Computational Methods to Analyze Small Angle X-ray Scattering Data of Biological Molecule & Free Energy Calculations to Validate Structural Ensembles

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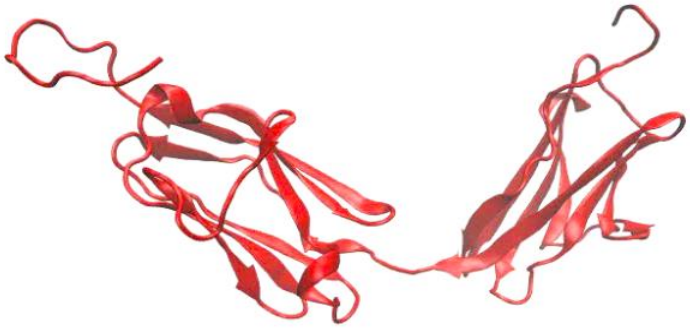
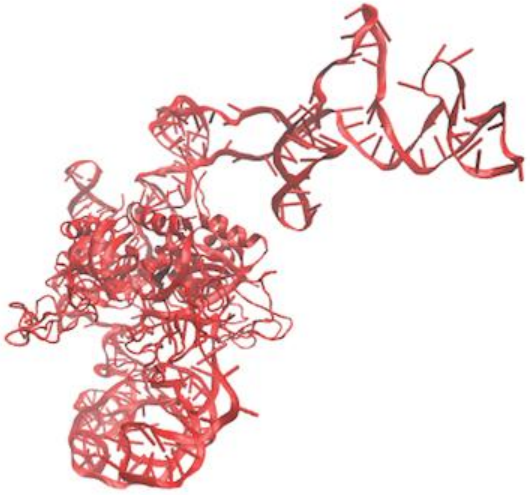


