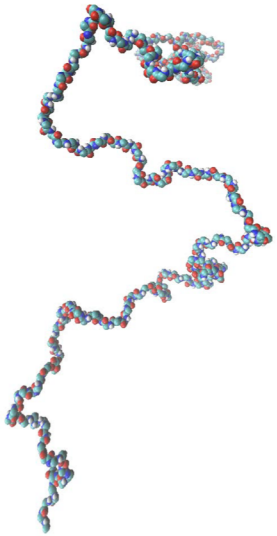
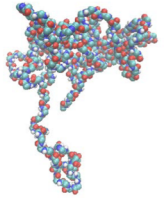
Advanced topics 4: Flexible systems and intrinsically disordered proteins





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BioSAXS 5
Nov. 6, 2019 Argonne Nat. Lab

Advanced topics 4: Flexible systems and intrinsically disordered proteins

References:

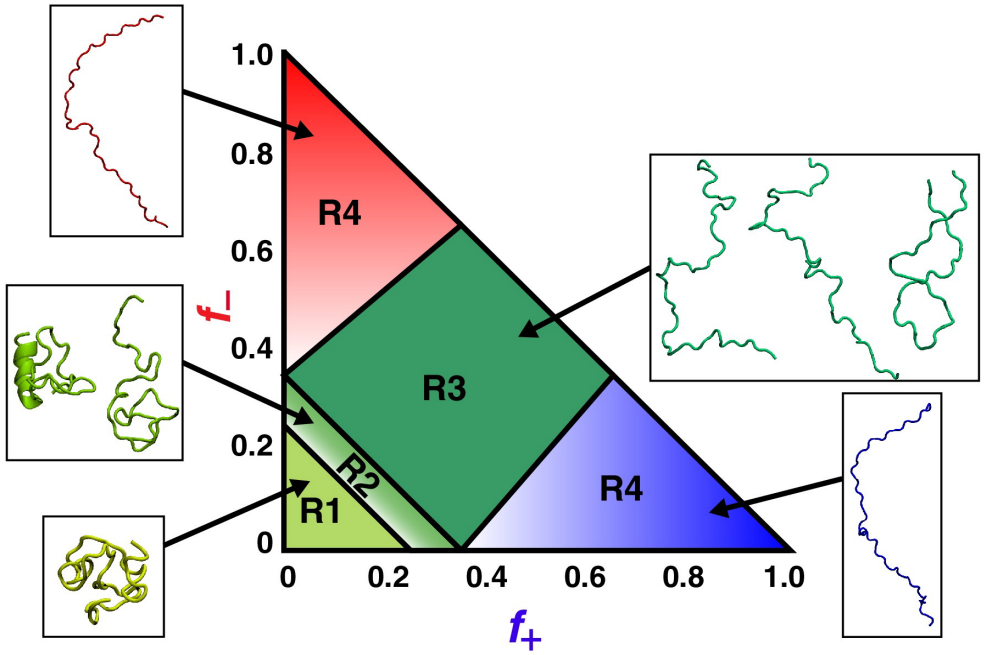
Small-angle Scattering

1. Riback, J.A., et al., *Innovative scattering analysis shows that hydrophobic disordered proteins are expanded in water.* Science, 2017. **358**(6360): p. 238-241.
2. Rambo, R.P. and J.A. Tainer, *Characterizing flexible and intrinsically unstructured biological macromolecules by SAS using the Porod-Debye law.* Biopolymers, 2011. **95**(8): p. 559-71.
3. Bernado, P. and D.I. Svergun, *Analysis of intrinsically disordered proteins by small-angle X-ray scattering.* Methods Mol Biol, 2012. **896**: p. 107-22.
4. Receveur-Brechot, V. and D. Durand, *How random are intrinsically disordered proteins? A small angle scattering perspective.* Curr Protein Pept Sci, 2012. **13**(1): p. 55-75.

Disordered proteins

1. Das, R.K., K.M. Ruff, and R.V. Pappu, *Relating sequence encoded information to form and function of intrinsically disordered proteins.* Curr Opin Struct Biol, 2015. **32**: p. 102-12.
2. van der Lee, R., et al., *Classification of intrinsically disordered regions and proteins.* Chem Rev, 2014. **114**(13): p. 6589-631.

Unfolded and disordered proteins



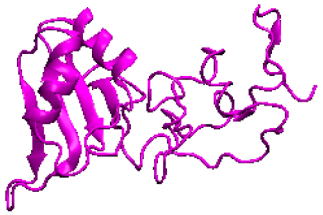
Das, Ruff, & Pappu
(2015) Curr Op Str Biol

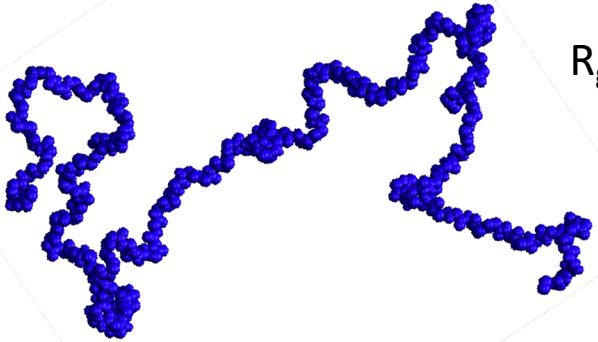
Relevance:

- Does protein folding typically initiate with a rapid hydrophobic collapse?
- ~1/3 of the proteome is intrinsically disordered: **IDP**(roteins), **IDR**(egions)
- Folding upon binding
- phase separation & disease
- Is water a good or poor solvent for proteins?

Scattering: compact versus unfolded polypeptides

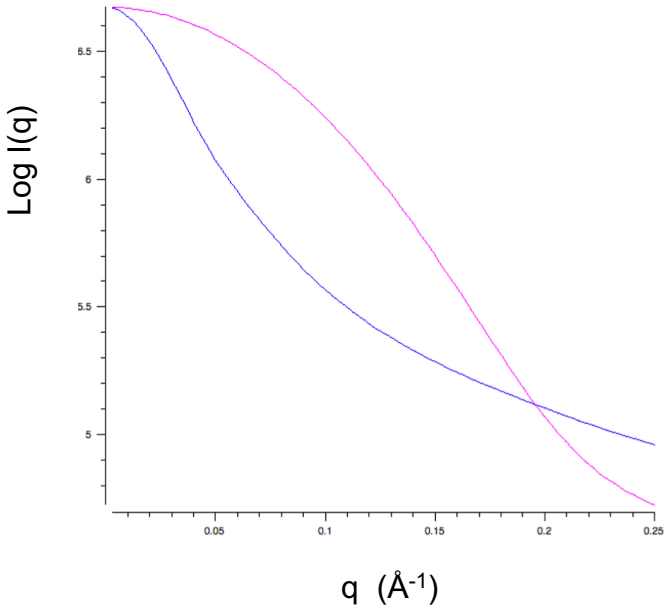
R_g=19 Å



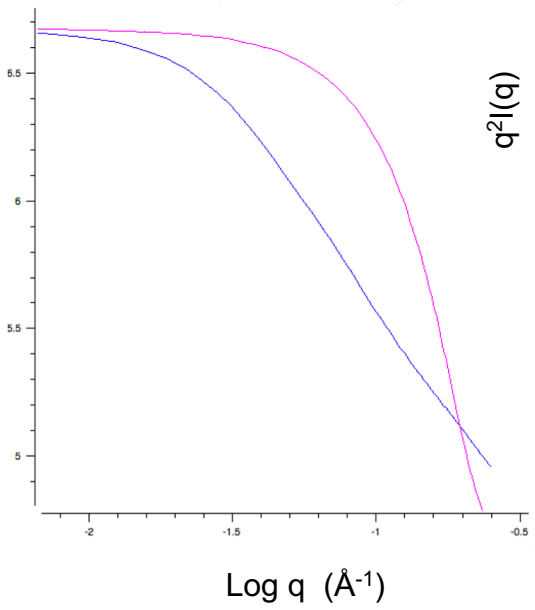


$R_g^{ensemble}$=49 Å

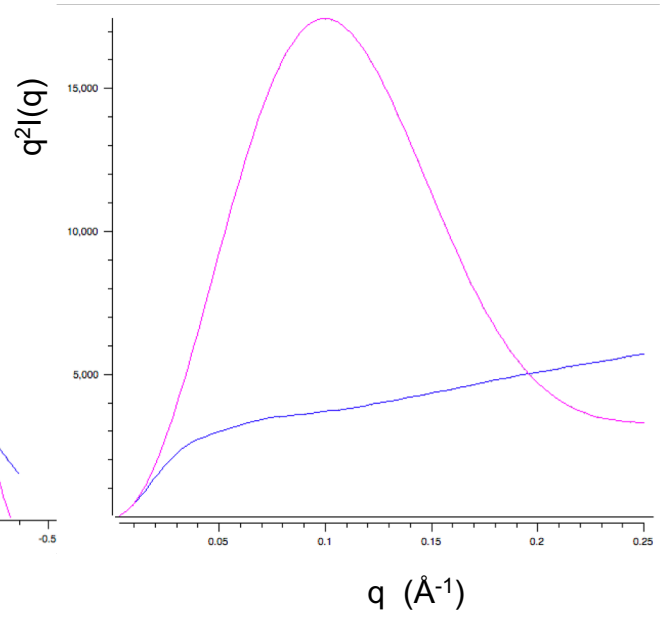
log-linear



log-log plot

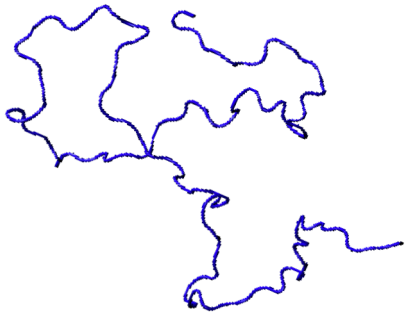


Kratky plot



Rescaling to remove size but keep shape information: Dimensionless Kratky Plot

Rg=100 Å



Rg=40 Å

Rg=10 Å

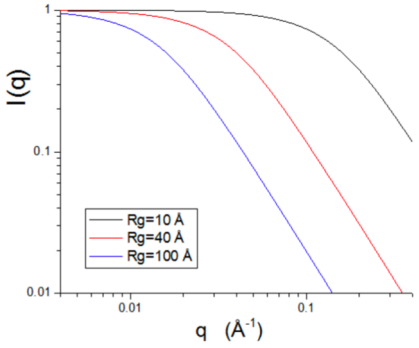
Debye formula for random walk:

