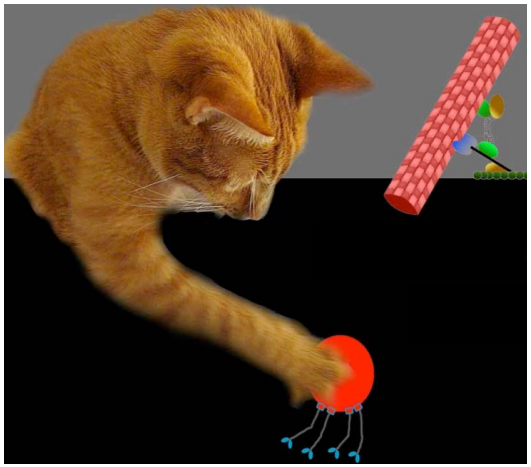
Contrast as a powerful tool for mitigating the ambiguous interpretation of scattering data



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Presented at a workshop on “determining the needs and challenges of isotopically labeled soft materials”

Held at Oak Ridge National Laboratory

Oak Ridge, Tennessee

27-29 April 2026

What I want to Discuss

- The development of tools for labeling and solvent contrast and data analysis now used in SANS and NR. I will not discuss spectroscopy, but clearly these methods benefit as well.
- Some examples of how they are used to push the envelop of new data challenging the *status quo.*
- How can we push farther to develop new tools to expand the use of neutron scattering into more complex and non-ideal systems?
- Make recommendations of the services and new tools that such a facility could provide for “young guns” to challenge the *status quo*.

Measurement of scattering intensity tells us, in principle, what is in the sample but is ambiguous about structure

- Lost phase information from the scattering amplitude needs to be recovered.
- Methods analogous to isomorphous replacement.
- Two methods are possible to at least partially recover phase, due to Babinet's principle.
  - Component labeling.
  - Background: dispersion or matrix contrast.
  - Powerful approach: combination of background contrast with component labeling.

The tools that we now use in neutron scattering came out of the "grand challenge of macromolecular structure.

- Contrast variation: Earliest attempts with background contrast with SAXS.
  - 1952: Bragg & Perutz determined the volume of haemoglobin from changes in low order x-ray diffraction when salt is added to hydrated crystals.[1]
  - 1965: Stuhrmann & Kriste introduce a theory to extract shape and internal structure from CV-SAXS, $I(Q) = \Delta\rho^2 I_{shp}(Q) + \Delta\rho I_{xtrm}(Q) + I_{int}(Q)$, implying that CV-SAXS intensity is parabolic with ρ_{sol}.[2]
  - 1972: Luzzati *et al.* discuss the implication of contrast variation and the Guinier approximation for CV-SAXS and the measurement of the radius of gyration.[3]
  - 1974: Stuhrmann *et al.* demonstrate the use of H_2O/D_2O solvent mixtures in CV-SANS and formalize the use of the Guinier approximation as, $R_g^2 = R_{sg}^2 + \frac{\alpha}{\Delta\rho} - \frac{\beta}{\Delta\rho^2}$; $\alpha = {\int \rho_{int}(r) r^2 dv_r}/{V_{shp}}$, $\beta = \left({\int \rho_{int}(r) dv_r}/{V_{shp}}\right)^2$.

1: Bragg, W. L. and M. F. Perutz (1952). "The structure of haemoglobin." *Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences* **213**(1115): 425–435.

2: Stuhrmann, H. B. and R. G. Kirste (1965). "Elimination der intrapartikulären Untergrundstreuung bei der ßöntgenkleinwinkelstreuungan kompakten Teilchen (Proteinen) (Elimination of Intraparticle Background Scattering in Small-Angle X-ray Scattering from Compact Particles (Proteins))." *Zeitschrift für Physikalische Chemie Neue Folge*, **46**(S): 247–250

3. Mateu, L., et al. (1972). "On the structure of human serum low density lipoprotein." *Journal of Molecular Biology* **70**(1): 105–116.

4. Stuhrmann, H. B., et al. (1975). "Neutron Scattering Study of Human Serum Low Density Lipoprotein (Solvent exchange)." *Proceedings of the National Academy of Science USA* **72**(6): 2270–2273.

Deuterium the key for neutron probes of soft matter and bioscience.

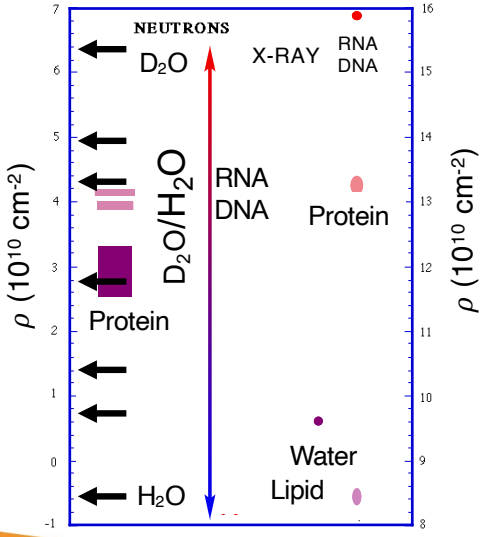
- Macromolecular labeling by H->D substitution.
  - 1959: Crespi & Kratz[1,3] (algae and bacteria) & Van Horn and Ware[2] (bacteria) demonstrate growth in deuterated media with >98% deuteration.
  - 1972: Kirste *et al. Demonstrates polymer radius of gyration random coil configuration in the glassy state of a protonated/deuterated mixture with SANS.*[4]
- H->D Exchange.
  - 1947: Linderstrøm-Lang (proteins).[5]
  - 1965: Von Hippel (nucleic acids).[6]

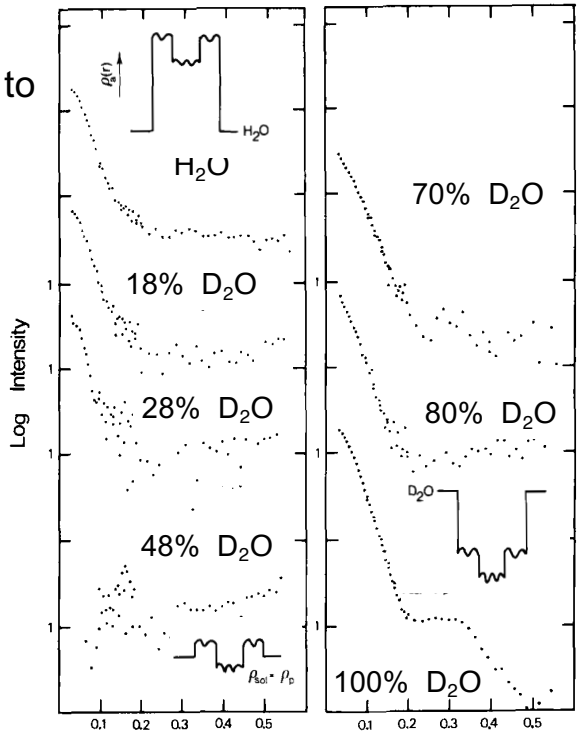
1. Crespi, H. L., et al. (1959). "Culture of Algae and other MIcro-organisms in Deuterium oxide." *Nature* **184**(4687): 729–728.
2. Van Horn, E. and G. C. Ware (1959). "Growth of Bacterium coli and Staphylococcus albus in Heavy Water." *Nature* **184**(4689): 833–833.
3. Chorney, W., et al. (1960). "The growth of algae in deuterium oxide." *Biochimica et Biophysica Acta* **37**(2): 280–287.
4. Kirste, R. G., et al. (1973). "Die Bestimmung des Tragheitsradius von Polymethyl methacrylat im Glaszustand durch Neutronenbeugung. (Determination of the Radius of Gyration of Polymethyl Methacrylate in the Glassy State by Neutron Diffraction)." *Die Makronzolekulare Chemie* **162**: 299–303.
5. Englander, S. W., et al. (1997). "Hydrogen exchange: the modern legacy of Linderstrøm-Lang." *Protein Sci* **6**(5): 1101–1109.
6. Printz, M. P. and P. H. Vonhippel (1965). "HYDROGEN EXCHANGE STUDIES OF DNA STRUCTURE." *Proc Natl Acad Sci U S A* **53**(2): 363–370.

It's 1977: What is the structure of the newly discovered chromosome basic unit, the nucleosome? A nexus of tools, methods, technology and the ILL.

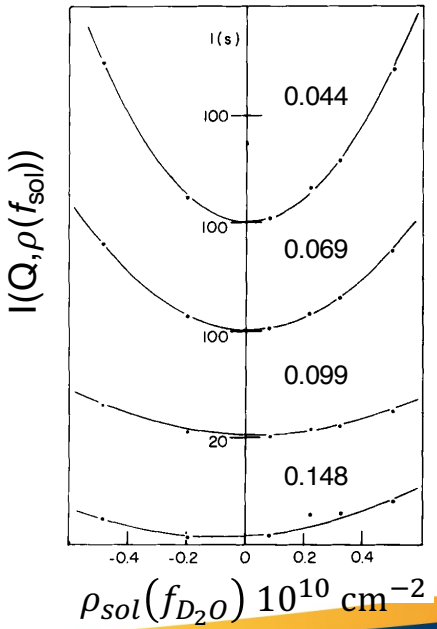
- 5 nm particle known to contain DNA and positively charged histone proteins.
- What is the arrangement of histone and DNA?

