{2\nu} (1 + a_e N^{-\Delta_1} + \dots)$$

$$\langle R_h^{-1} \rangle = D_h N^{-\nu} (1 + a_h N^{-\Delta_1} + b_h N^{-(1-\nu)} + \dots)$$

Finite Size Dependency: Multi Chain Simulations of PolyL



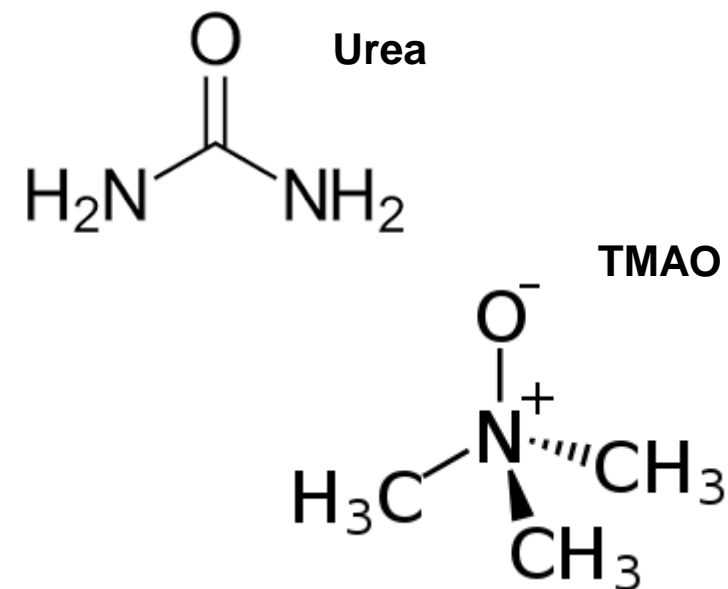
16 polyL chains
concentrations C_{prot}
3 μM , 0.18 mM, and 100 mM





CHAINS IN MIXED SOLVENTS (COSOLVENT) (MOLECULAR TRANSFER MODEL (MTM))

- **PolyQ in water plus TMAO**
- **PolyL in water plus Urea/TMAO**





CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

$$E_{CG}(\{\mathbf{r}\}, [C]) = E_{CG}(\{\mathbf{r}\}, 0) + \Delta G_{tr}(\{\mathbf{r}\}, [C]),$$

$$\Delta G_{tr}(\{\mathbf{r}\}, [C]) = \sum_{i=1}^{N_{res}} \delta g_{tr}^{bb}([C]) \frac{\alpha_i^{bb}(\{\mathbf{r}\})}{\alpha_{Gly-i-Gly}^{bb}} + \sum_{i=1}^{N_{res}} \delta g_{tr,i}^{sc}([C]) \frac{\alpha_i^{sc}(\{\mathbf{r}\})}{\alpha_{Gly-i-Gly}^{sc}},$$

$$\left. \begin{array}{l} \delta g_{tr}^{bb}([C]) \\ \delta g_{tr,i}^{sc}([C]) \end{array} \right\} \text{Transfer free energies from} \\ \text{water to a cosolvent solution [C]}$$

$$\left. \begin{array}{l} \alpha_i^{bb}(\{\mathbf{r}\}) \\ \alpha_i^{sc}(\{\mathbf{r}\}) \end{array} \right\} \text{solvent accessible surface} \\ \text{area (SASA)}$$

- **PolyQ in water plus TMAO**
- **PolyL in water plus Urea/TMAO**



CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

$$E_{CG}(\{\mathbf{r}\}, [C]) = E_{CG}(\{\mathbf{r}\}, 0) + \Delta G_{tr}(\{\mathbf{r}\}, [C]),$$

$$\Delta G_{tr}(\{\mathbf{r}\}, [C]) = \sum_{i=1}^{N_{res}} \delta g_{tr}^{bb}([C]) \frac{\alpha_i^{bb}(\{\mathbf{r}\})}{\alpha_{Gly-i-Gly}^{bb}} + \sum_{i=1}^{N_{res}} \delta g_{tr,i}^{sc}([C]) \frac{\alpha_i^{sc}(\{\mathbf{r}\})}{\alpha_{Gly-i-Gly}^{sc}},$$