$$I(q) = \frac{2I_o\left(e^{-(qRg)^2} - 1 + (qRg)^2\right)}{(qRg)^4}$$

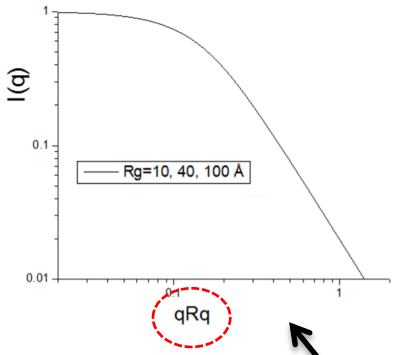
$$x = qRg$$

$$I(q) = \frac{2I_o\left(e^{-x^2} - 1 + x^2\right)}{x^4}$$

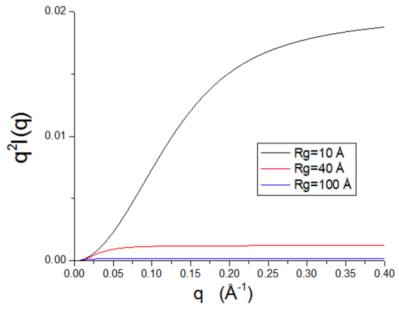
log-log Plot



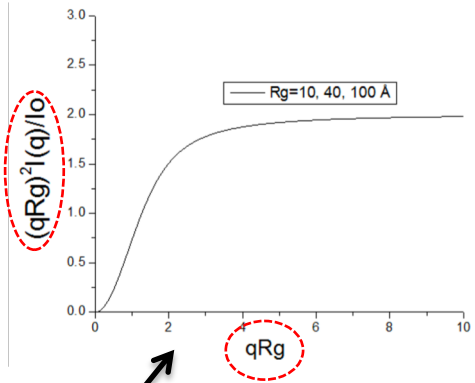
Dimensionless log-log Plot



Kratky plot



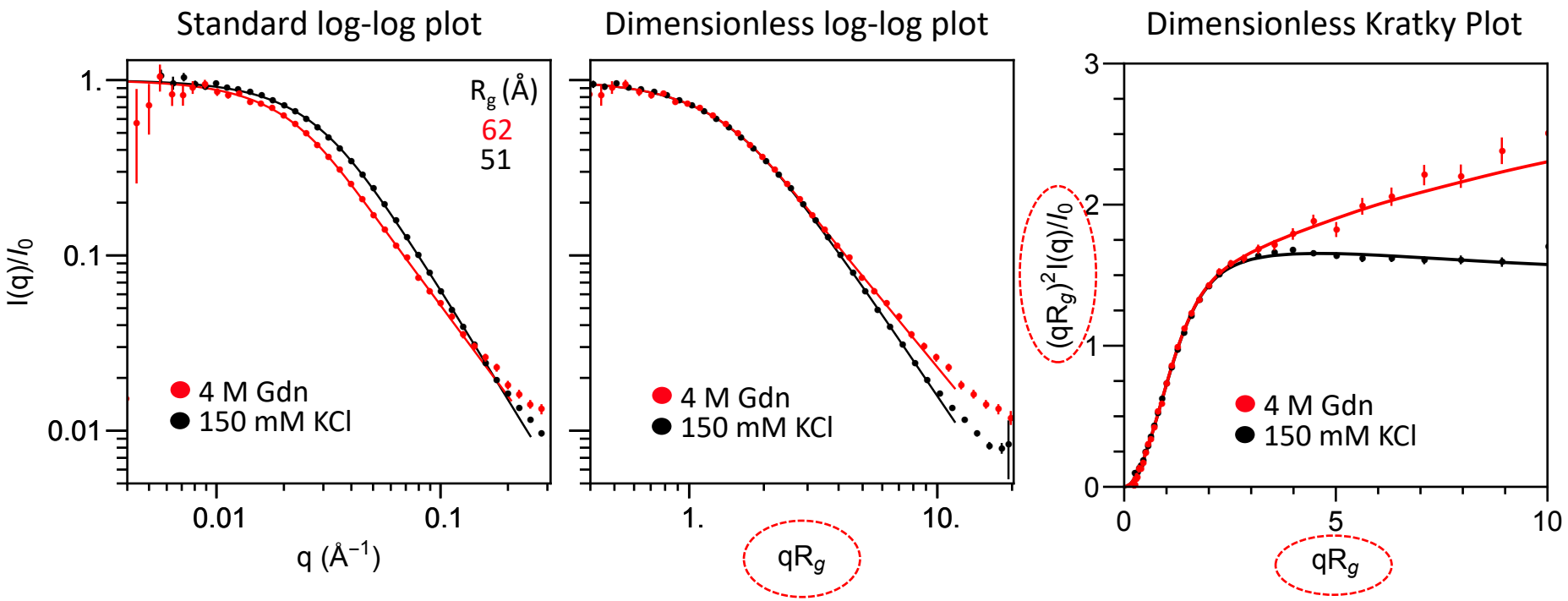
Dimensionless Kratky plot



Report only on shape

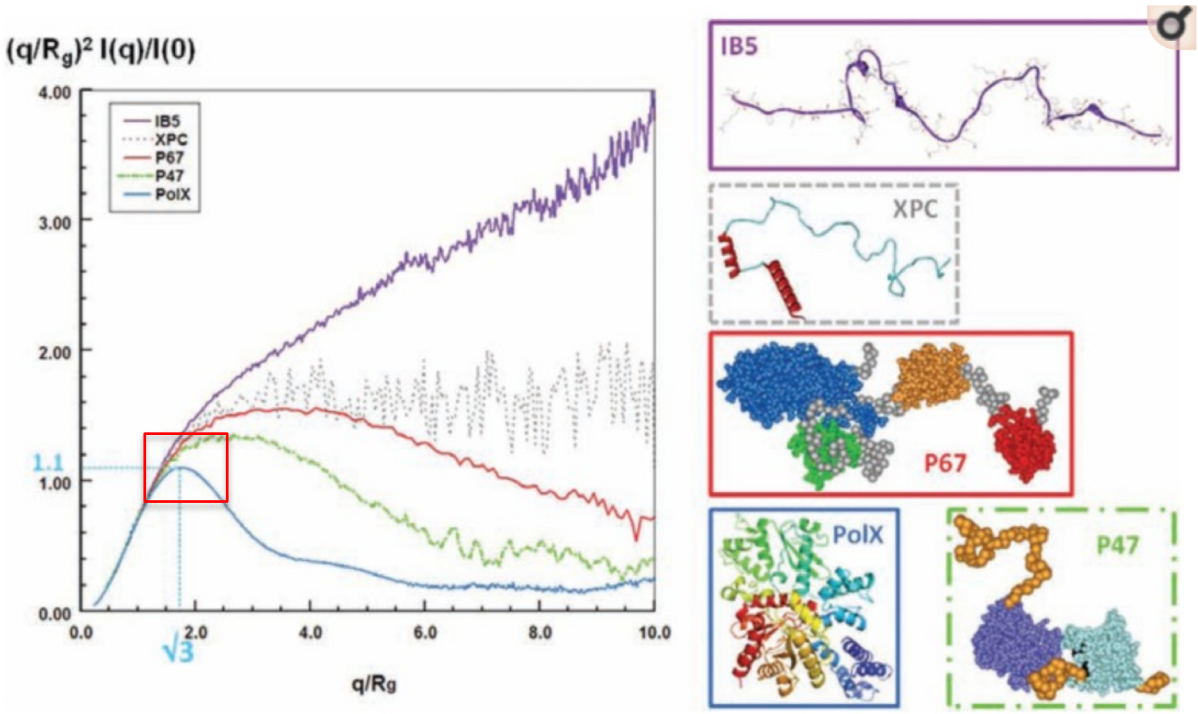
More on scaling and shape

IDP in water and high denaturant concentration



Shapes are different – but how?