- Approach: Contrast variation: change D_2O/H_2O solvent content to alter contrast with DNA & Histone





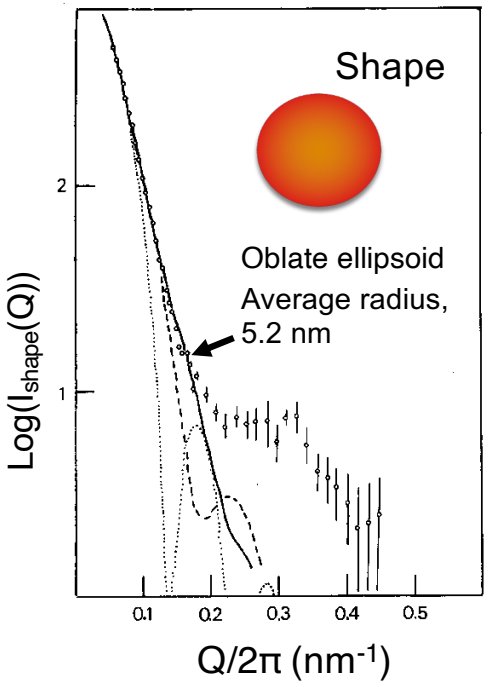
- Contrast variation: Intensity must be parabolic with ρ_{sol}.
- Parabolic parameters: isolate shape, internal structure & DNA/protein distribution.

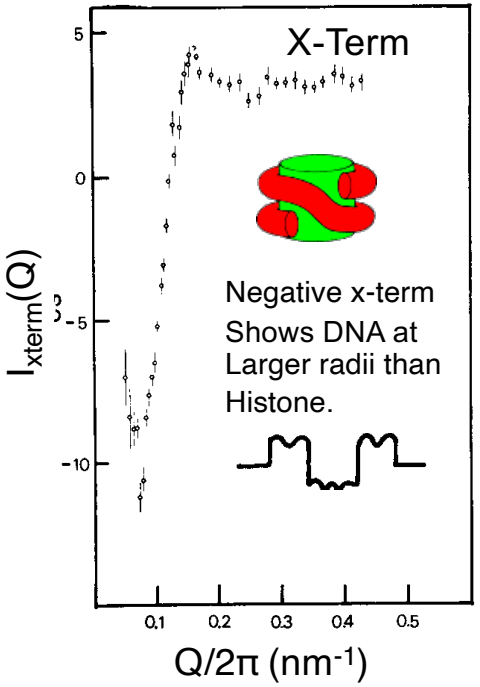


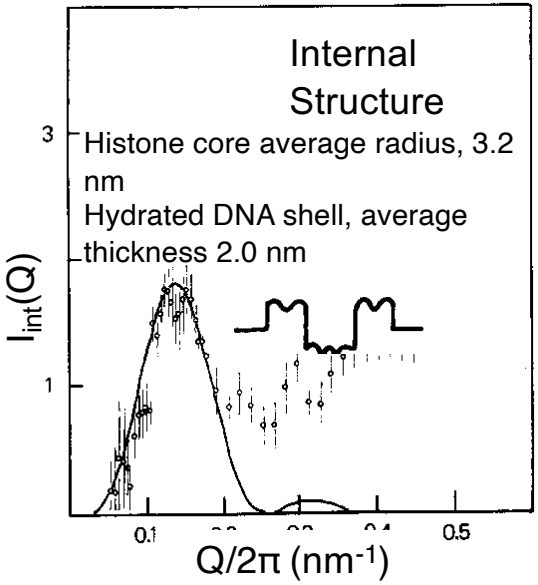
Hjelm, R. P., et al. (1977). "Small angle neutron scattering studies of chromatin subunits in solution." Cell **10**(1): 139–151.

Hjelm, R.P., et al. (1978). "Utilization of Neutron Scattering for Analysis of Chromatin and Nucleoprotein Structure." *Methods in Cell Biology* **18**(3): 295–325.

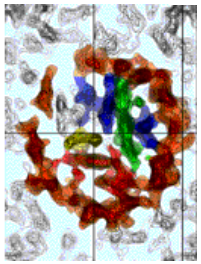
Contrast variation yields scattering contributions from the (A) shape envelope, (C) the internal structure & (B) A/C correlations.







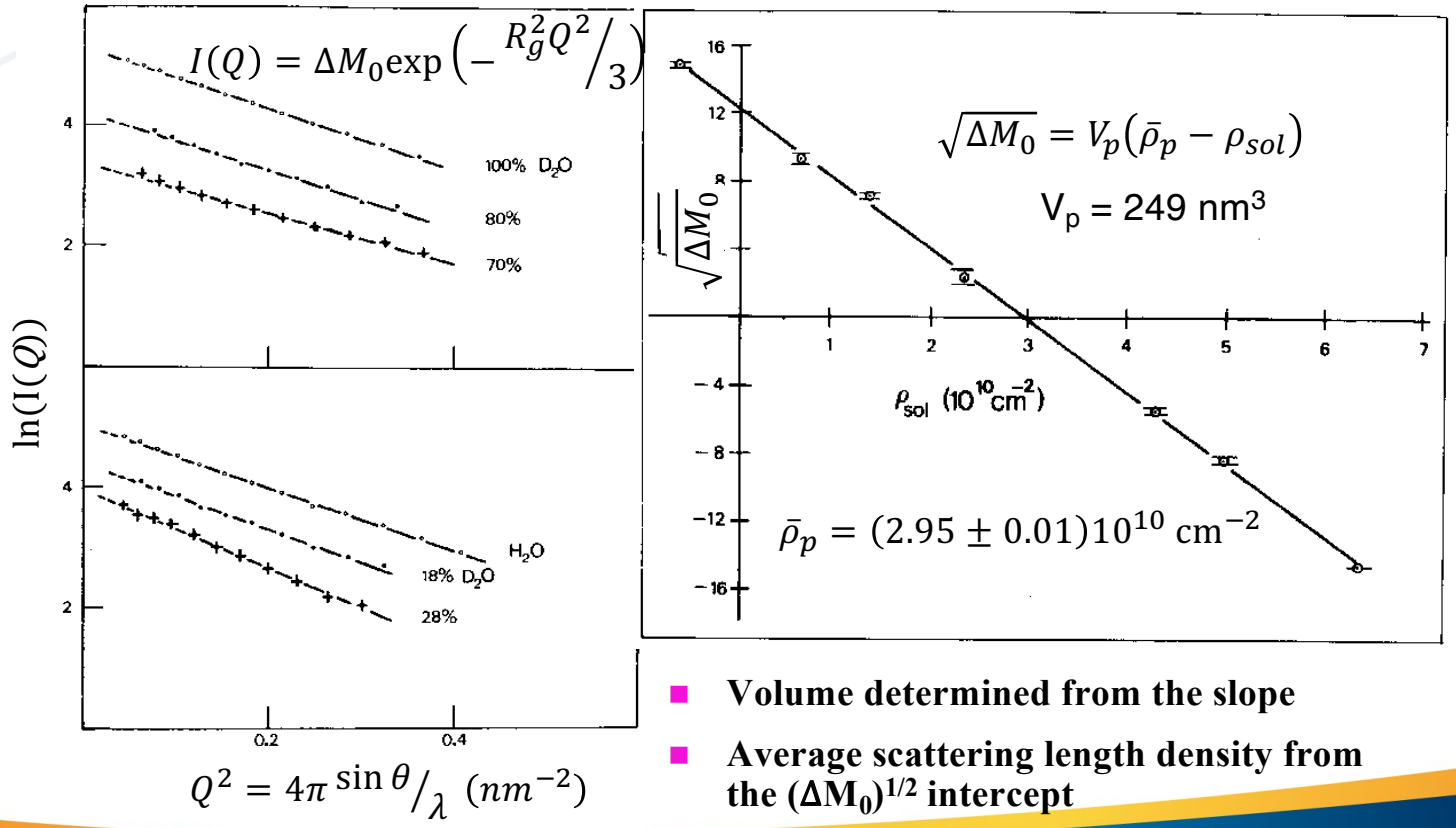
$$I(Q) = \Delta\rho^2 I_{shp}(Q) + \Delta\rho I_{xtrm}(Q) + I_{int}(Q)$$



XRCryst:
Richmond *et al. Nature* **311**, 532–537 (1984). https://doi.org/10.1038/311532a0
Luger, K. *et. al.*, *Nature* **389**, 251–260 (1997). https://doi.org/10.1038/38444