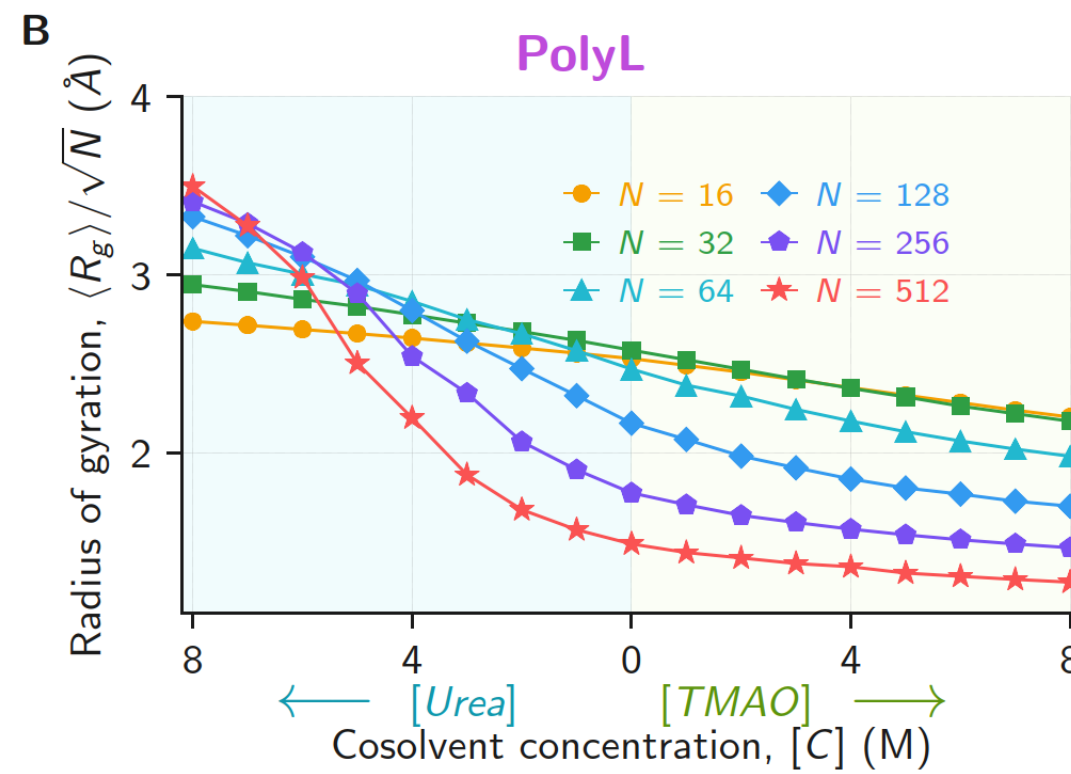
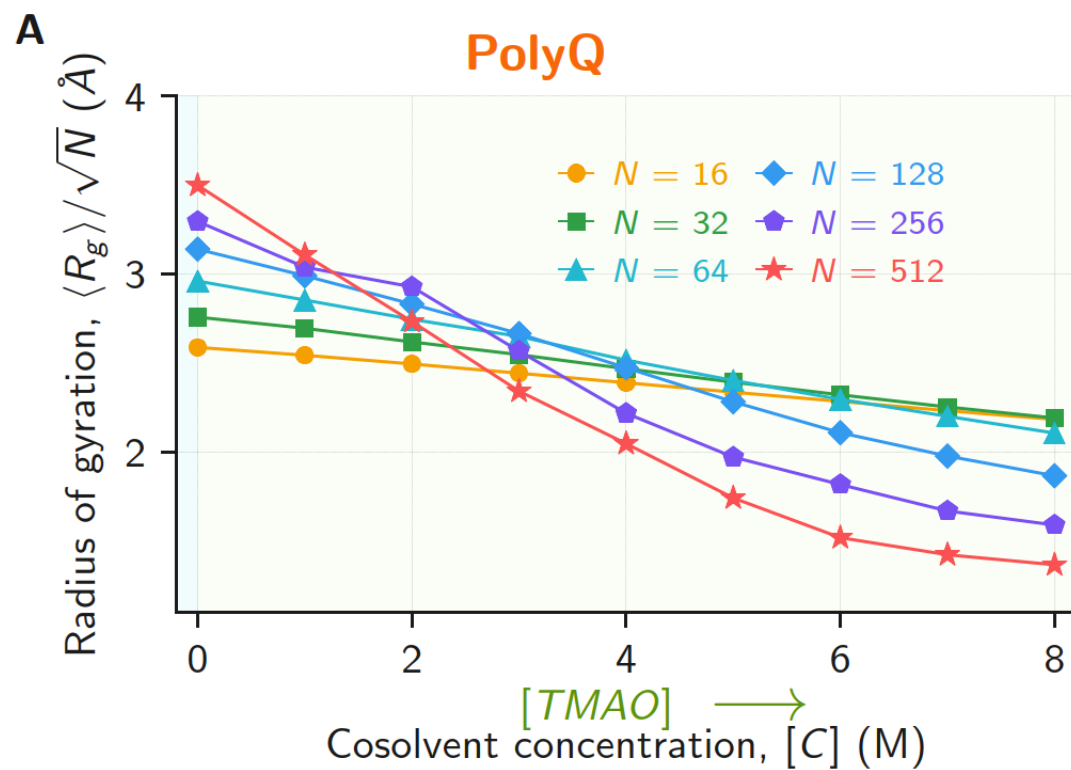
- Mean field concept for solvent contributions in the energy function
- Co-nonsolvency etc cannot be studied