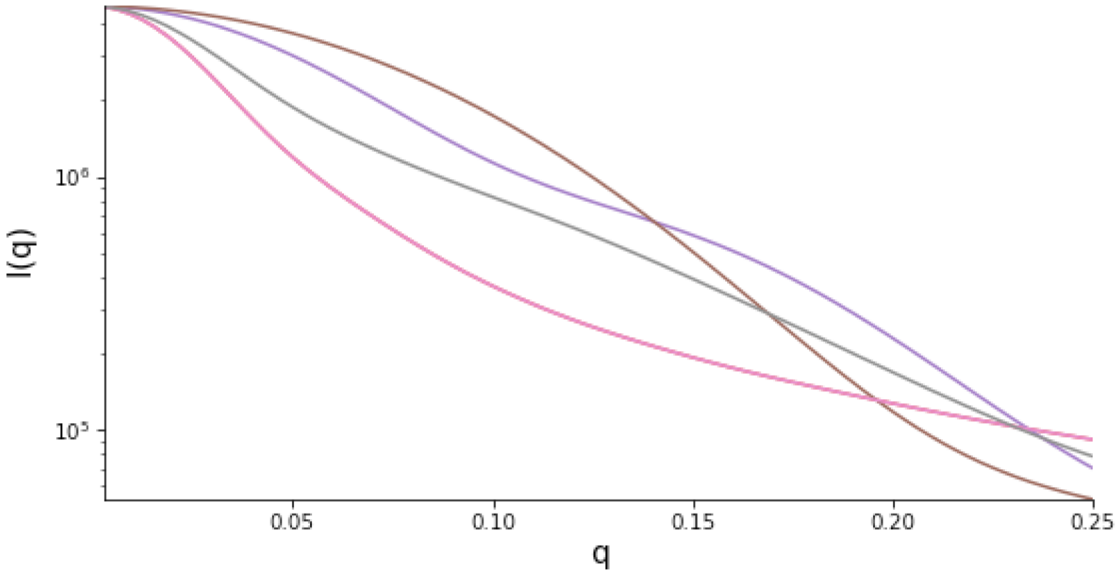
Useful examples from globular to unfolded

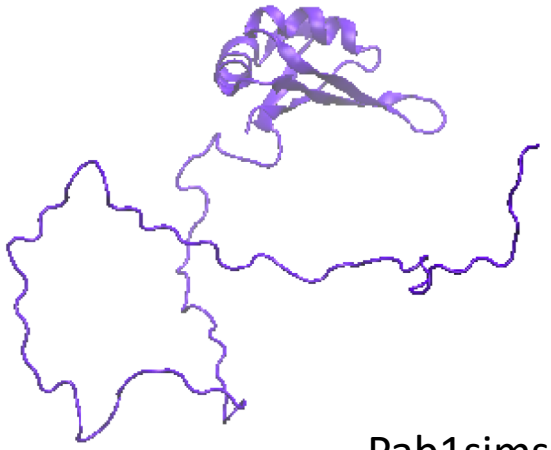


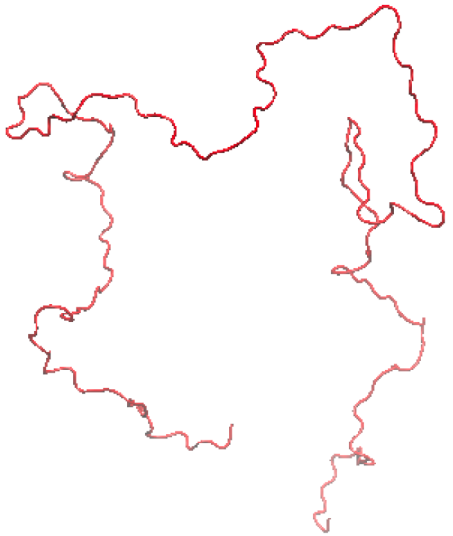
Normalized Kratky plots. The scattering pattern of globular proteins in a normalized Kratky plot exhibits a bell-shaped profile with a clear maximum value of 1.104 for $qR_g=\sqrt{3}$, regardless of the size of the protein, and are all nearly superimposable in the q range $0<qR_g<3$. Conversely, for a random chain, the curve rises with increasing angle, to nearly reach a plateau between 1.5 and 2 and may further increase at q>0.2-0.3 $Å^{-1}$, depending on the persistence length and the internal structure of the protein. Bell-shaped profile of a globular protein (PolX, blue line); curve of a protein consisting of several domains tethered by linkers with rather compact conformations ($p47^{phox}$, dotted green line) or extended conformations ($p67^{phox}$, continue red line); curve of a fully disordered protein with very short elements of secondary structure (XPC dotted grey line); and curve of a fully disordered and extended protein with short segments of polyproline repeats (salivary protein IB5, continue purple line).

Receveur-Brechot, V. and D. Durand, *How random are intrinsically disordered proteins? A small angle scattering perspective.* Curr Protein Pept Sci, 2012. **13**(1): p. 55-75.

Examples of mixed systems



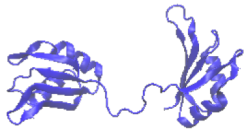


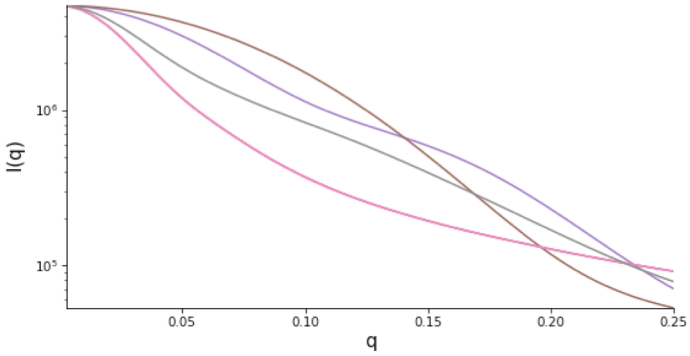


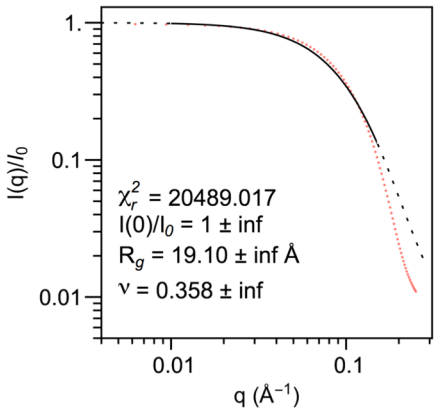
Pab1sims.vmd

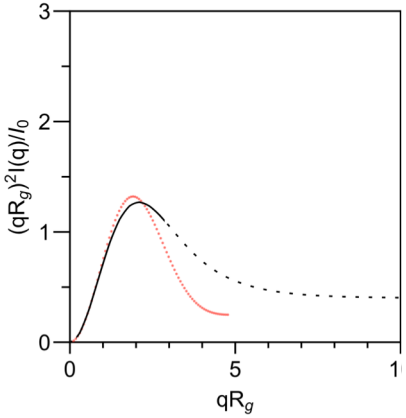
Examples of mixed systems

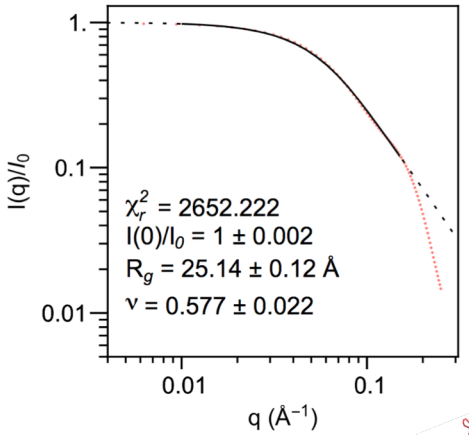
(shapes, Fit)