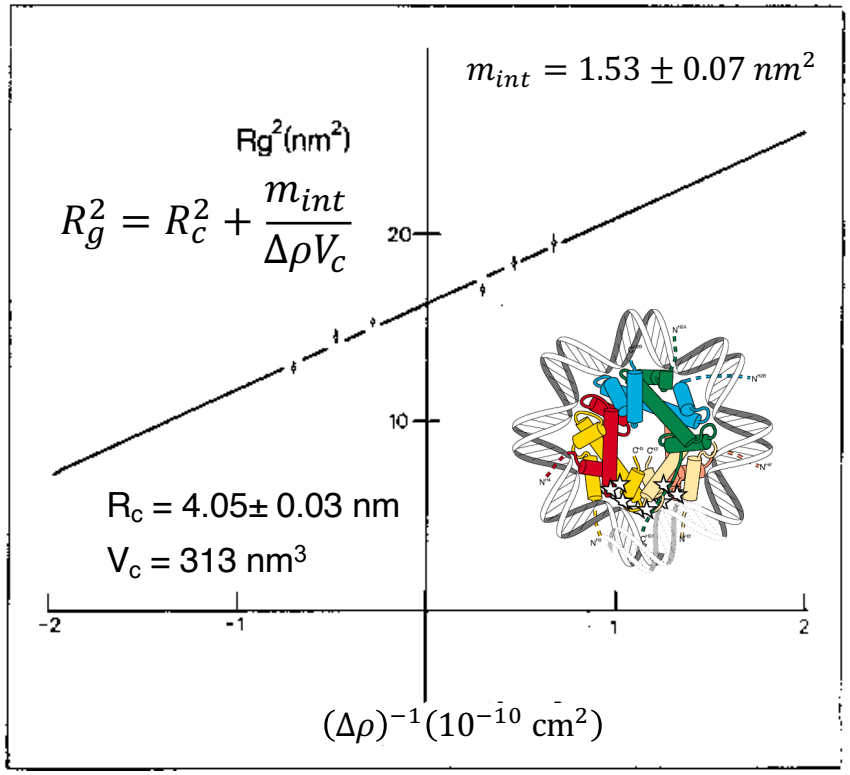
Hjelm, R.P.; Ibel, K. *et al*., Cell, **10**, (1), 139 – 151 (1977)

Guinier analysis of the nucleosome yields particle volume and density



- **Volume determined from the slope**
- **Average scattering length density from the $(\Delta M_0)^{1/2}$ intercept**

Guinier analysis of nucleosome contrast variation data yields particle shape R_g & internal structure second moment



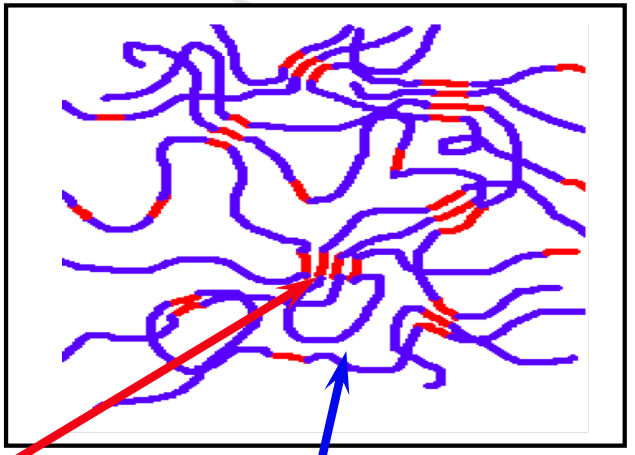
- **Intercept ($\Delta\rho=\infty$) gives R_c, the radius of gyration of the shape envelope.**
- **Slope gives, $R_{int}^2 = m_{int}^2\,\bar{\rho}_p$, where, m_{int}^2, is the second moment of the internal structure and V_c is the volume of the shape envelope.**
- **Positive m_{int}^2 implies that DNA (larger ρ) is at larger radius than histone.**

Application to more complex problem of a domain phase separated polymer by Swelling.

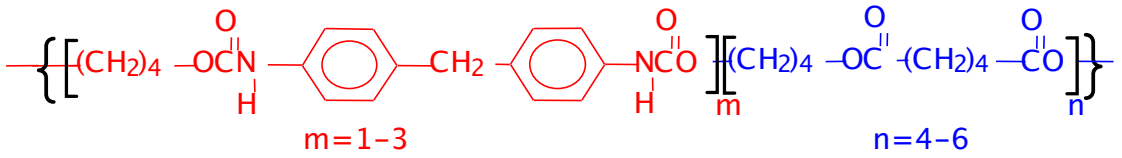
- Network structure:
  - Mechanical properties.
  - Thermodynamics of hard and soft segment interactions through solvent interactions—self assembly.
  - Domain composition and structure.
  - Polymer chain conformations.
  - Directly applicable to Estane.
- Contrast variation:
  - D/H solvent mixtures allows manipulation of contrast.
  - Phase morphology.
  - Phase composition.
  - Solvent distribution in phases.
- Studies of unmodified polyurethane.

Estane™ Binder: Phase Separated, Cross-linked Polymer

- **Segmented polyurethane containing immiscible, rigid aromatic, hard segments (23 wt%, and flexible, soft segments.**
- **Chemical linkages constrain phase separation into domains ~ 10 nm.**
- **Hard segment-rich domains are multifunctional, reinforcing cross-links.**

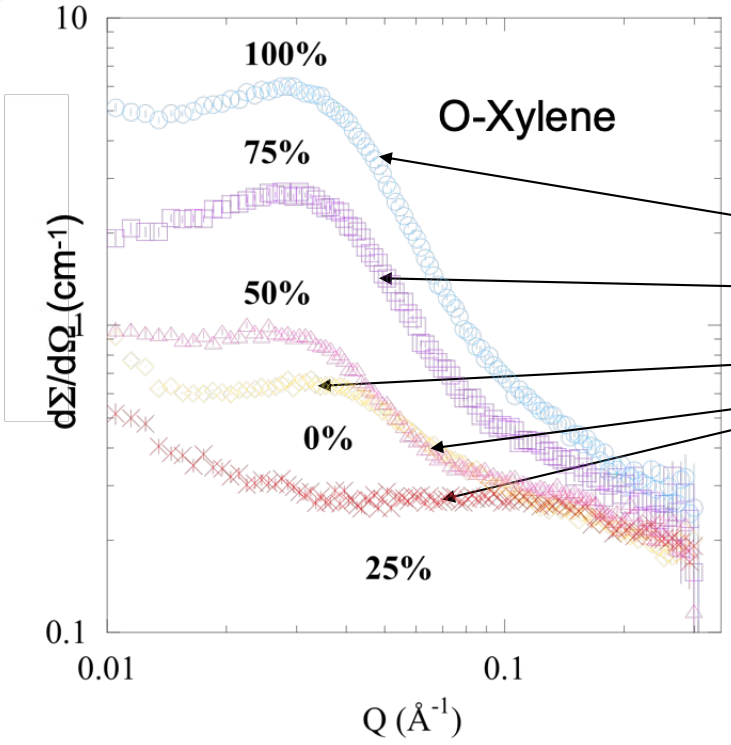


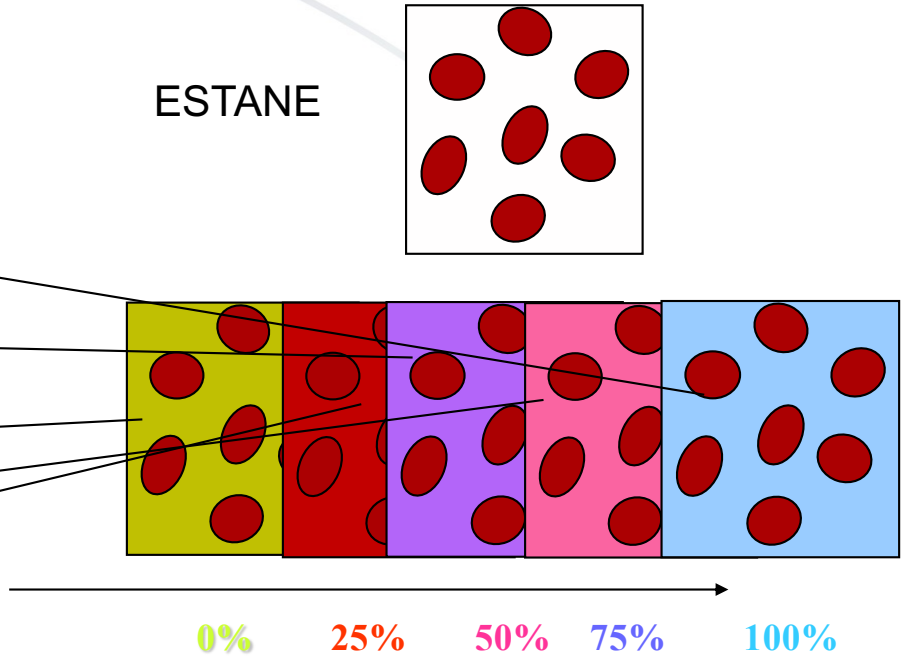
Polyurethane (MDI plus chain extender) Hard Segment Polyester Soft Segment



Hoffman, D.M.; Caley, L.E. ACS Div. of Organic Coatings and Plastics Chem. **1981**, 44, 686.

… Provides a Determination of Difference in Chemical Composition between Matrix and Discontinuous Domains.