Ig58/59

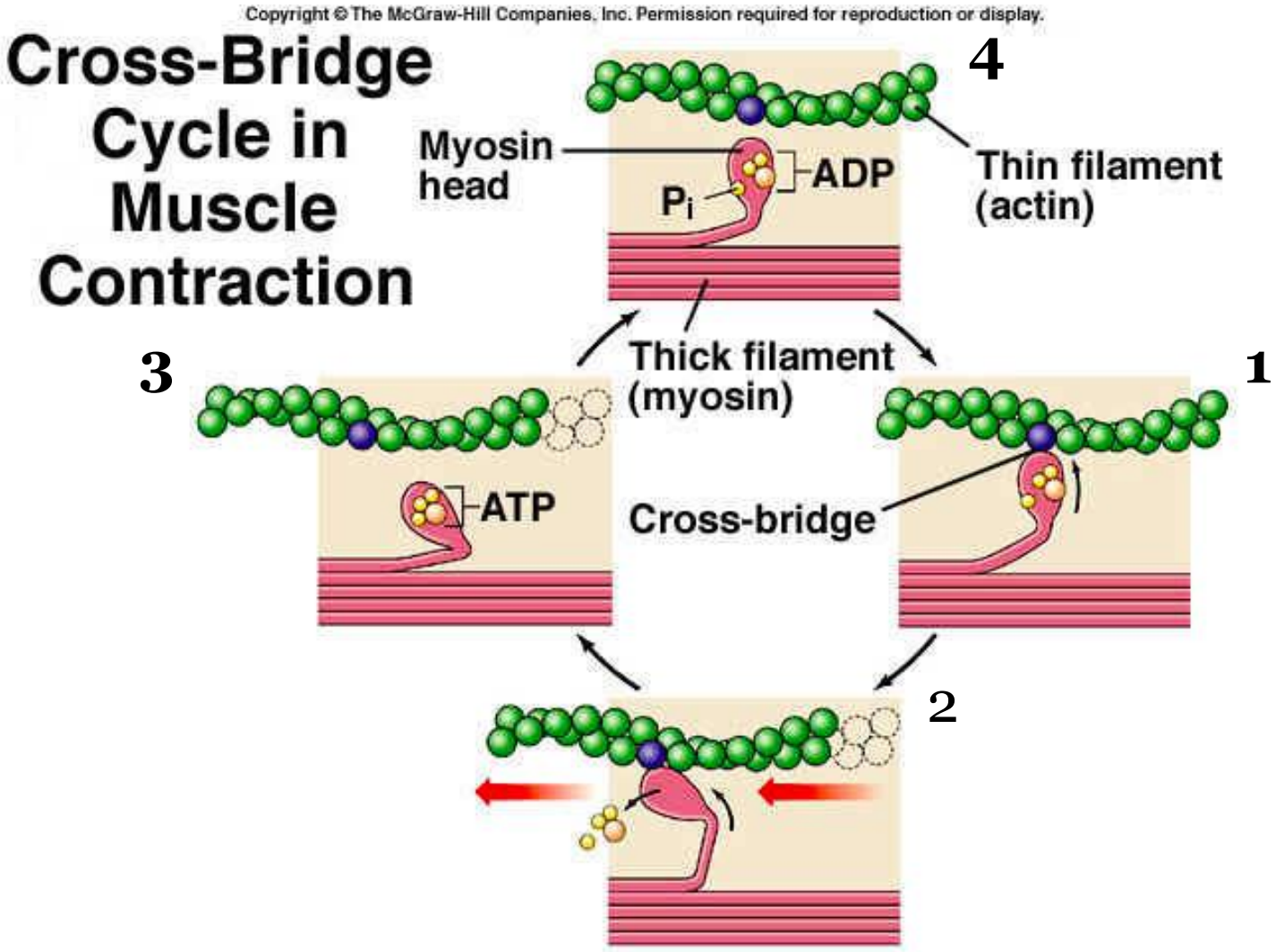


Hfq + rpoS RNA

Overview

- Background – Obscurin
- Advanced analysis
  - Ig58/59
- Free energy calculations
  - Ig58/59
  - CHD1
  - rpoS RNA/HFQ

Muscle contraction



Obscurin

- Cell maintenance + organization + signaling
- Ig58/59
  - Mutation (Arg8Gln)

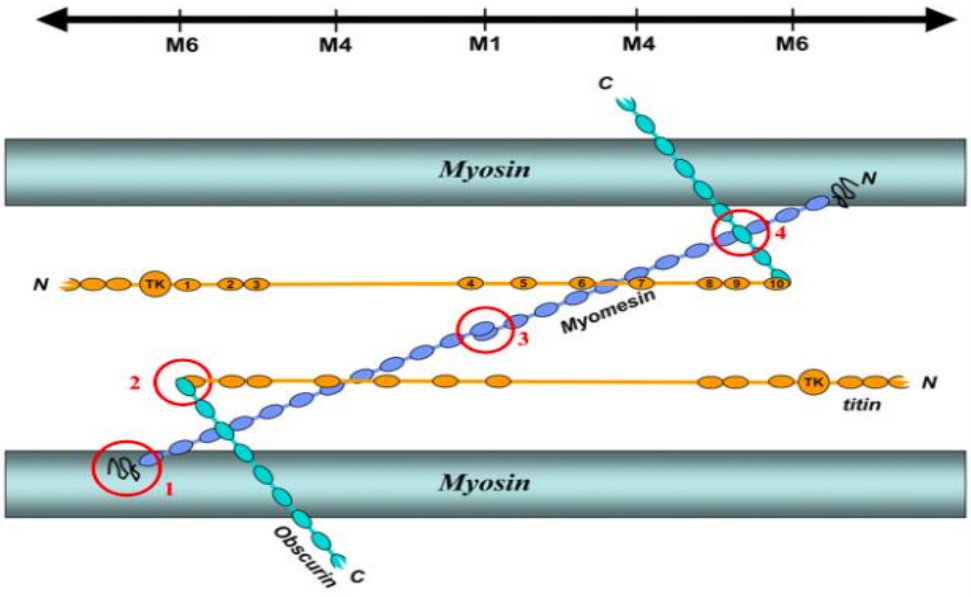


Figure adapted from Fukuzawa *et al.* (2008)