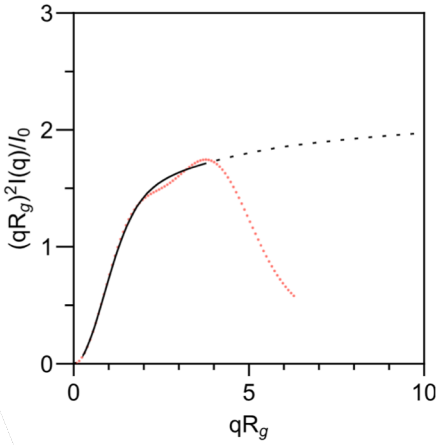


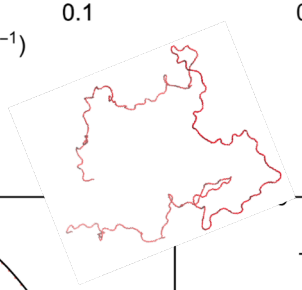


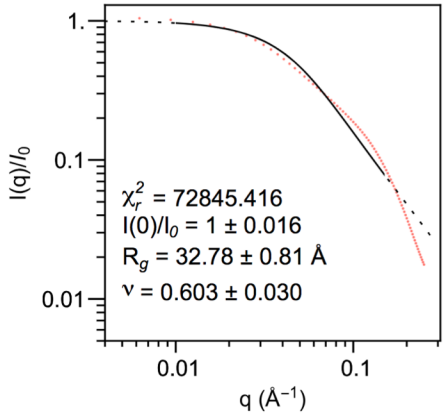


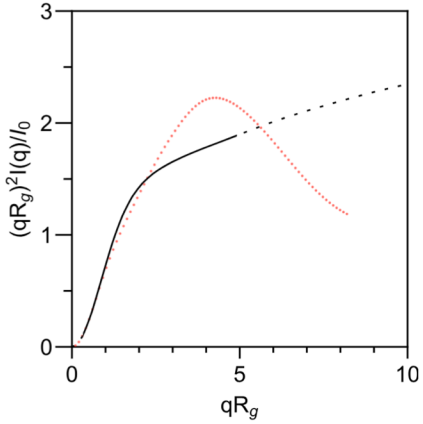


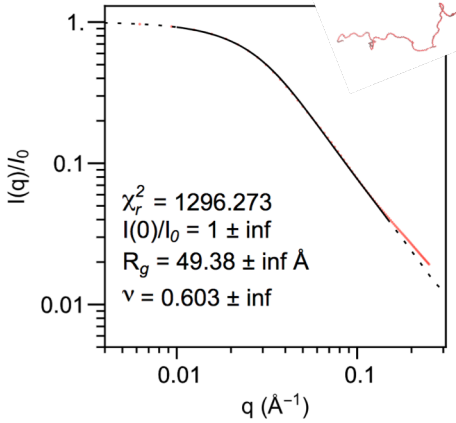


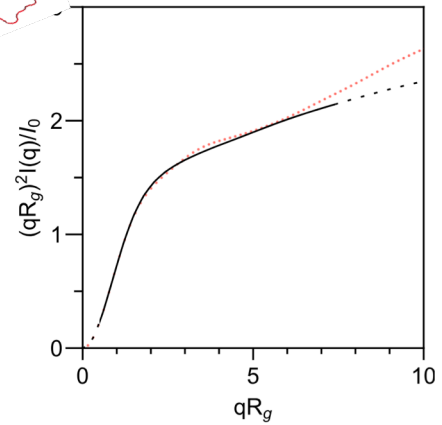




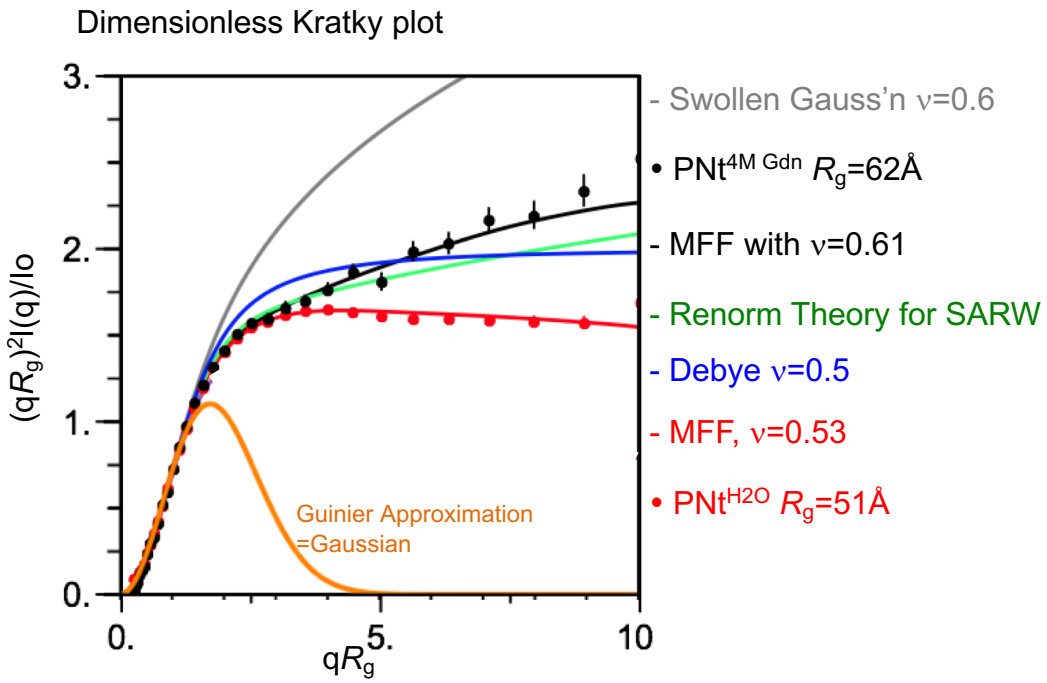








Scattering from Random Walks (allowed to cross) and self-avoiding RW (not allowed to cross)



Debye formula for random walk:

$$I_{Debye}(q) = \frac{2I_o\left(e^{-(qRg)^2} - 1 + (qRg)^2\right)}{(qRg)^4}$$

Swollen Gaussian coil model

$$P_{SwollenGausCoil}(R_{ij}) \propto e^{-\left(3R_{ij}^2 \,/\, 3\langle R_{ij}^2\rangle\right)}$$

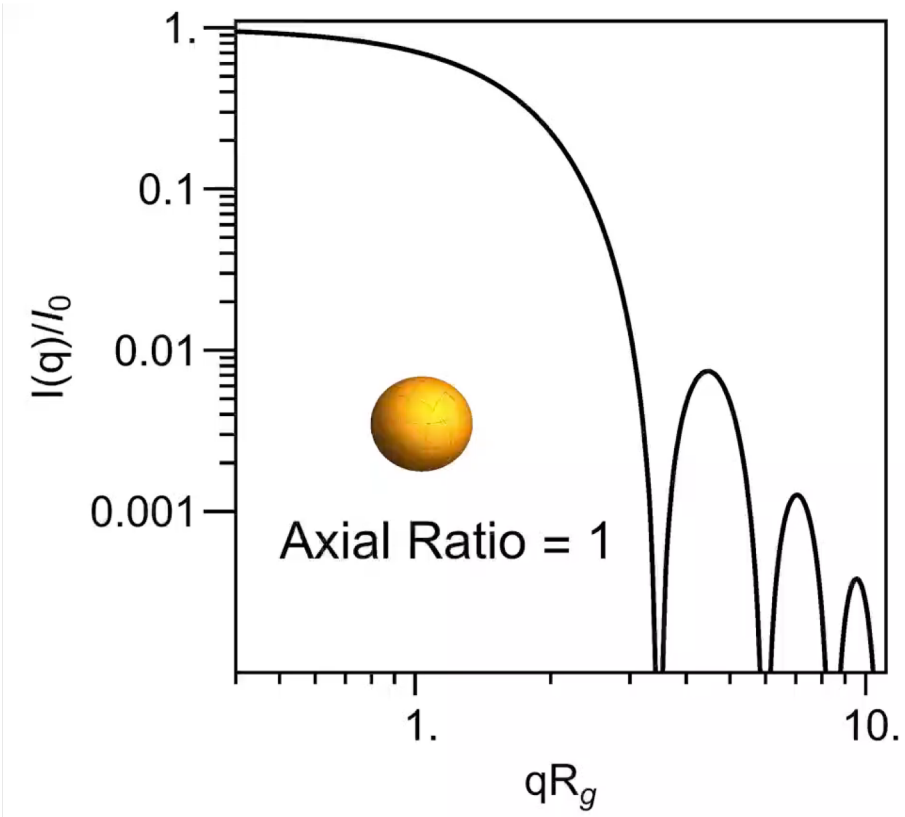
$$\left\langle R_{ij}^2 \right\rangle = a^2 \left|i-j\right|^{2\nu}$$

a=segment length

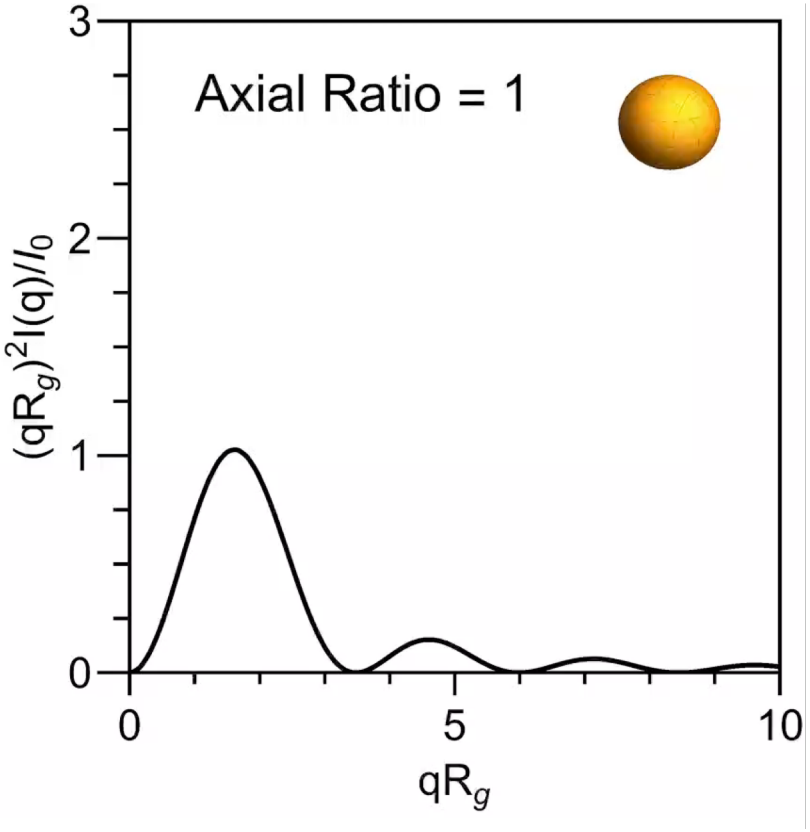
Molecular Form Factors (MFF) describe shape of objects