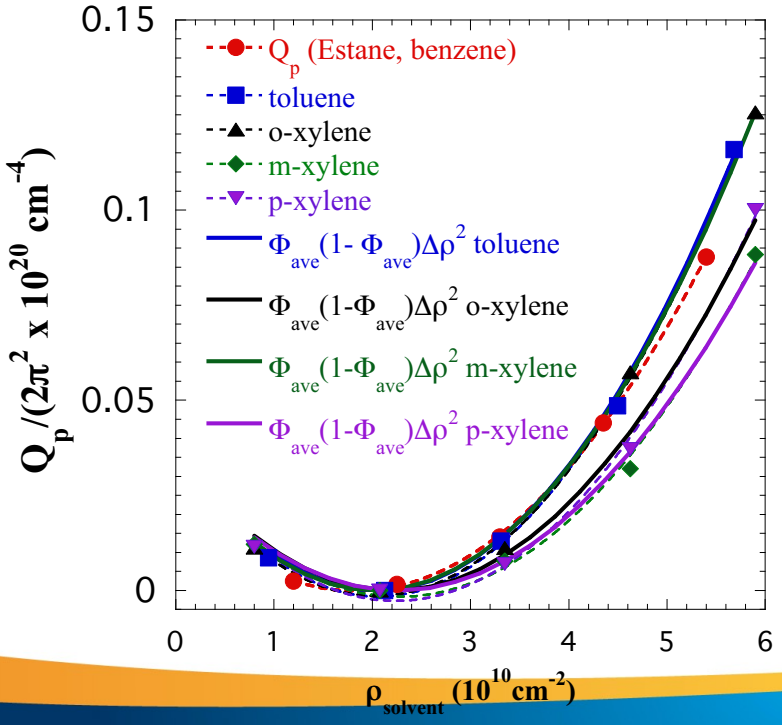


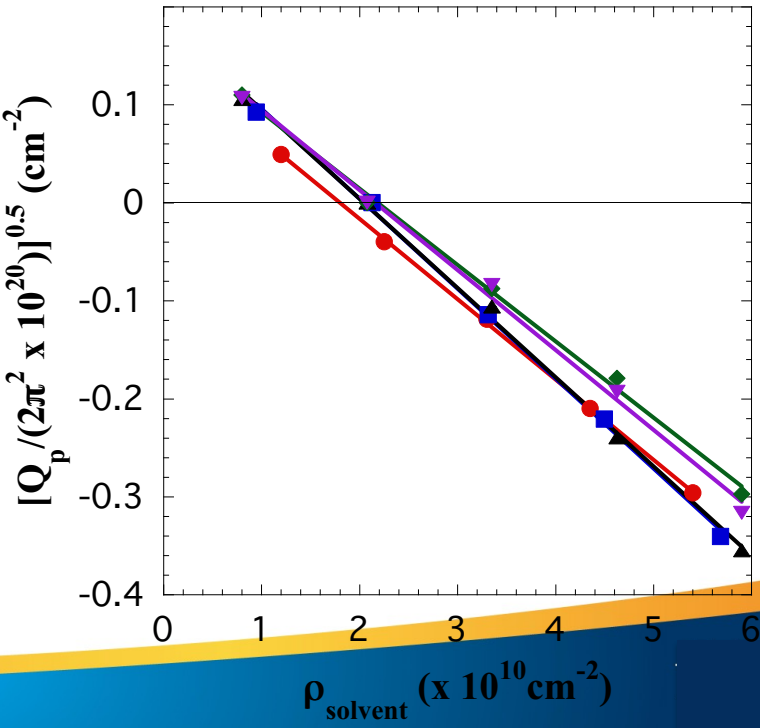


The Porod Invariant is key to teasing domain composition from CV-SANS

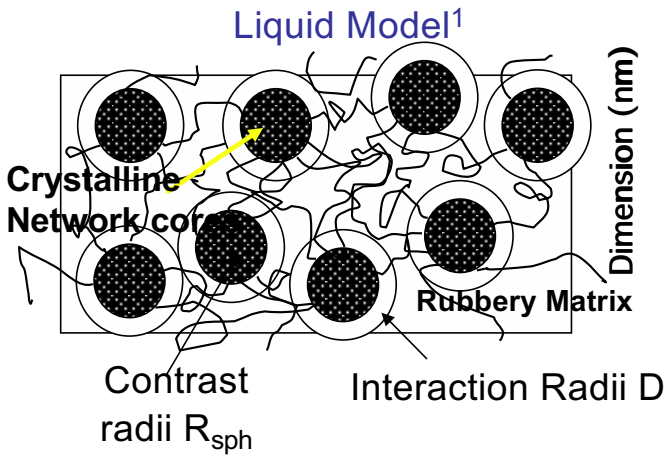
- The Porod Invariant: $Q_p = \int_0^\infty Q^2 \frac{d\Sigma(Q)}{d\Omega} dQ = 2\pi^2 \Phi(1-\Phi)\Delta\rho^2$.
- Plots of $Q_p/2\pi^2$ show the anticipated parabolic form.
- The simultaneous fits of the data give estimates of $\bar{\rho}$ & Φ_{ave}.
- $\frac{d\left(Q_p/(2\pi^2)\right)}{d\rho_{sol}} = \sqrt{\frac{1-\Phi_{ave}}{\Phi_{ave}}}(1-\varphi_{sol}-\phi_{Hm}-\phi_{Sm})$ & $\bar{\rho} = \frac{(\varphi_S-\phi_{Sm})\rho_S+(\varphi_H-\phi_{Hm})\rho_H}{1-\varphi_{sol}-\phi_{Hm}-\phi_{Sm}}$.

Mang, J. T., et al. (2008). "Small-Angle Neutron Scattering of a Solvent-Swollen Segmented Polyurethane as a Probe of Solvent Distribution and Polymer Domain Composition." *Macromolecules* **41**(12): 4358–4370.

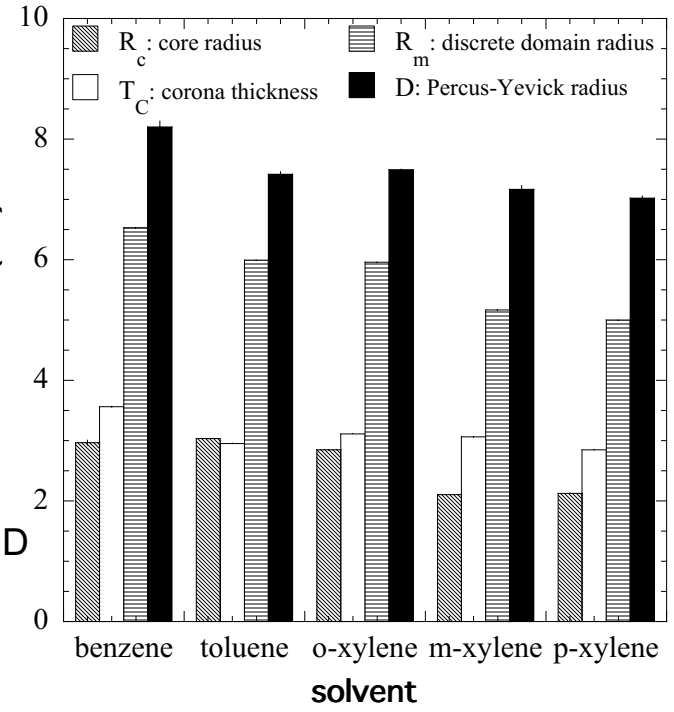


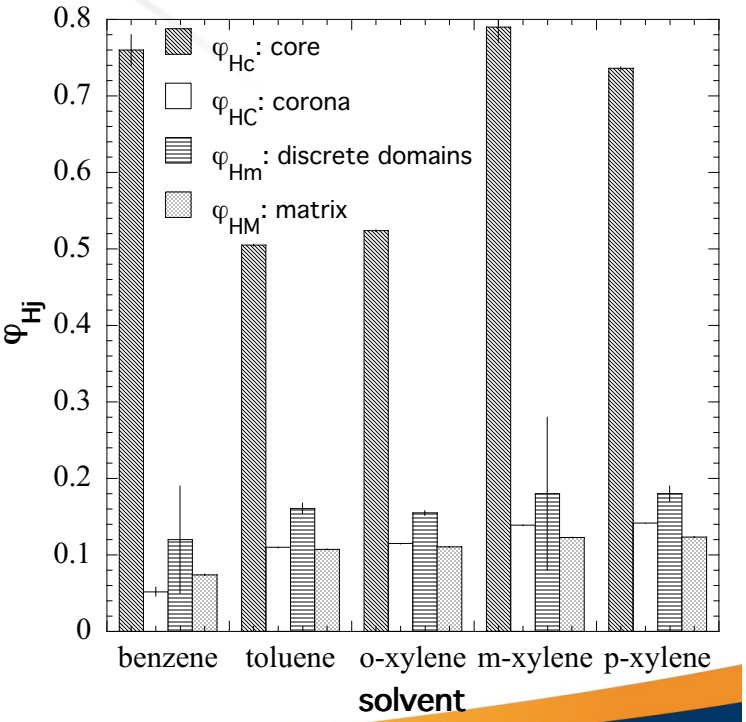


Polyurethane modeled as a network of cores, surrounded by a corona of polymer, entangled with a rubbery matrix.