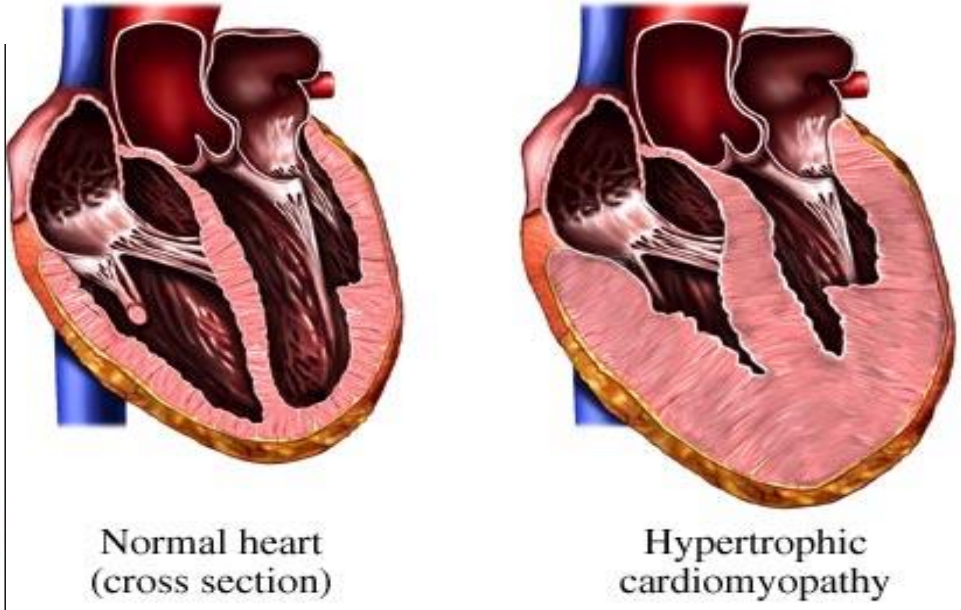
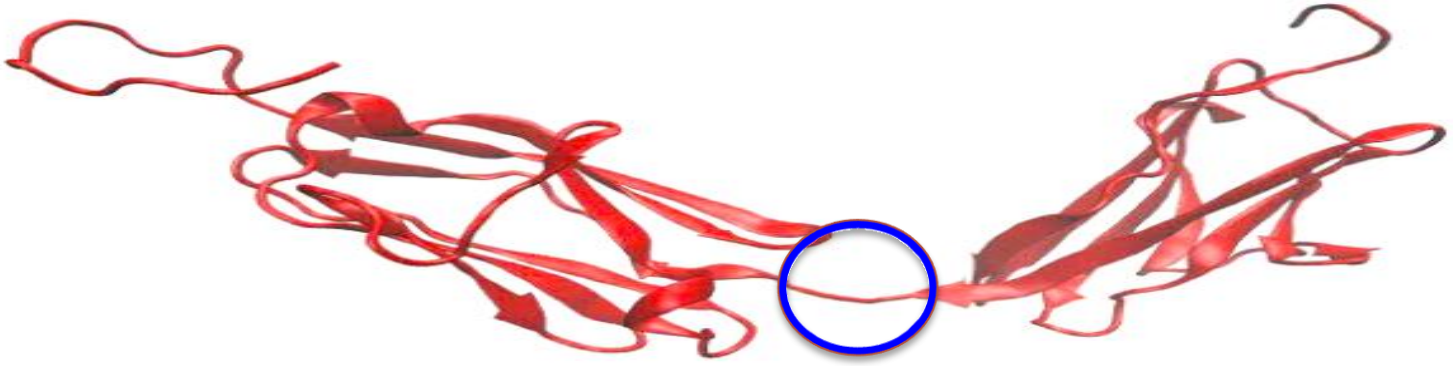