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO



CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

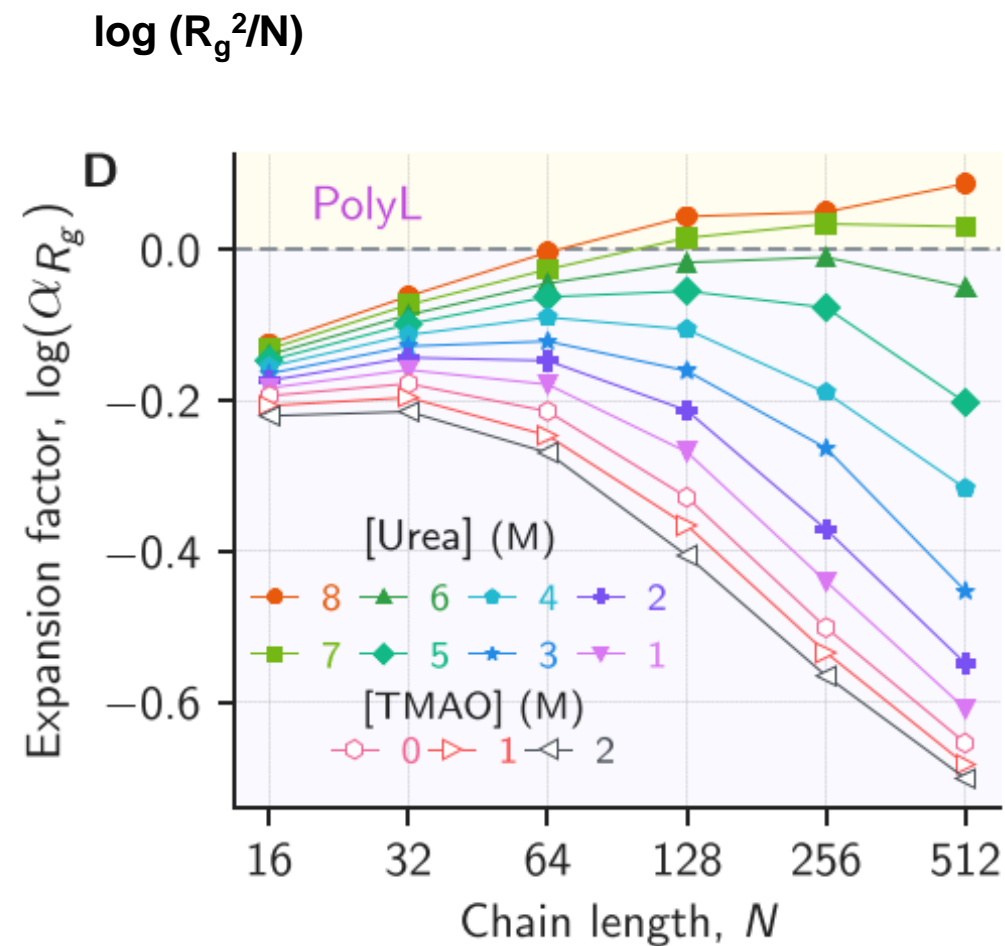
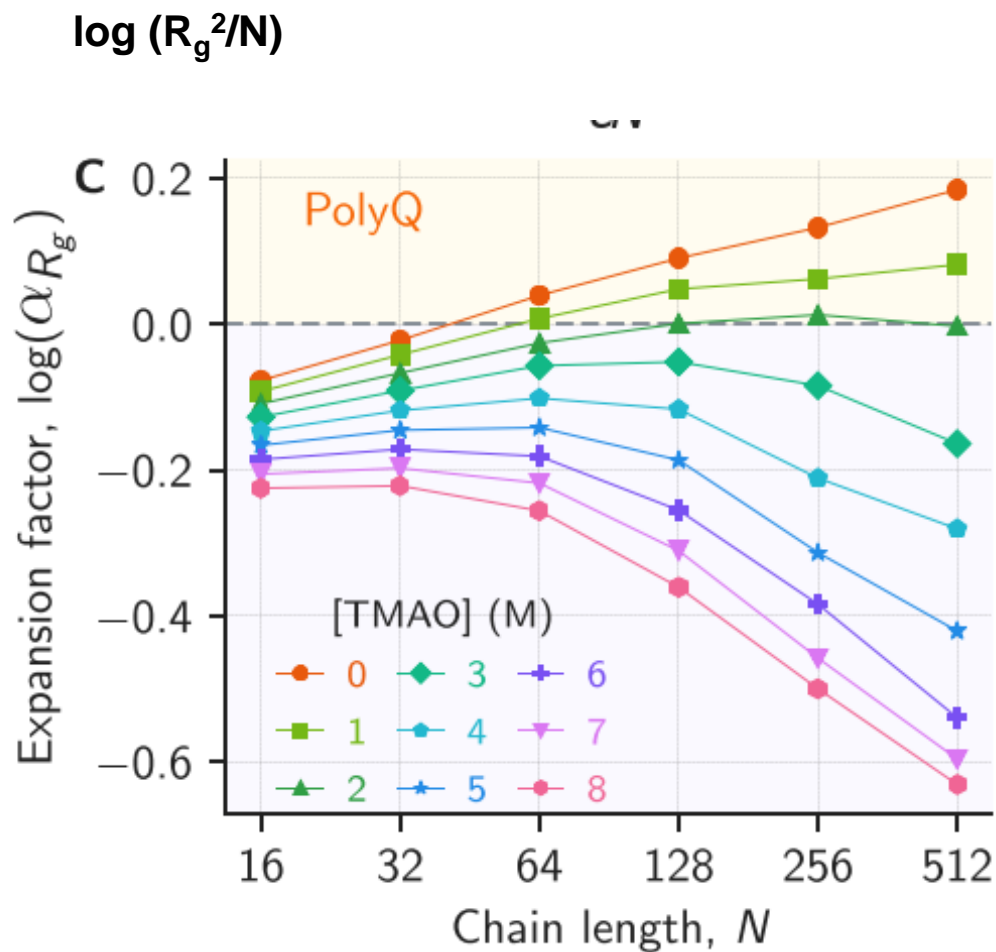
- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO



CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)



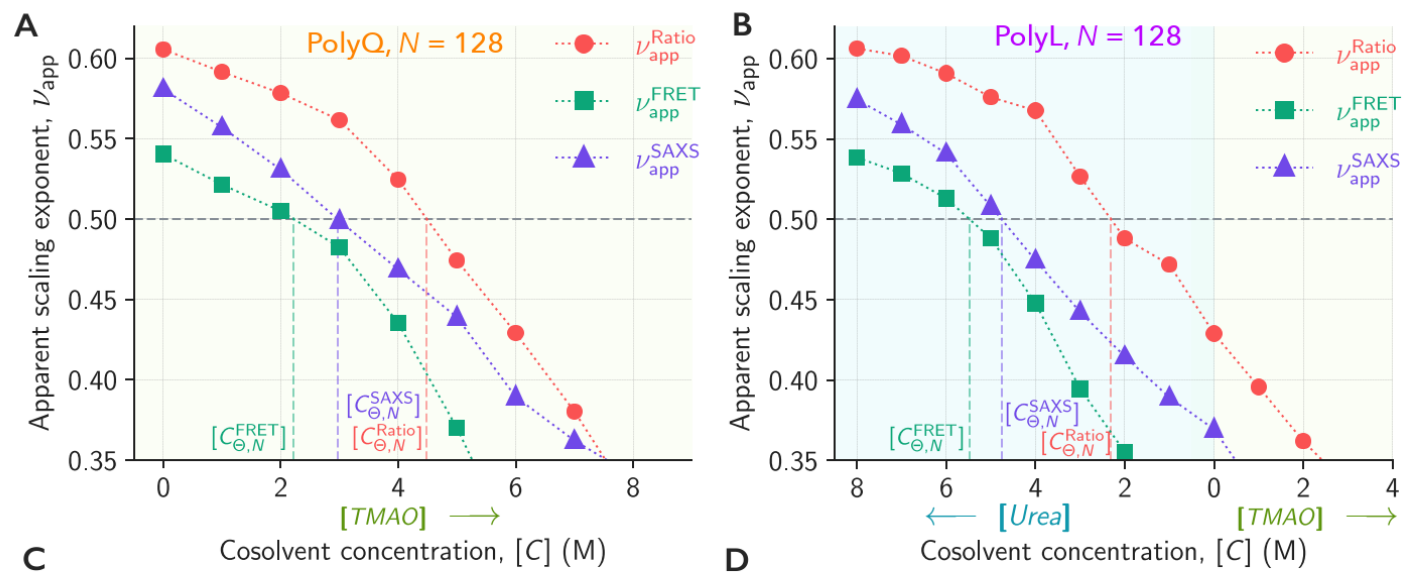
- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO





CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO





CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

BOLD ANSATZ:

TREAT COSOLVENT CONCENTRATION AS
SHIFT IN TEMPERATURE FOR COIL-GLOBULE TRANSITION

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO

$$\langle R_{ee}^2(N) \rangle^{1/2} \sim N^{1/2} f_{\pm}(N\tau^{1/\phi})$$

Intrinsic stiffness of model IDPs relevant

Single chain properties for long chains needed to estimate propensity for LLPS

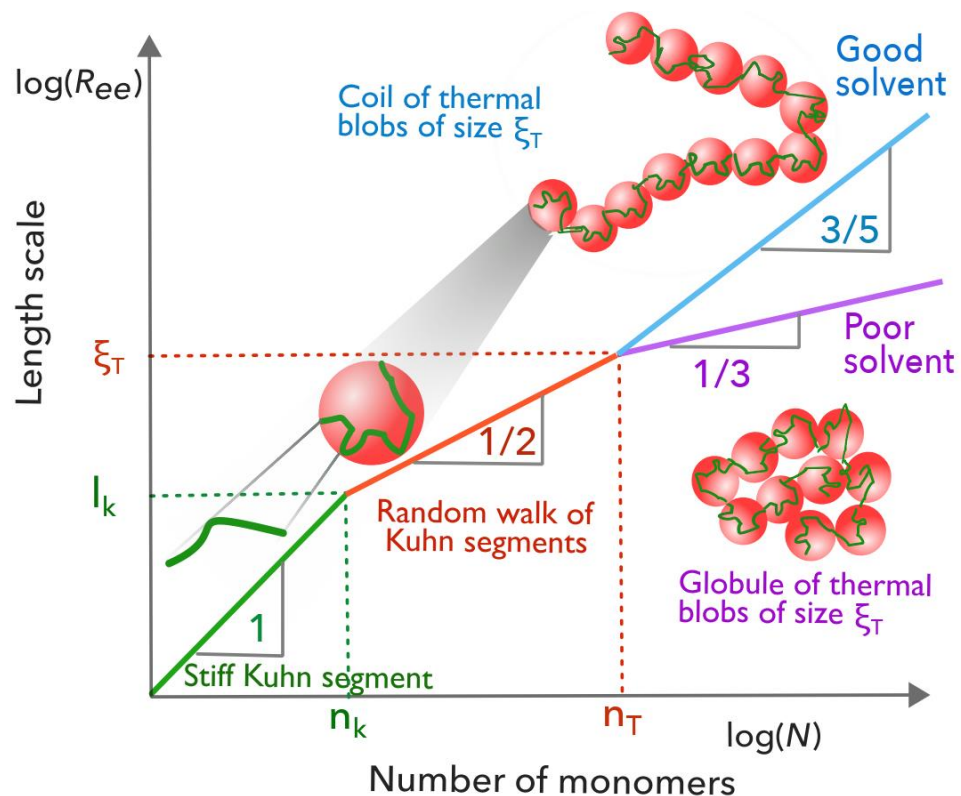
**Θ type scaling adapted to cosolvent concentration can be applied
(model dependent?)**



CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

BOLD ANSATZ:
TREAT COSOLVENT CONCENTRATION AS
SHIFT IN TEMPERATURE FOR COIL-GLOBULE TRANSITION

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO



$$\langle R_{ee}^2(N) \rangle^{1/2} \sim N^{1/2} f_{\pm}(N\tau^{1/\phi})$$

$$\tau = (T - \Theta) / \Theta$$

$$\Phi = 2$$

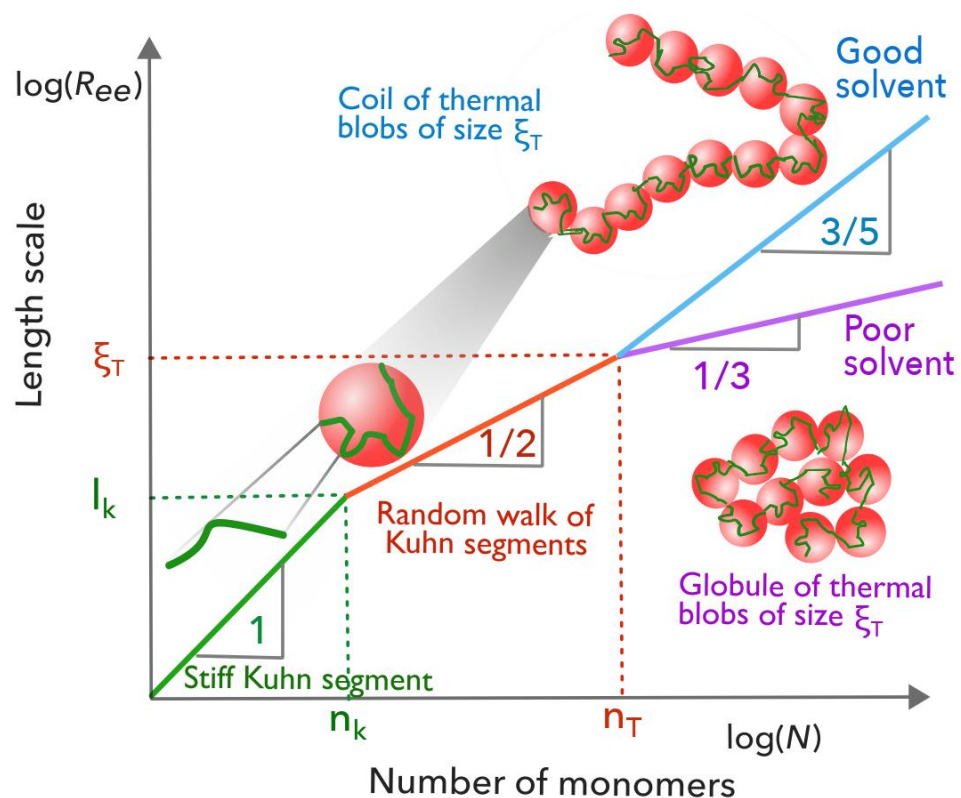
$$f(x) \begin{cases} \rightarrow |x|^{(3/5-1/2)}, & x \rightarrow +\infty \\ \rightarrow \text{const}, & x \rightarrow 0 \\ \rightarrow |x|^{(1/3-1/2)}, & x \rightarrow -\infty \end{cases}$$



CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

BOLD ANSATZ:
TREAT COSOLVENT CONCENTRATION AS
SHIFT IN TEMPERATURE FOR COIL-GLOBULE TRANSITION

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO



$$\langle R_{ee}^2(N) \rangle^{1/2} \sim N^{1/2} f_{\pm}(N\tau^{1/\phi})$$

$$\tau = (T - \Theta) / \Theta$$

to

$$\tau = (c_{\text{cos}} - c_{\text{cos}\Theta}) / c_{\text{cos}\Theta}$$

$$\Phi = 1/2$$

works only for $l \gg l_k$



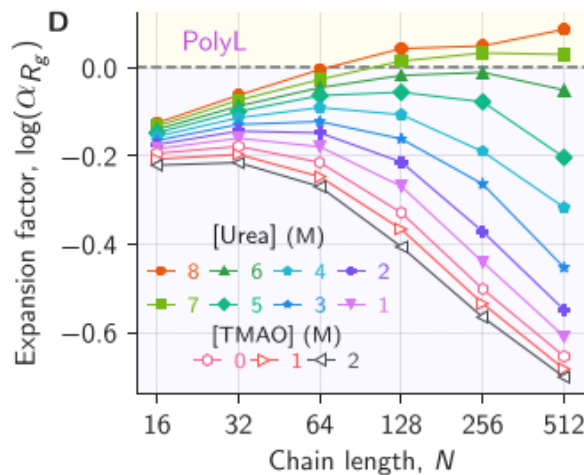
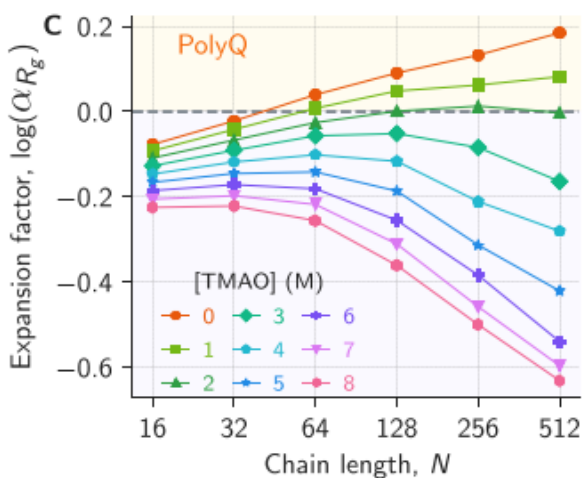
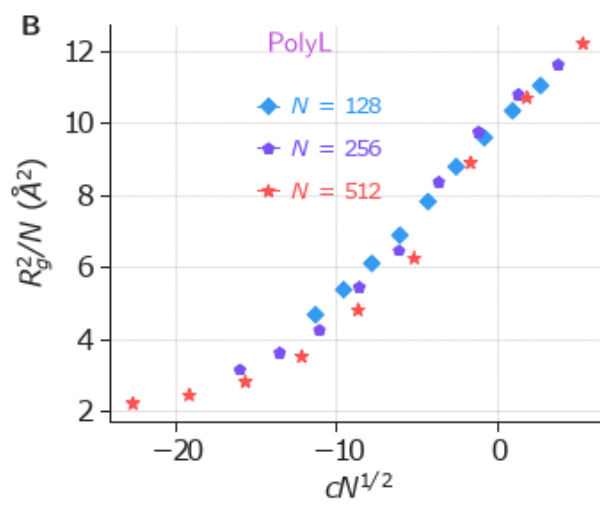
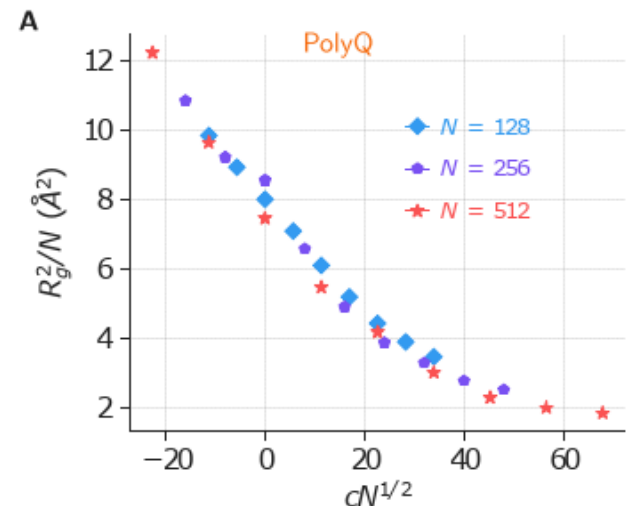
CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

BOLD ANSATZ:

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO

ON AS GLOBULE TRANSITION

$$\langle R_{ee}^2(N) \rangle^{1/2} \sim N^{1/2} f_{\pm}(N\tau^{1/\phi})$$



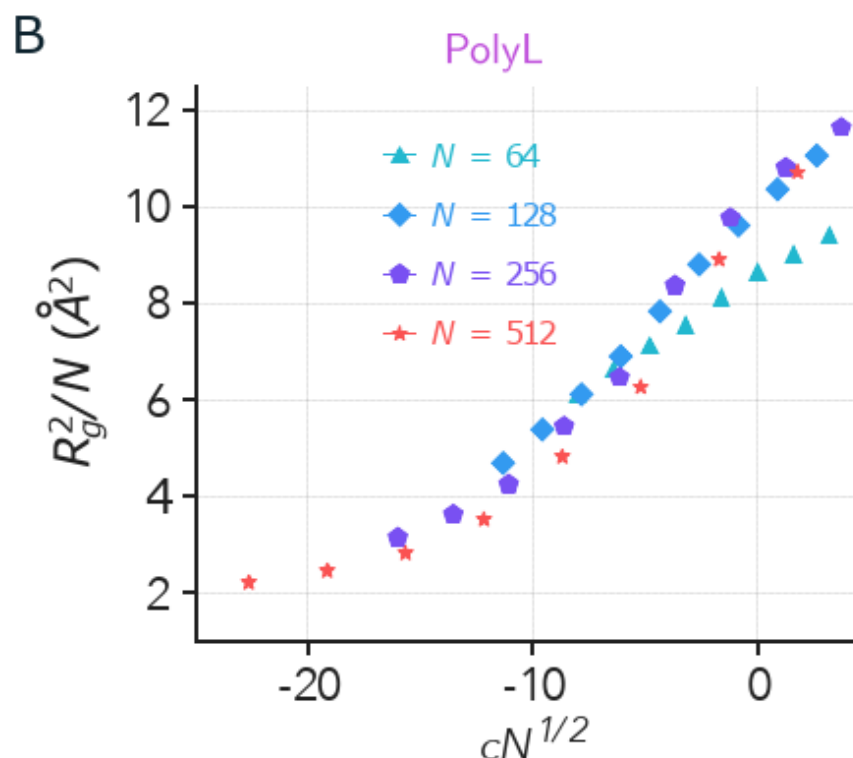
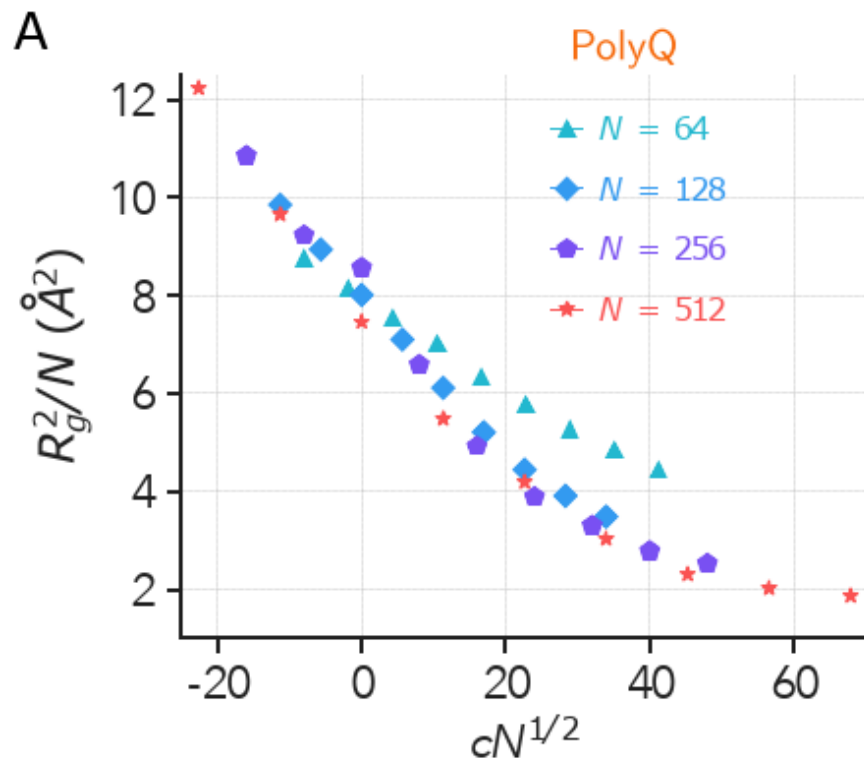
- $\tau = (T - \Theta) / \Theta$ to $c = (c_{\text{cos}} - c_{\text{cos}\Theta}) / c_{\text{cos}\Theta}$
- $\Phi = 1/2$
- Works only for $l \gg l_k$