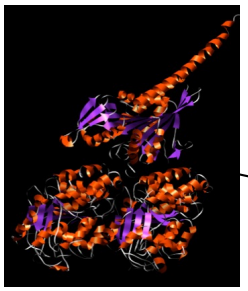


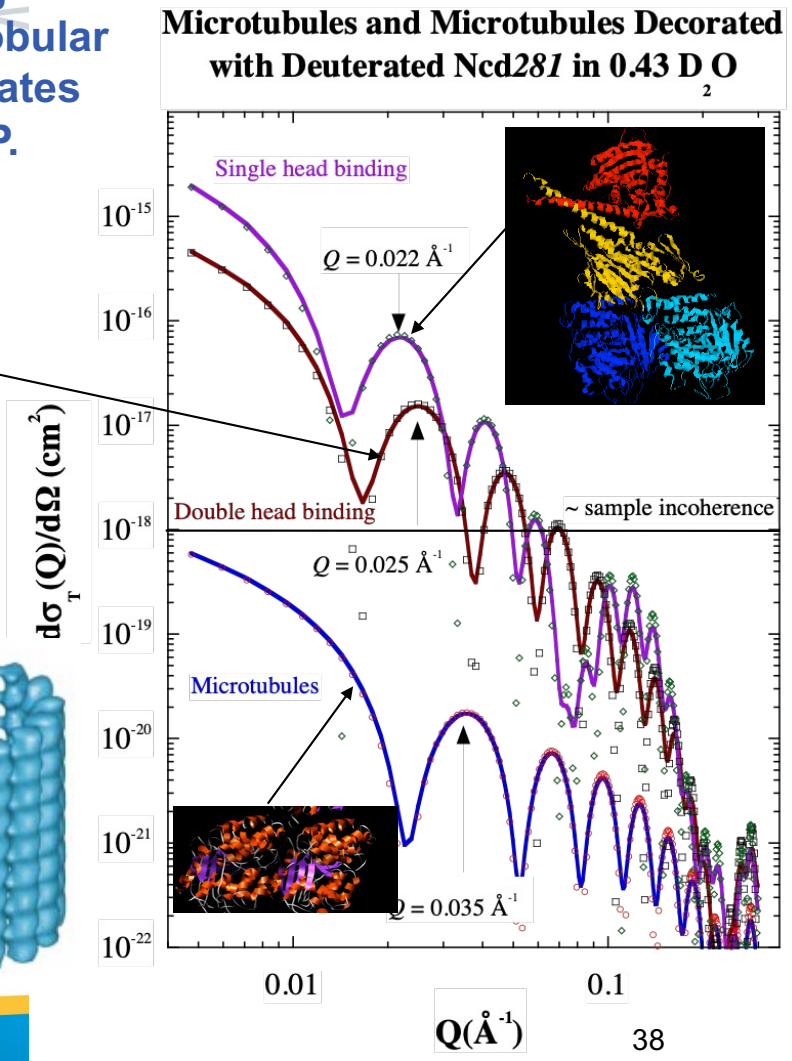
[1] Laity et. al. *Polymer* **2004**, *45,* 7273.



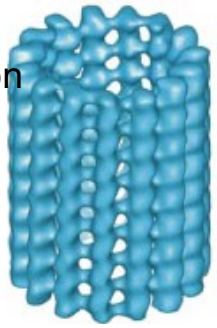


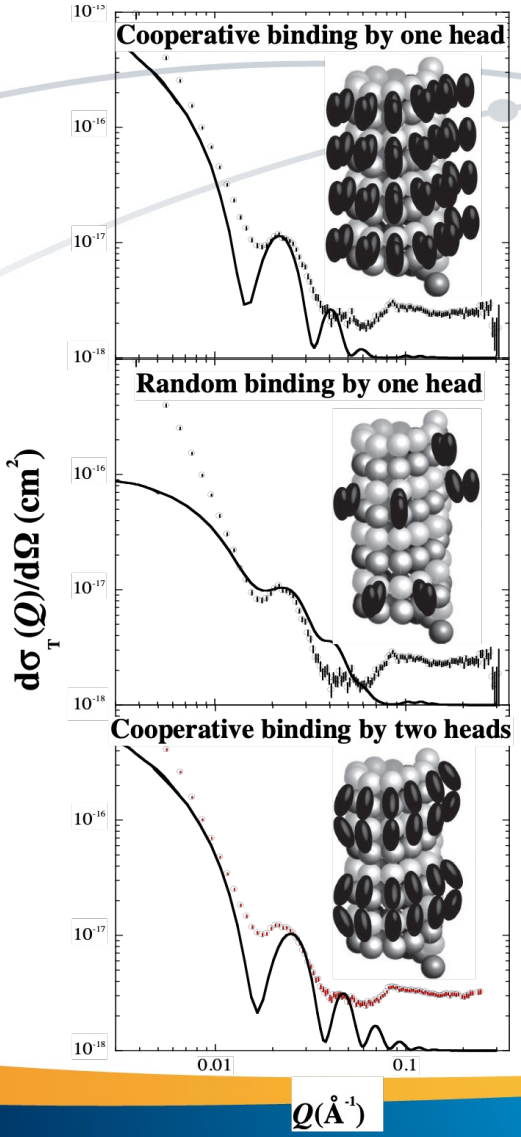
Molecular motors move cellular cargo along microtubules by cyclic movement of the globular dimer head group through a sequence of states involving the binding of ATP then ATP->ADP.





- What is the binding of the motor in the ATP bound state:
  - Cooperative binding.
  - Both heads bound to the MT.
  - One head bound, the other stacked on top.
- Approach:
  - Deuterate the molecular motor.
  - SANS of complex in 43% D_2O/H_2O.
  - Examine scattering signatures.



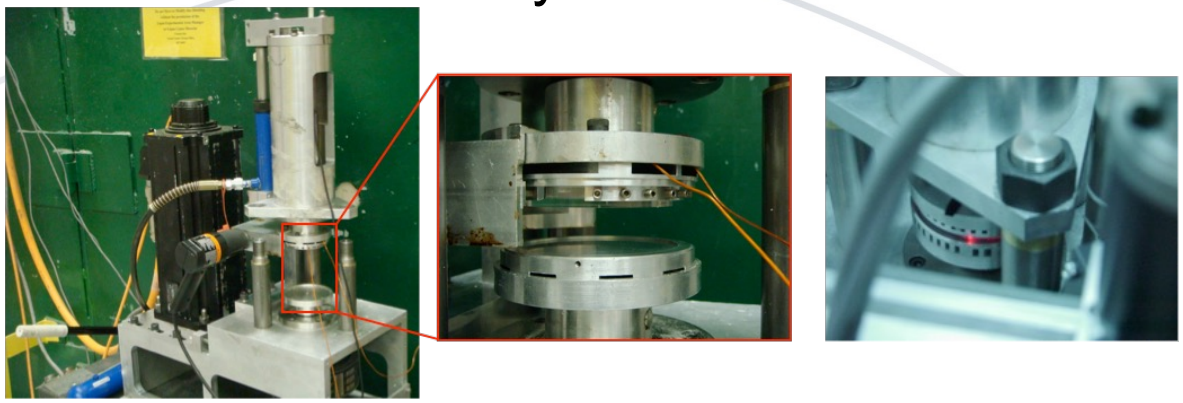


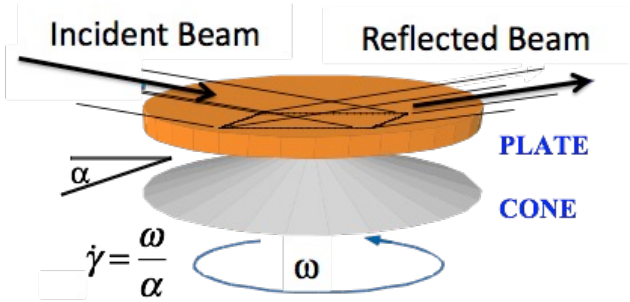
Result: single head. stacked cooperative dimer binding

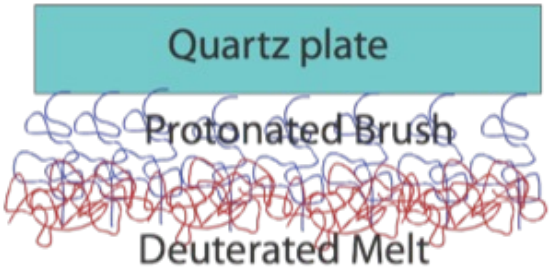
- Motor-MT complex in solution emulating one state of the cyclic motion.
- 43% D_2O/H_2O: scatter from MT disappears.
- Comparison of data with computed signatures consistent with single head cooperative binding.

Hjelm, R.P., Stone, D.B., Fletterick, R.J. & Mendelson, R.A. (2010). Decoration of microtubules in solution by kinesin-14, Ncd. Acta Crystallagraphica, D66, 1218-1223.

Enabling polymer response to stress: LANSCE shear cell in cone & plate geometry for neutron reflectometry to emulate real conditions in the melt.