E.g., ellipsoid, axial ratio

Standard Log-log plot

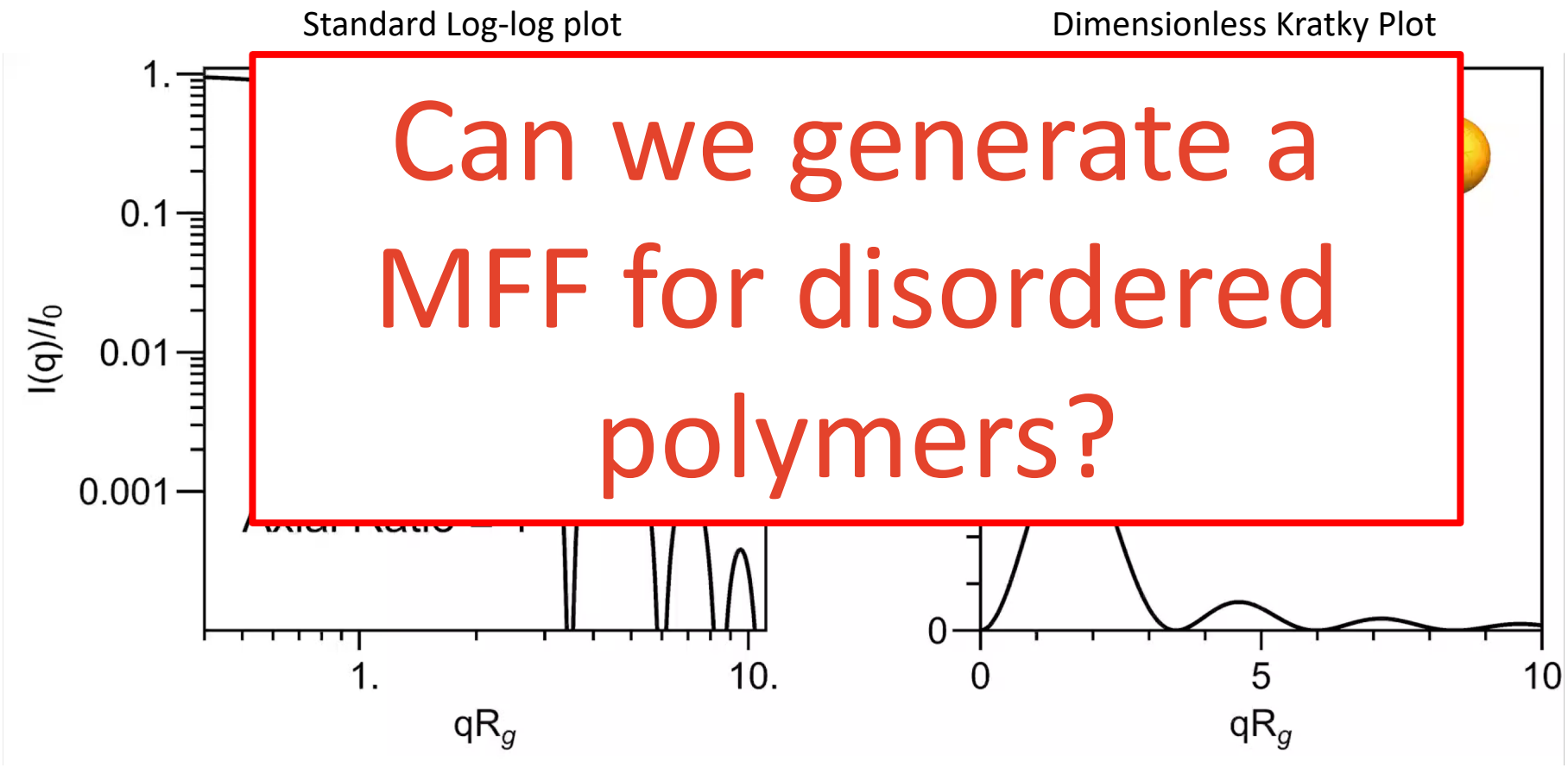


Dimensionless Kratky Plot



Molecular Form Factors (MFF) describe shape of objects

E.g., ellipsoid, axial ratio



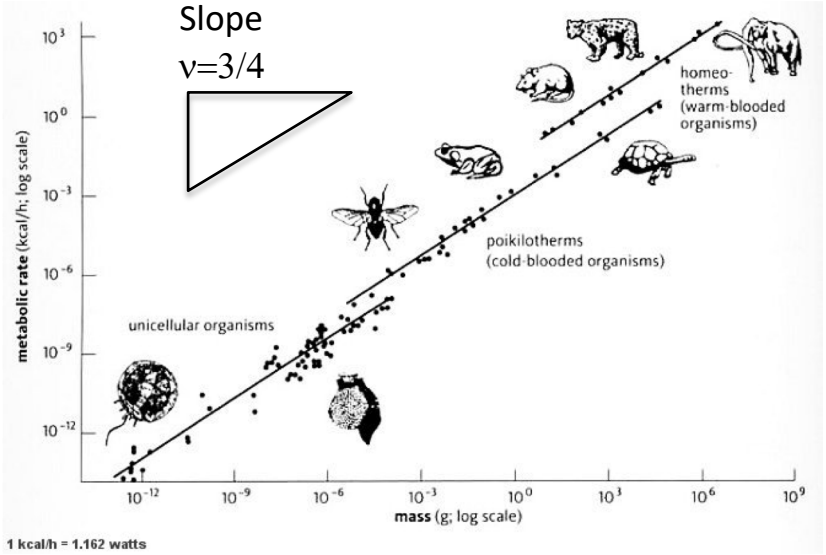
Can we generate a MFF for disordered polymers?

Allometry and scaling laws in Biology

General form for a biological variable for animals

Variable $\propto$ (Size)$^{\nu}$

Kleiber's Law: Metabolic Rate $\propto$ (Mass)$^{3/4}$



http://universe-review.ca/I10-83-metabolic.jpg

Spheres

Mass $\propto R^3$

or

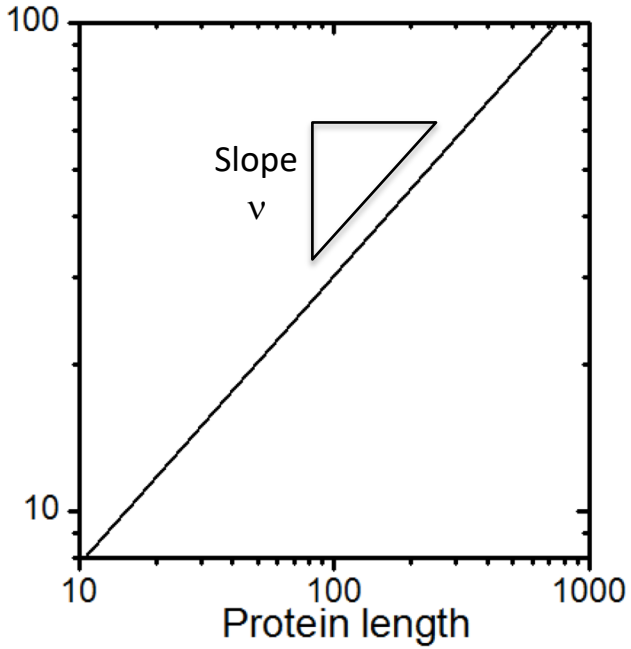
$R \propto M^{1/3}$

$\nu=1/3$

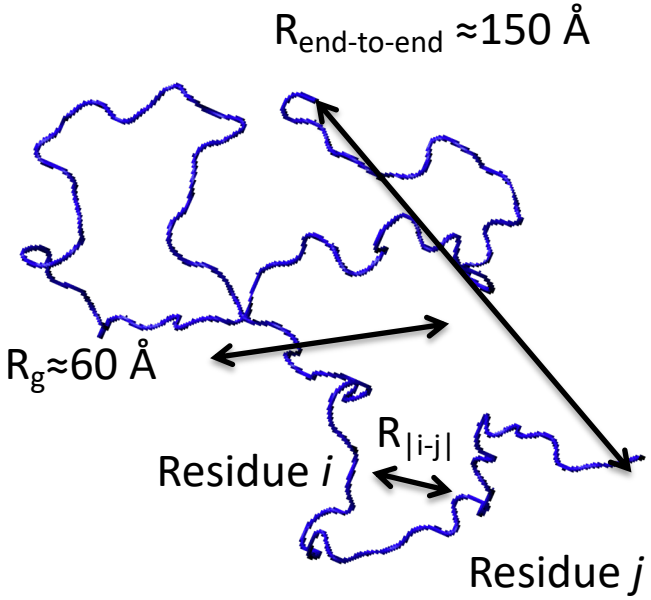
Polymer Physics: scaling laws and Flory exponent ν
Good and poor solvents

Solvent quality: Flory exponent ν

$R_g \propto N^{\nu}$
or
$R_{|i-j|} \propto |i-j|^{\nu}$



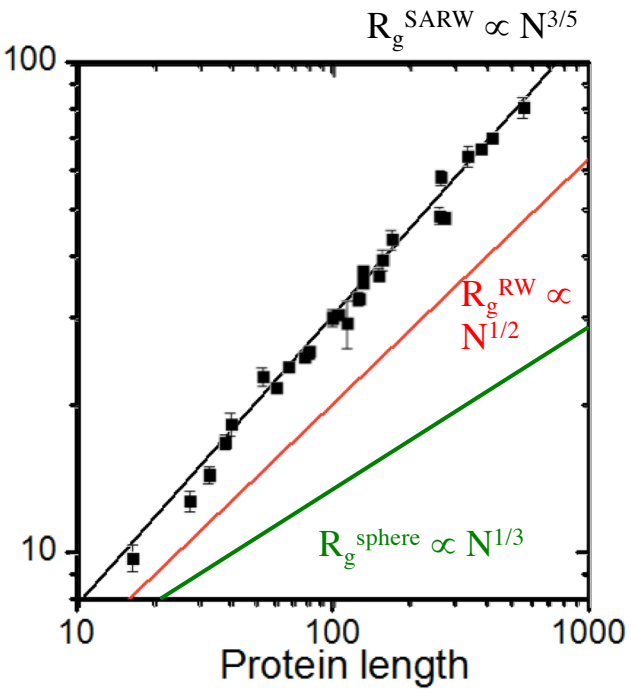
Or intra-residue spacing |i-j|



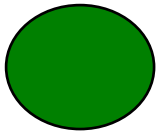
Polymer Physics: scaling laws and Flory exponent ν
Good and poor solvents

Solvent quality: ν

$R_g \propto N^{\nu}$



ν		
3/5	Self Avoiding random walk (SARW) $E_{prot\text{-}water} > E_{prot\text{-}prot}$	**Self-avoiding** Random Walk
Good solvent (contracted) $0.5<\nu\leq0.6$		
1/2	Random Walk behavior: Θ solvent $E_{prot\text{-}prot} \sim E_{prot\text{-}water}$	Random Walk (non-physical)
Poor (collapsed) $1/3<\nu<1/2$		
1/3	Globule $E_{prot\text{-}prot} > E_{prot\text{-}water}$	