CHAINS IN MIXED SOLVENTS (COSOLVENT) MOLECULAR TRANSFER MODEL (MTM)

BOLD ANSATZ:
TREAT COSOLVENT CONCENTRATION AS
SHIFT IN TEMPERATURE FOR COIL-GLOBULE TRANSITION

- PolyQ in water plus TMAO
- PolyL in water plus Urea/TMAO



$$\sim N^{1/2} f_{\pm}(N\tau^{1/\phi})$$

$$\text{or } c = (c_{\text{cos}} - c_{\text{cos}\theta}) / c_{\text{cos}\theta}$$

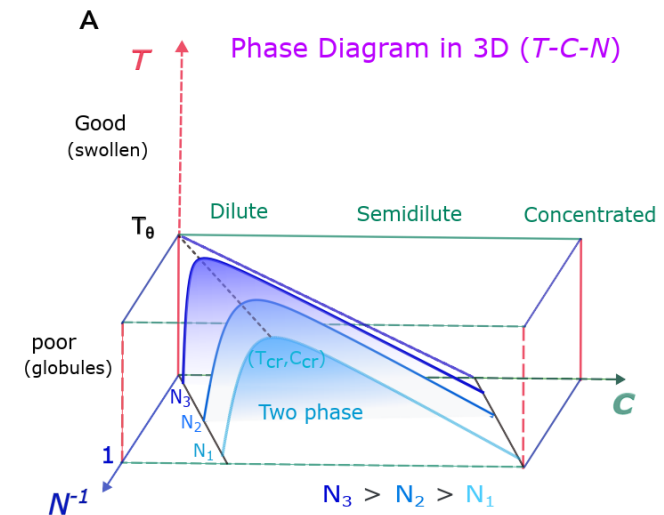
$$\text{or } l \gg l_k$$



CLASSICAL POLYMER THEORY AND

INTRINSIC STIFFNESS AND θ REGIME OF MODEL IDP_s AND RELATION TO LLPS

- Experimental situation
 - Single chain analysis used to infer solvation behaviour
 - Good solvent => no contribution to LLPS
 - Poor solvent => tendency to trigger LLPS
- Two homopolymers as example
 - polyQ: hydrophilic polyglutamine in water
 - polyL: hydrophobic polyglutamine in water
 - polyQ, polyL in mixed solvents



Single chain properties have to be taken with great care