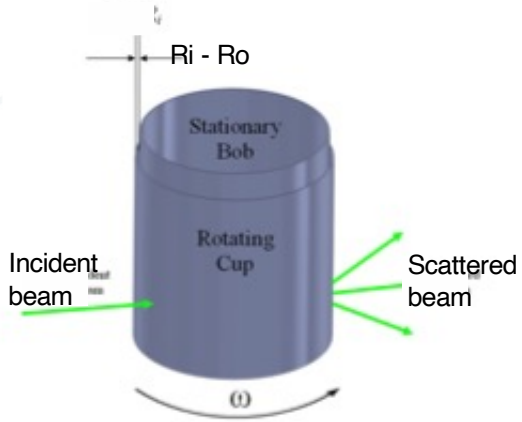


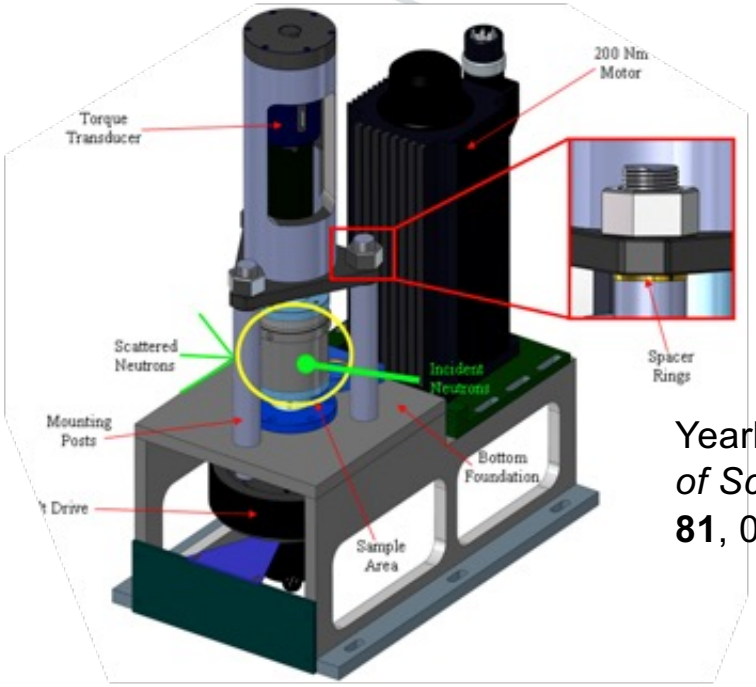
Simultaneous torque (200 Nm), shear rate (800 Hz) & reflectometry Measurements at up to 240 C under inert atmosphere.

L.A,.Sasa,, *et al., Review of Scientific Instruments,* **81**, 055102:1-6 (2010).

L.A. Sasa, *et al. Phys. Rev. E:*, **2011**, *84*, 021803:1-6.

…and in the bulk: LANSCE shear cell in Couette configuration for rheo-SANS. Together provide critical tests of tube theory.





Yearley, E. *et al.*, *Review of Scientific Instruments,* **81**, 045109-1 - 7 (2010).

Simultaneous torque (200 Nm), shear rate (800 Hz) & SANS measurements at up to 240 C under inert atmosphere.

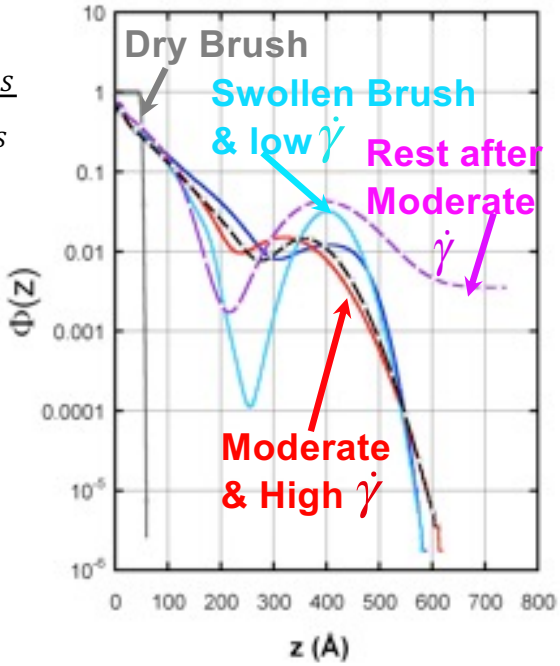
Design mitigates slippage and vortex instabilities

Polymer profiles from reflectometry with $\dot{\gamma}$: brush collapse with reversible after moderate shear

$$\phi(z) = \frac{\rho(z) - \rho_{dPS}}{\rho_{PS} - \rho_{dPS}}$$

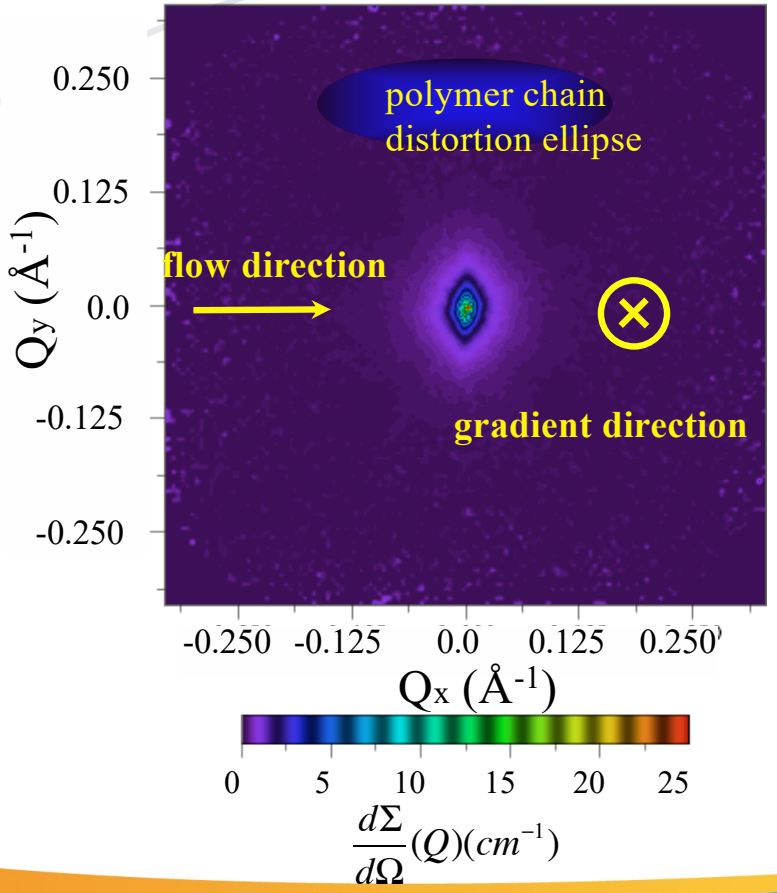
$$R(Q_z) = \frac{4\pi^2}{Q_z^4}\left|\int_{-\infty}^{\infty}\frac{d\rho}{dz}\exp(iQ_z z)dz\right|^2$$

- Brush swollen in contact with melt.
- No change for $\dot{\gamma}$ = 1.4 Hz .
- Brush collapses for $\dot{\gamma}$ = 5, 10.1, 15.1, 20.2 & 30.2 l
- Returns to quiescent state after moderate $\dot{\gamma}$.
- But not after higher $\dot{\gamma}$.
- Chains must overlap.
- **But no correlation with shear rate**

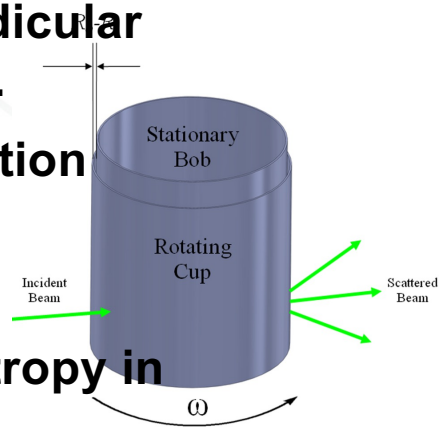


L.A. Sasa, *et al. Phys. Rev. E:*, **2011**, *84*, 021803:1-6.