$V=\frac{4}{3}\pi R^3$

Chemically denatured Proteins:
R_g scales as a self-avoiding random walk

Kohn et al., (2005) *PNAS*

General scaling behavior of proteins

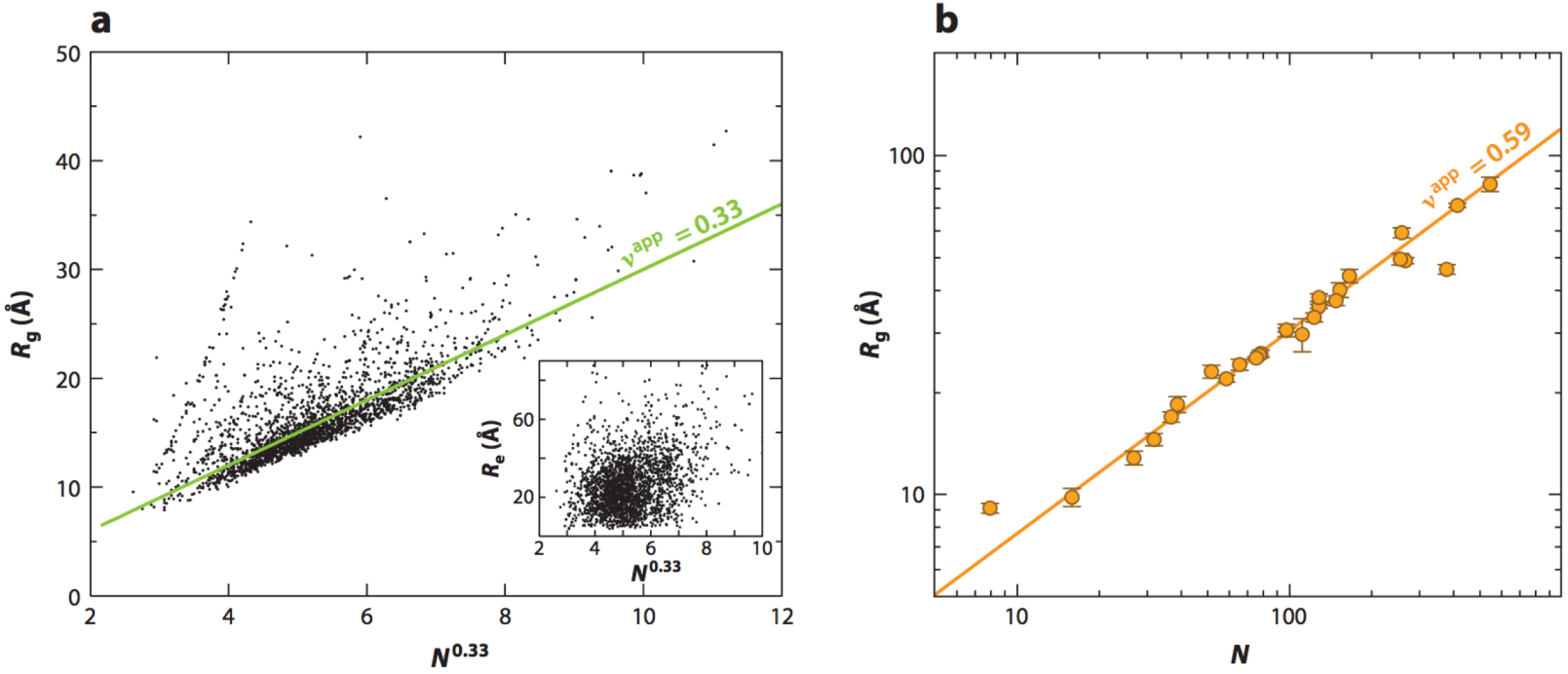


Figure 3

(*a*) Scaling of radius of gyration (R_g) with chain length N for folded proteins based on ~2,400 nonredundant structures taken from PDBSELECT25 (50). While the radius of gyration shows reasonable agreement with $\nu_{app} \approx 0.33$, the end-to-end distance shows a poor correlation (*inset*). (*b*) Scaling behavior for chemically denatured proteins based on data from Reference 74. The unfolded state under strongly denaturing conditions is well described by a self-avoiding random chain ($\nu_{app} \approx 0.59$).

Holehouse & Pappu (2018)
Annual Review Biophysics

Obtaining a MFF for disordered polymers
Polymer Physics: scaling laws and Flory exponent ν
Good and poor solvents

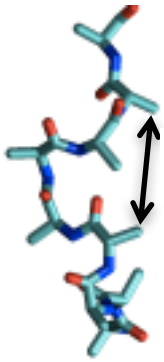
Debye formula for random walk

$$I_{Debye}(q) = \frac{2I_o\left(e^{-(qRg)^2} - 1 + (qRg)^2\right)}{(qRg)^4}$$

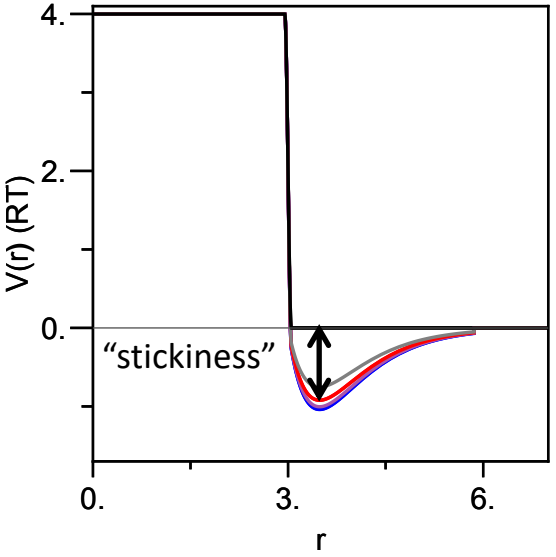
non-physical inter-penetration of chain

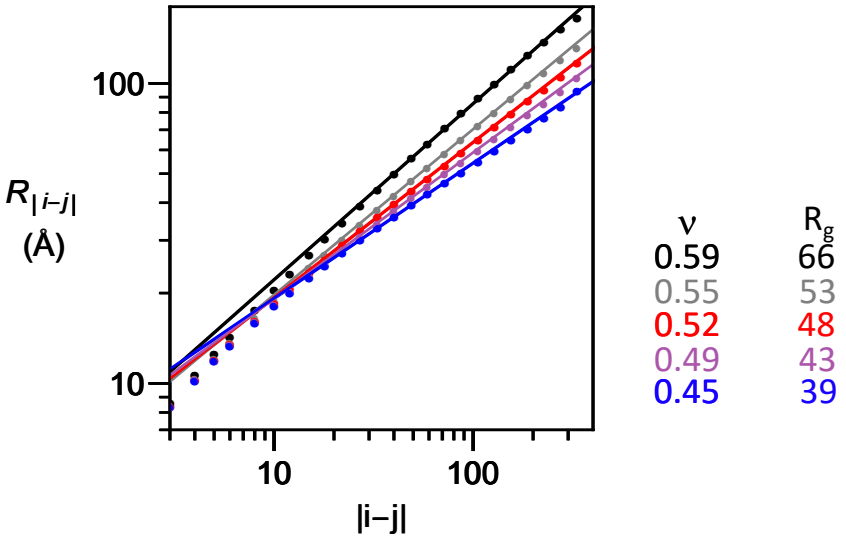
Can we generate a general MFF for realistic polymers?

Simulations generate ensembles used to make an MFF



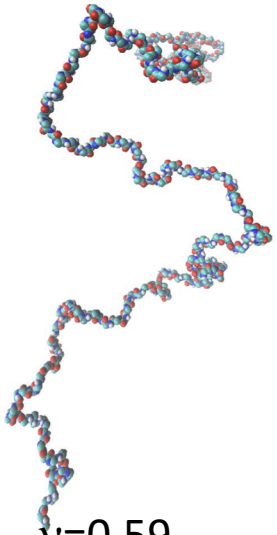
Vary Cβ–Cβ interaction





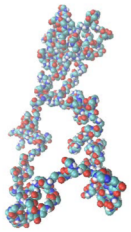
ν	R_g
0.59	66
0.55	53
0.52	48
0.49	43
0.45	39

Hard sphere



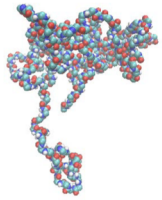
ν=0.59

A little sticky



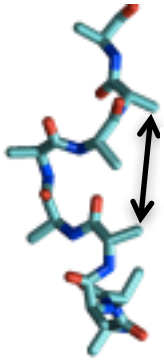
ν=0.52

A little more sticky



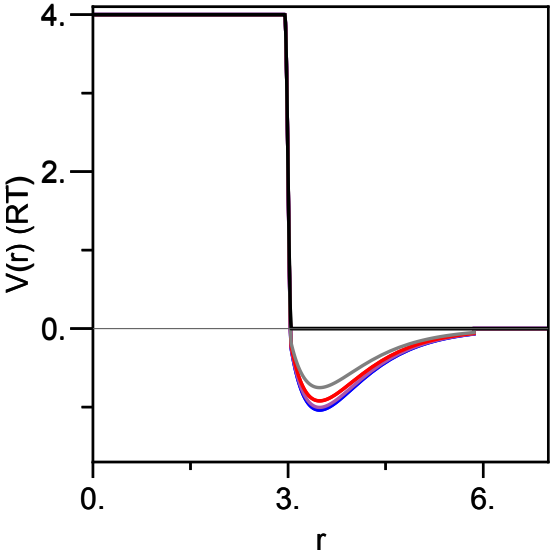
ν=0.45

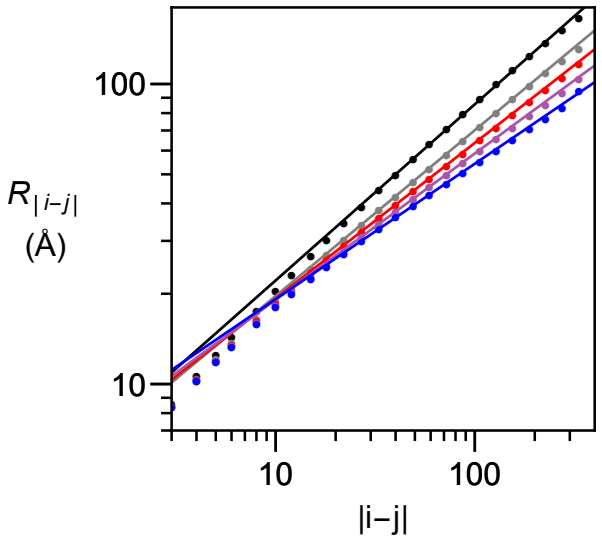
Simulations generate ensembles used to make an MFF



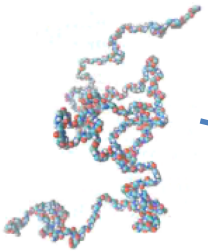
V(r)