Example of shear induced anisotropy (114 Kda, PS)10% h-PS in d-PS

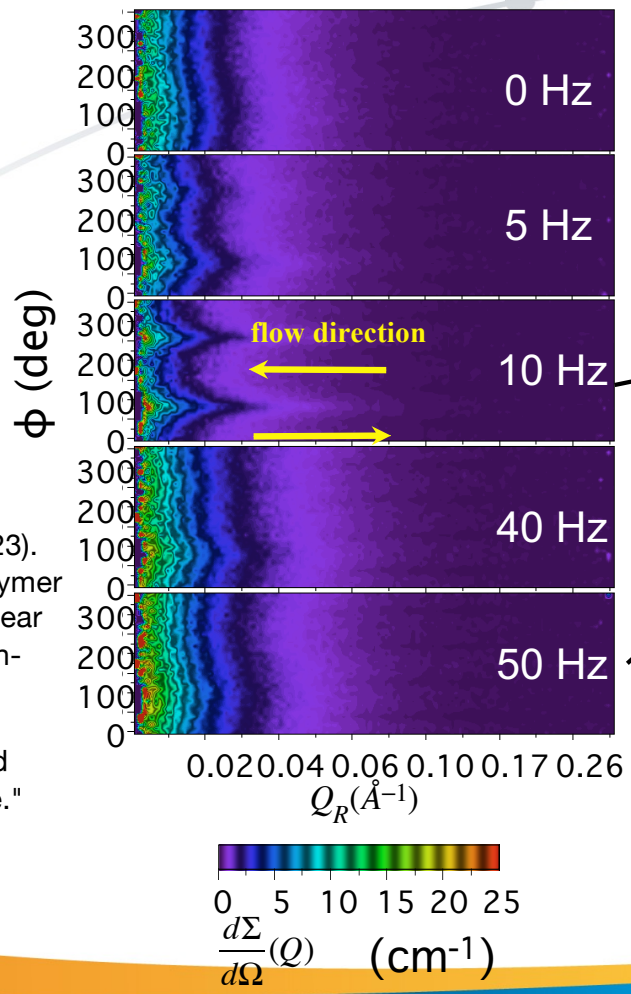


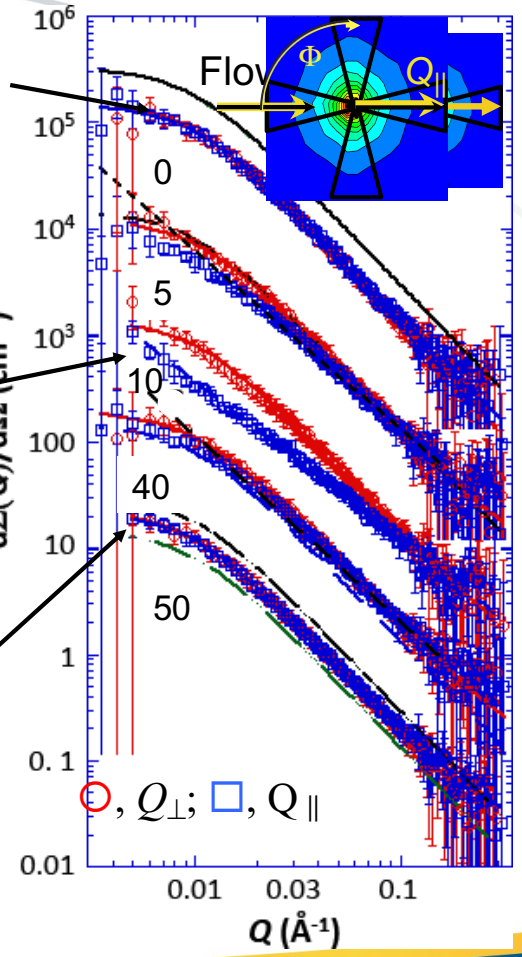
- Anisotropy, when present, perpendicular to flow direction.
- Indicates elongation along flow.
- $\dot{\gamma}$ =10 Hz shows maximum anisotropy in all samples.
- Rotation of elongation along gradient direction[1] not observable in this configuration.



- 1.Muller, R, Pesce, J.J. and Picot, C., "Chain Conformation in Shear Polymer Melts as Revealed by SANS", Macromolecules, 26, 4356-4362 (1993).

Example of shear induced anisotropy (114 Kda, PS)

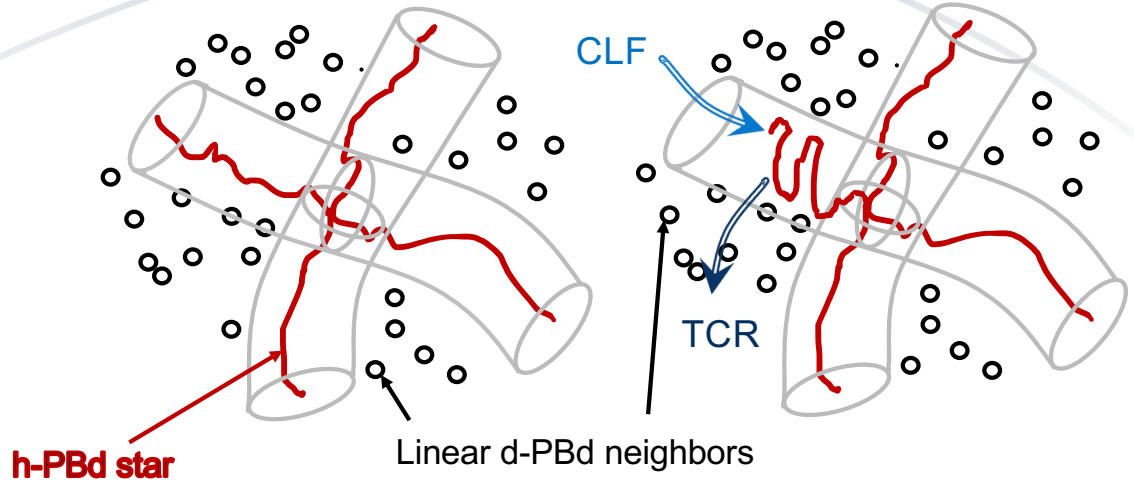




- Polymer at rest fit to the RPA and Debye random coil.
- Anisotropy increases to $\dot{\gamma}$ = 10 Hz then returns to Debye random coil.
- Polymer chain extended along flow power law < 2, but retains Debye random coil viewed perpendicular to flow.

Hjelm R.P., et al. (2023). "Molecular-scale polymer melts response to shear in the linear and noon-linear rheological domains: affect of molecular weight and complex architecture." *KGK* **2023**(2): 67–71.

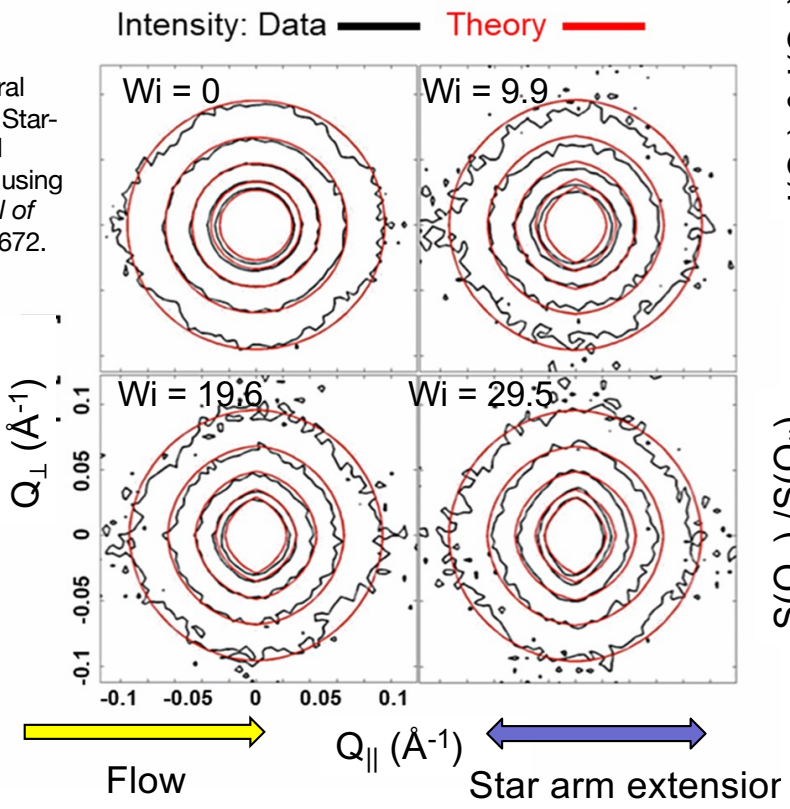
Branched polymer arms limit relaxation mechanisms in the linear domain

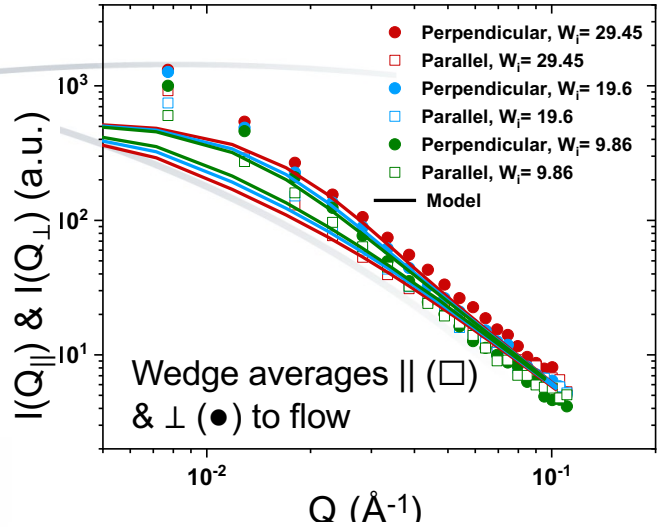


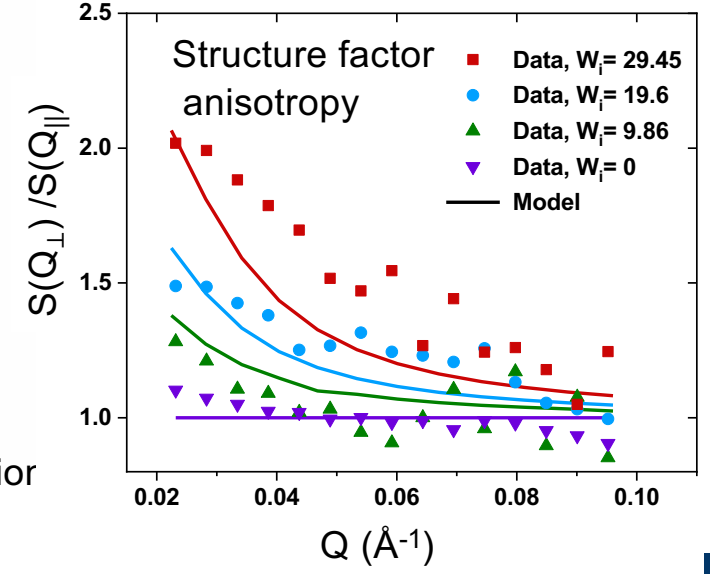
Where as for linear chains we have reptation, CLF and TCR over $Wi \lesssim \dot{\gamma}\tau_d$,the constrains of the other arms prevents reptation. Arms can only relax from stretched state in the linear domains by CLF (retraction) and TCR. CCR occurs at higher $\dot{\gamma}$.