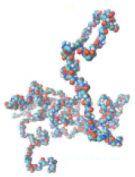
Change Cβ–Cβ interaction

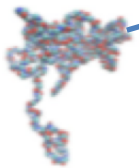




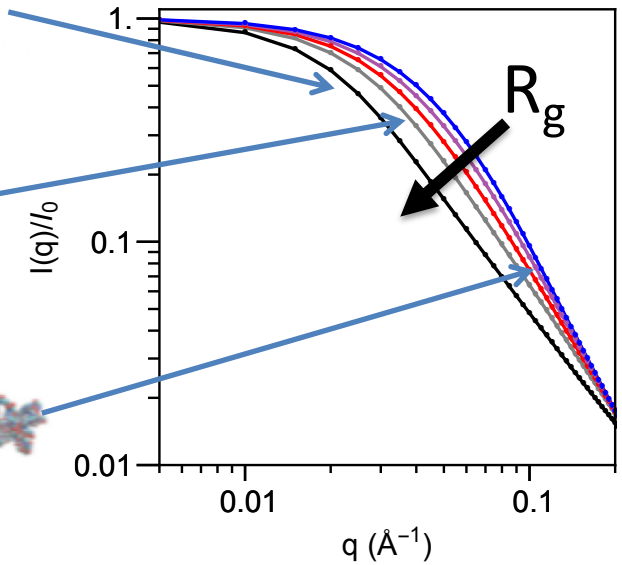
ν	R_g
0.59	66
0.55	53
0.52	48
0.49	43
0.45	39



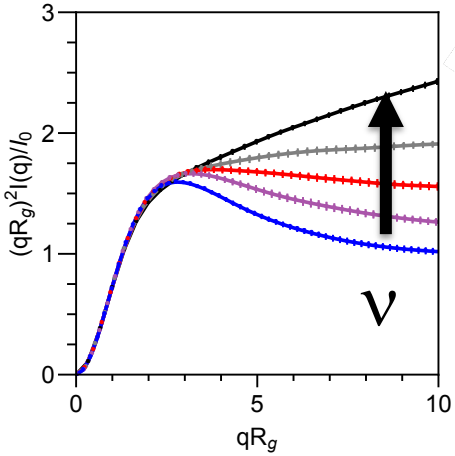


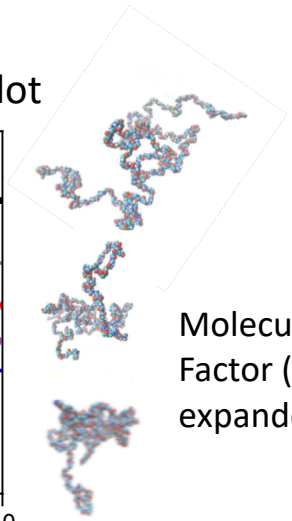


Standard Log-Log Plot



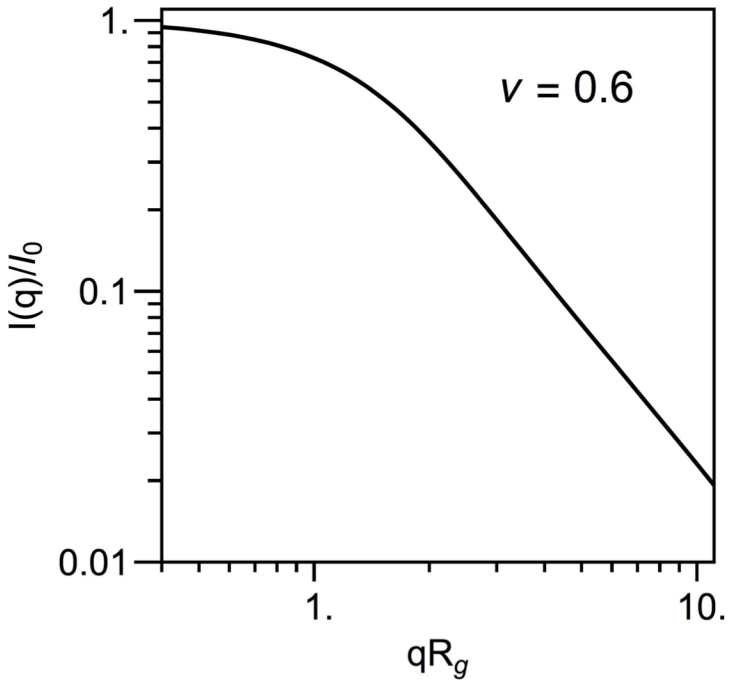
Dimensionless Kratky Plot

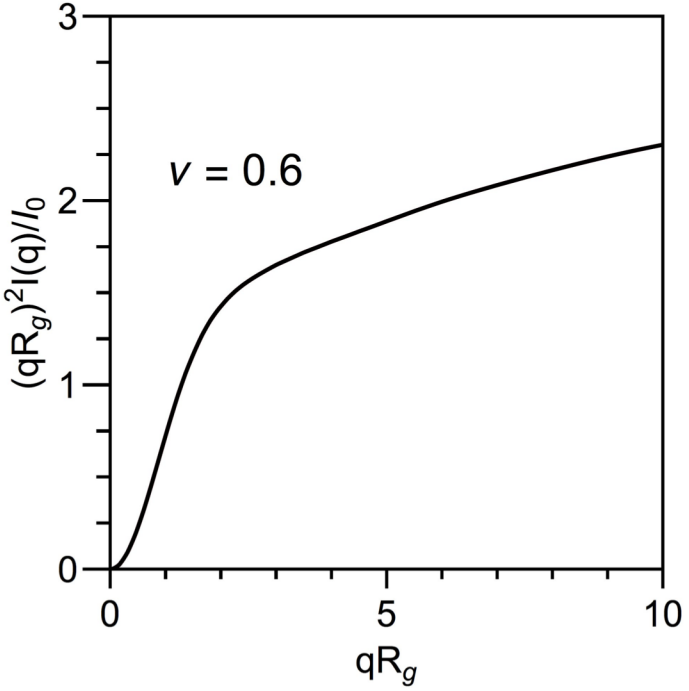




Molecular Form Factor (MFF) for expanded polymers

MFF for disorder systems: Obtain R_g and ν from a single SAS measurement





How well does it work?

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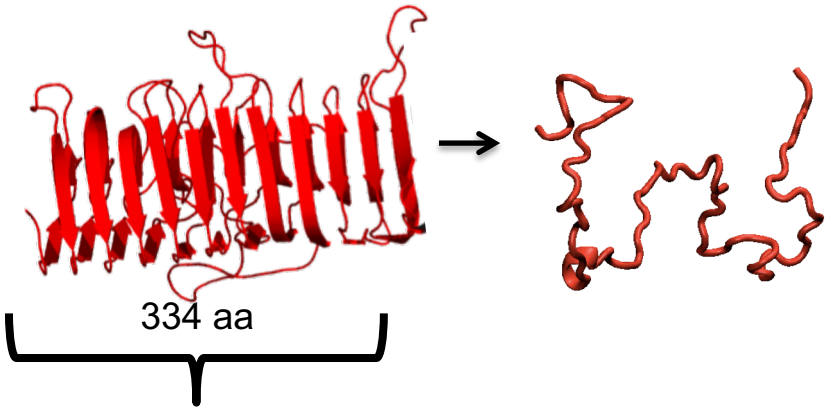
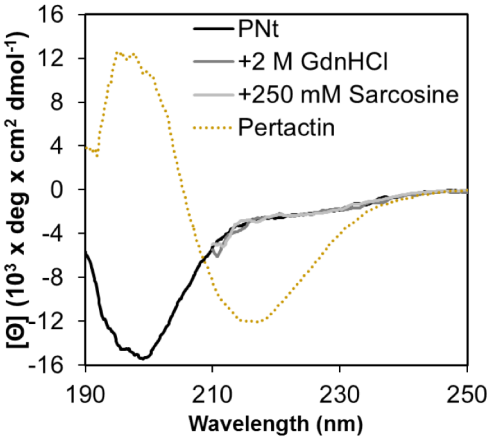
How well does our MFF work?
PNt, 334 residue, low charge hydrophobic IDP
P. Clark (U Notre Dame)