Data well into the non-linear, CCR domain

Andriano, L. T., et al. (2020). "Microstructural Characterization of a Star-Linear Polymer Blend under Shear Flow by using Rheo-SANS." *Journal of Rheology* **63**(3): 663–672.





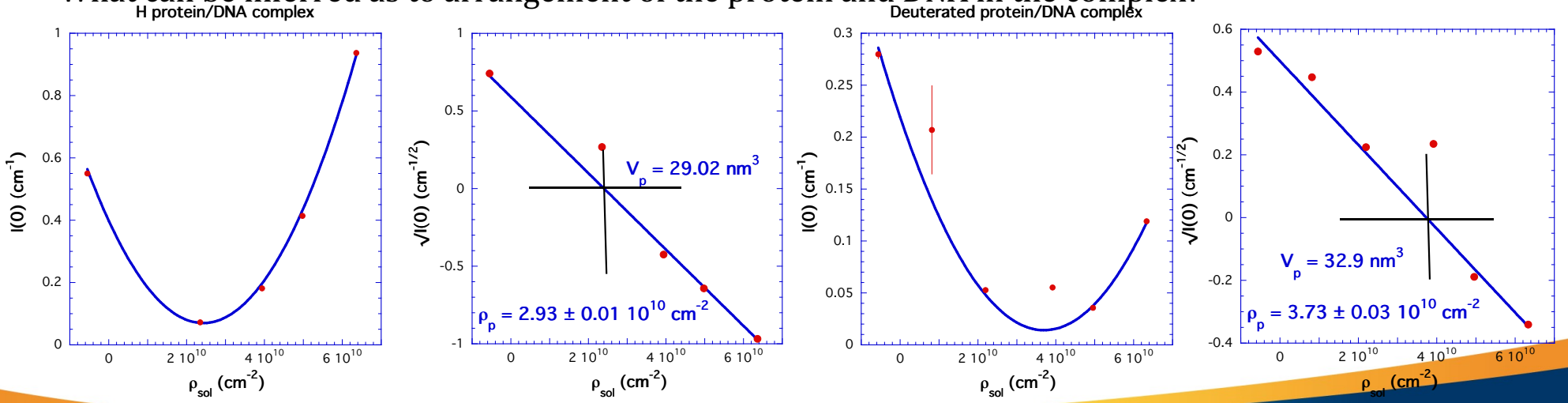


CV-SANS of the structure an IDP:HCNcore (E1-40Cor30) chimera complexed with DNA.

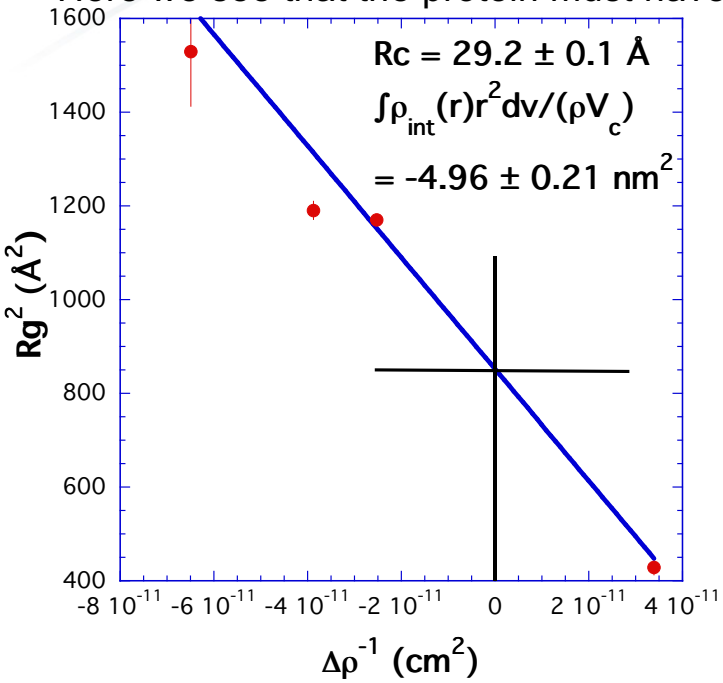
- UNM team has produced a hepatitis C nucleocapsid core protein with an elastin-like polypeptide, intrinsically disordered protein (IDP) to give a protein with chaperone activity and temperature dependent coacervate formation.
- Chaperones catalyze the formation of double stranded DNA from two complementary hair pin molecules.
- The process involves multiple steps.
- What is the structure of the complex when E1-40Cor30 forms a complex with one hair pin DNA.
- The result informs the incipient state of the chaperone process engineered chimera.

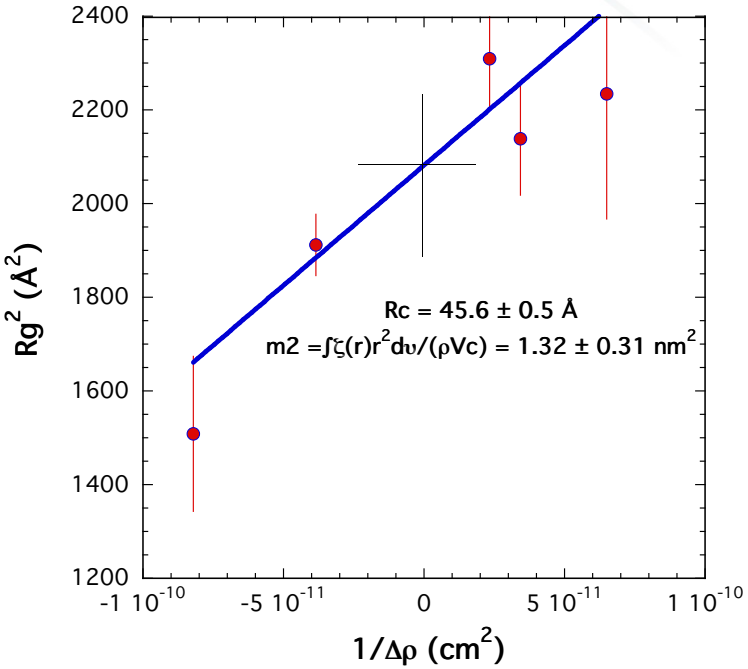
Case study: CV-SANS on a protein/DNA complex

- Bio-SANS measurement on H protein and D protein-DNA complex.
- Guinier analysis of data on an absolute scale gives I(0) as a function of ρ_{sol}.
- Data show presence uncomplexed protein and DNA.
- Modeling of the I(0) vs ρ_{sol} parabola indicate 40% complex and incomplete deuteration and H->exchange.
- What can be inferred as to arrangement of the protein and DNA in the complex?



- Luzzati *et al.* (1976) discuss the rigorous criteria for the analysis of CV-SAXS data: the dispersion morphology must be uniform to a degree if meaningful parameters are to be determined from the data.[1]
- However, the result here show the possibility that a combination of specific labeling and CV-SANS and careful analysis of the contrast dependent intensity allows for some assessment of the non-uniformity.
- The result is that at least a qualitative answer can be inferred.
- Here we see that the protein must have a distribution of the complex so that it is around the DNA.





1. Luzzati, V., et al. (1976). "Structure of human serum lipoproteins in solution: I. Theory and techniques of an X-ray scattering approach using solvents of variable density." *Journal of Molecular Biology* **101**(2): 115–127.

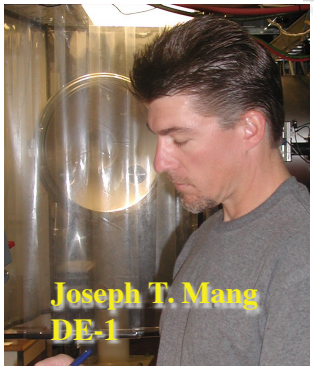
Conclusions

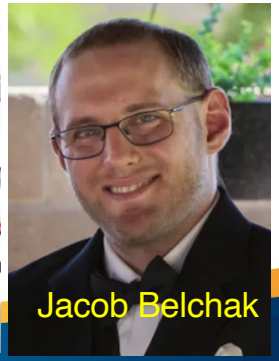
- Contrast variation provides for detailed assessment of structure and composition in homogeneous dispersions and of domains in complex polymer materials.
- Specific labeling in bioscience allows for detailed assessment of the relative positions of components associated with their process specific states.
- The combination of specific labeling and CV-SANS enables assessment of dispersion non-uniformity, allowing for at least some assessment of the structure in non-ideal cases.

What services might be provided

- Characterization:
  - the percentage of isotope substitution.
    - Mass spectroscopy.
    - NMR.
    - Infared spectroscopy.
  - exchange interactions.
    - Chromatography.
- Stock pile of common deuterated molecules for polymer science and bio-science.
- Support of collaborative work of new deuterated materials.
- Promote the synergism between facility and the user communities.

Highlighting the work of real people: physicists, chemists, materials scientist and engineers





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