Pertactin: 539 aa β-helix

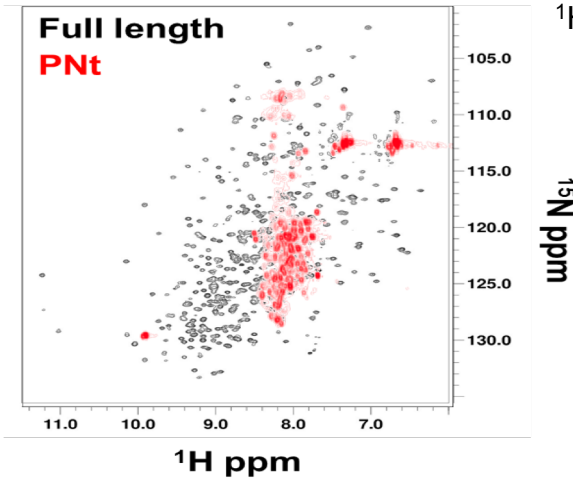


CD: unfolded

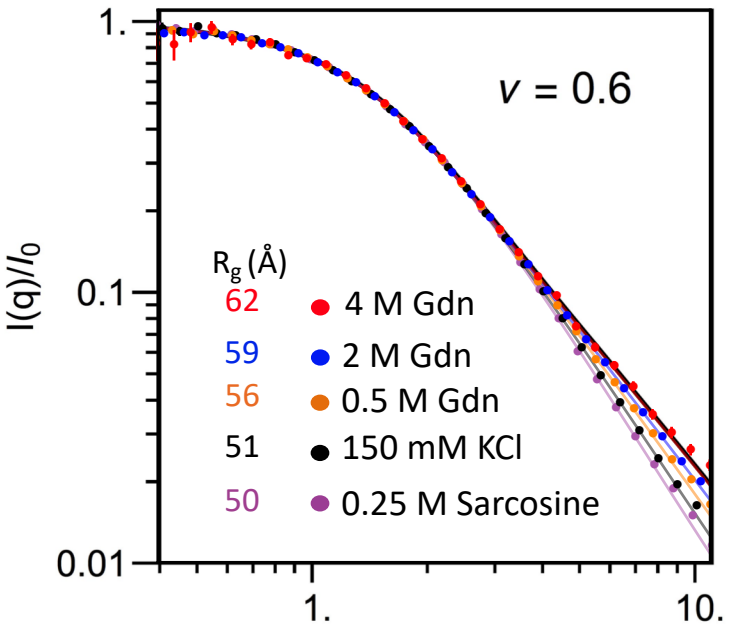


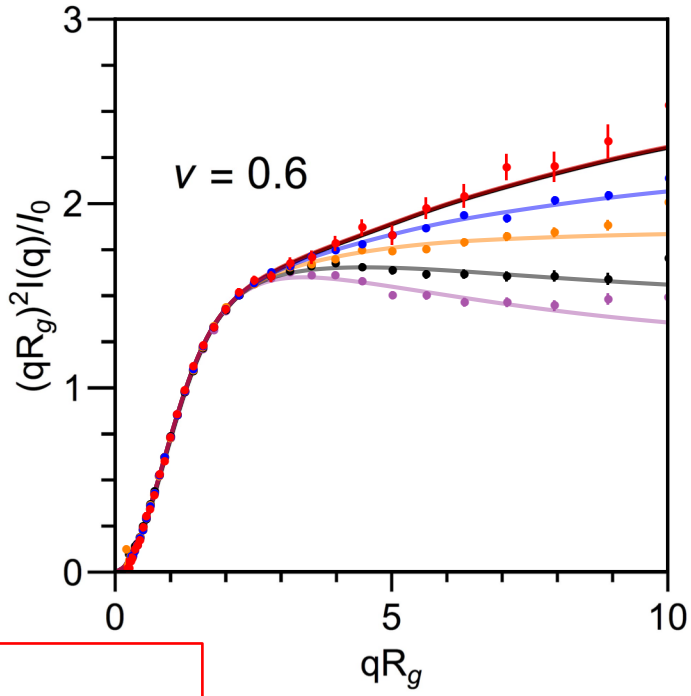
Chemical shifts typical of IDP

^{1}H ^{15}N HSQC:



Measuring R_g and ν for an IDP using the MFF





IT WORKS!

LET'S USE IT!

Fit out to qR_g~5 is generally sufficient
Hydration may be an issue at q>0.15Å^{-1}

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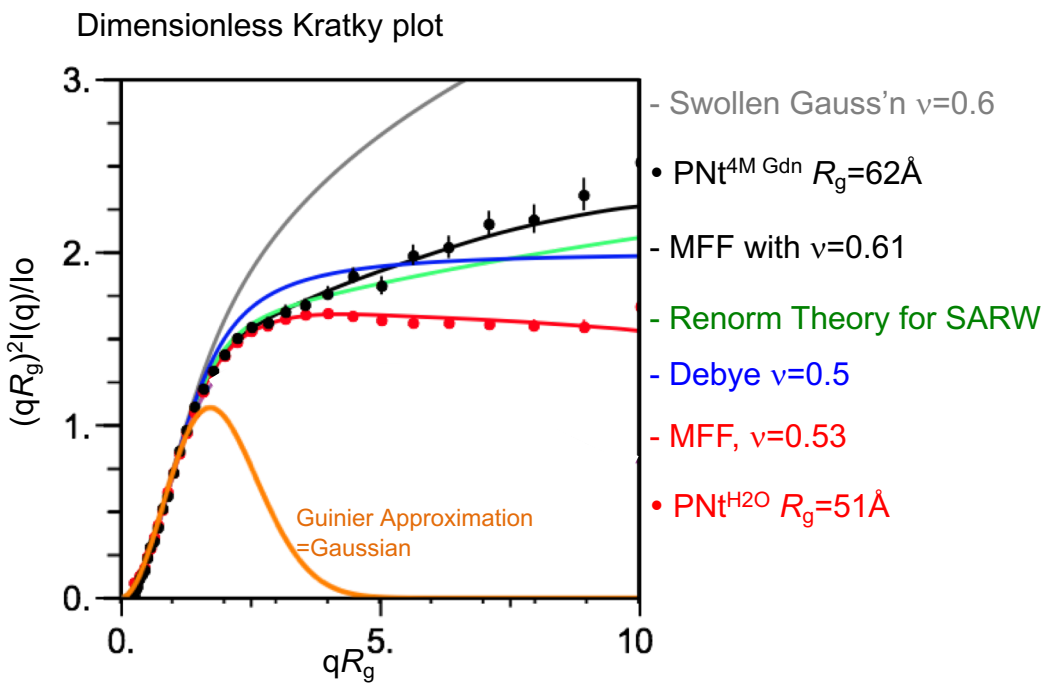
Scattering from Random Walks (allowed to cross) and self-avoiding RW (not allowed)



Debye formula for random walk:

$$I_{Debye}(q) = \frac{2I_o\left(e^{-(qRg)^2} - 1 + (qRg)^2\right)}{(qRg)^4}$$

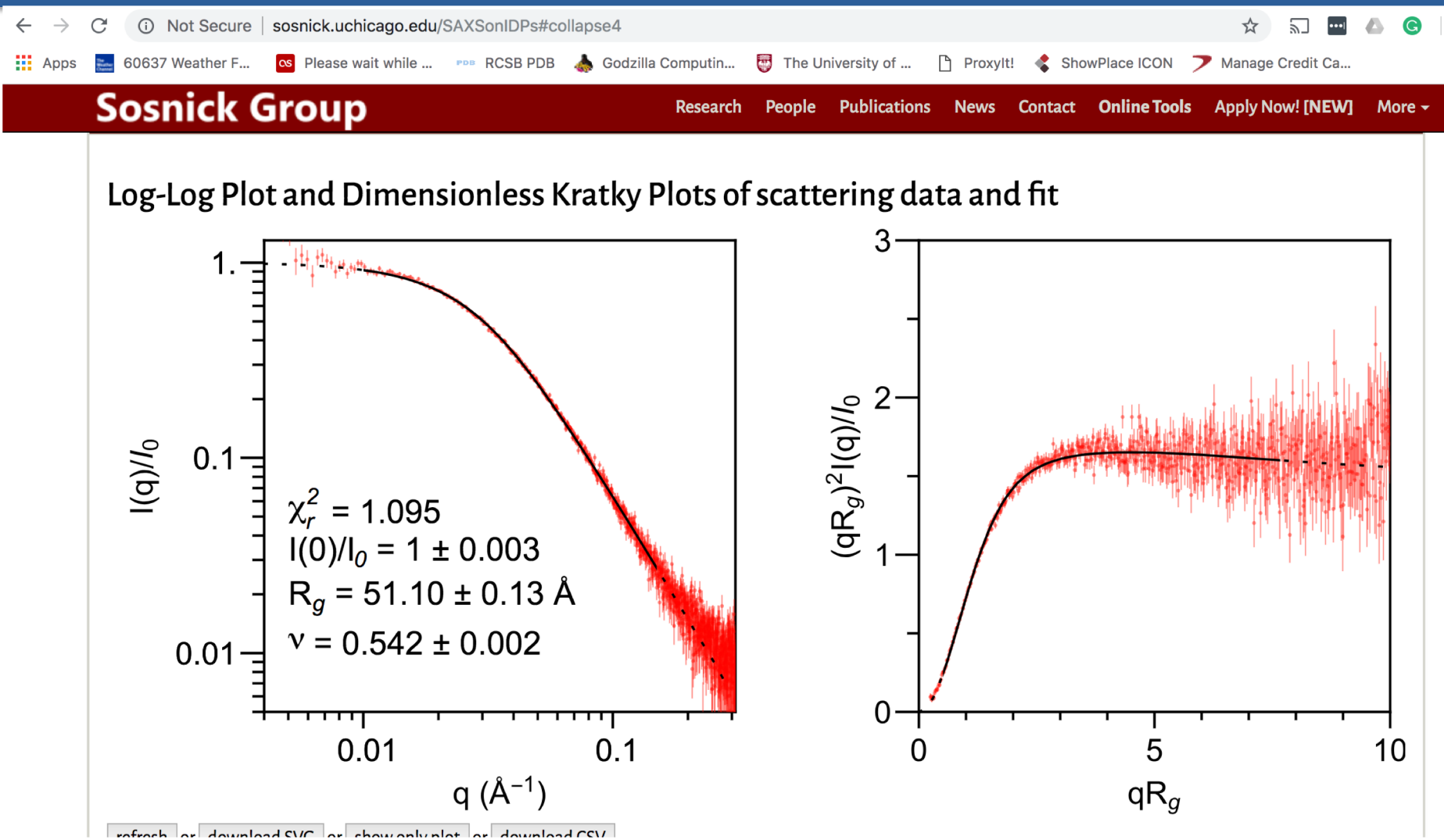
Swollen Gaussian coil model

$$P_{SwollenGausCoil}(R_{ij}) \propto e^{-\left(3R_{ij}^2 \,/\, 3\langle R_{ij}^2\rangle\right)}$$

$$\left\langle R_{ij}^2 \right\rangle = a^2 \left|i-j\right|^{2\upsilon}$$

a=segment length

Website for fitting disordered polymers
http://sosnick.uchicago.edu/SAXSonIDPs



Acknowledgements

Collaborators:
Notre Dame: Prof. Patricia Clark, Dr. Micayla Bowman

UChicago: Prof. Karl Freed,

Drs. Joshua Riback, John Jumper
Adam Zmyslowski

NIGMS

NSF GRP

Srinivas Chakravarthy BioCAT Beamline
APS: DOE, NIH