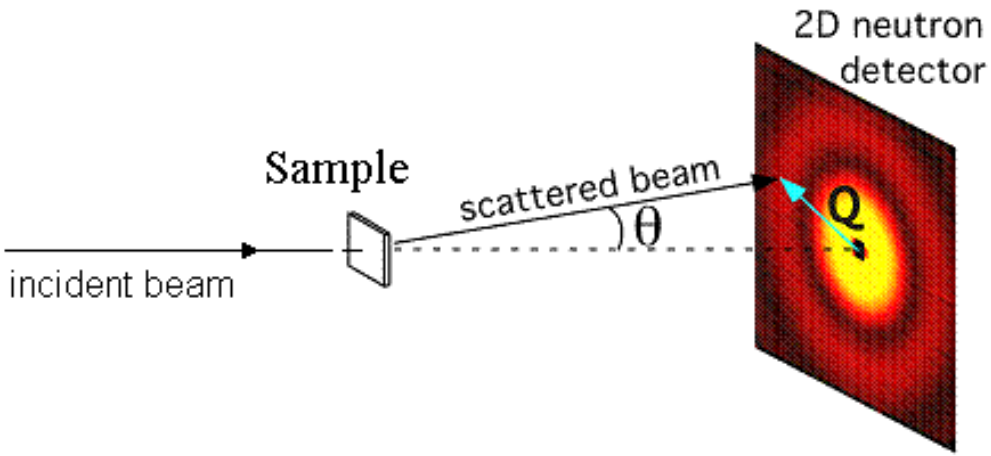
Figure adapted from Kontrogianni-Konstantopoulos *et al.* (2009)

Intrinsic Flexibility/Disorder

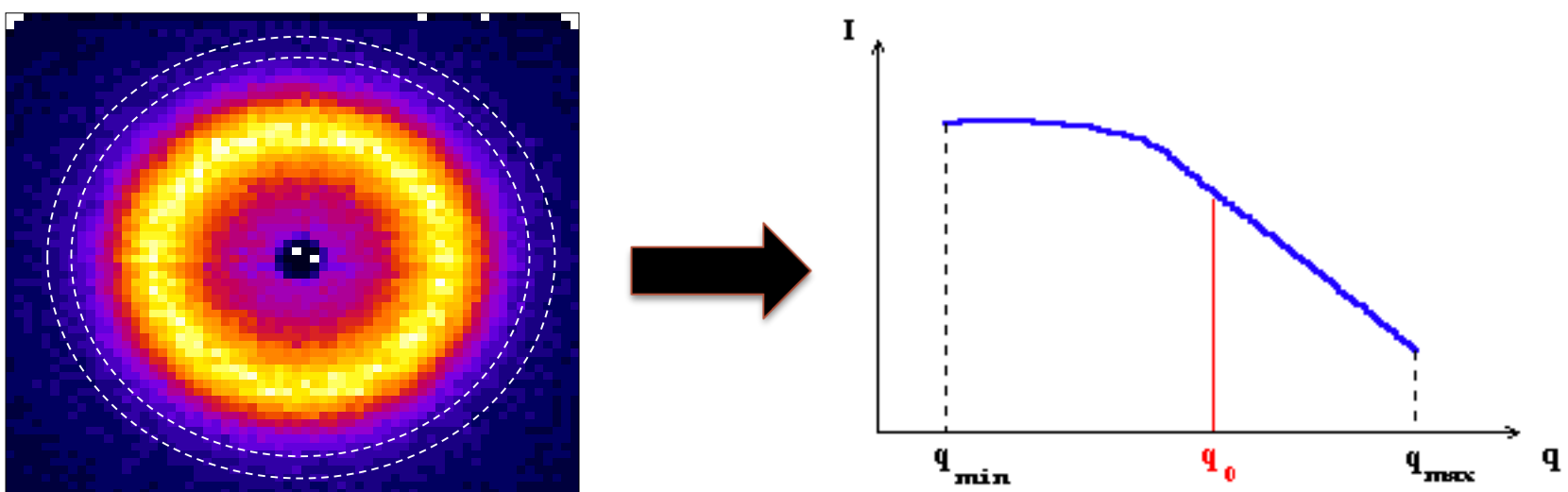
- Intrinsically disordered proteins
  - Lack fixed 3-D structure



Small Angle Scattering Theory

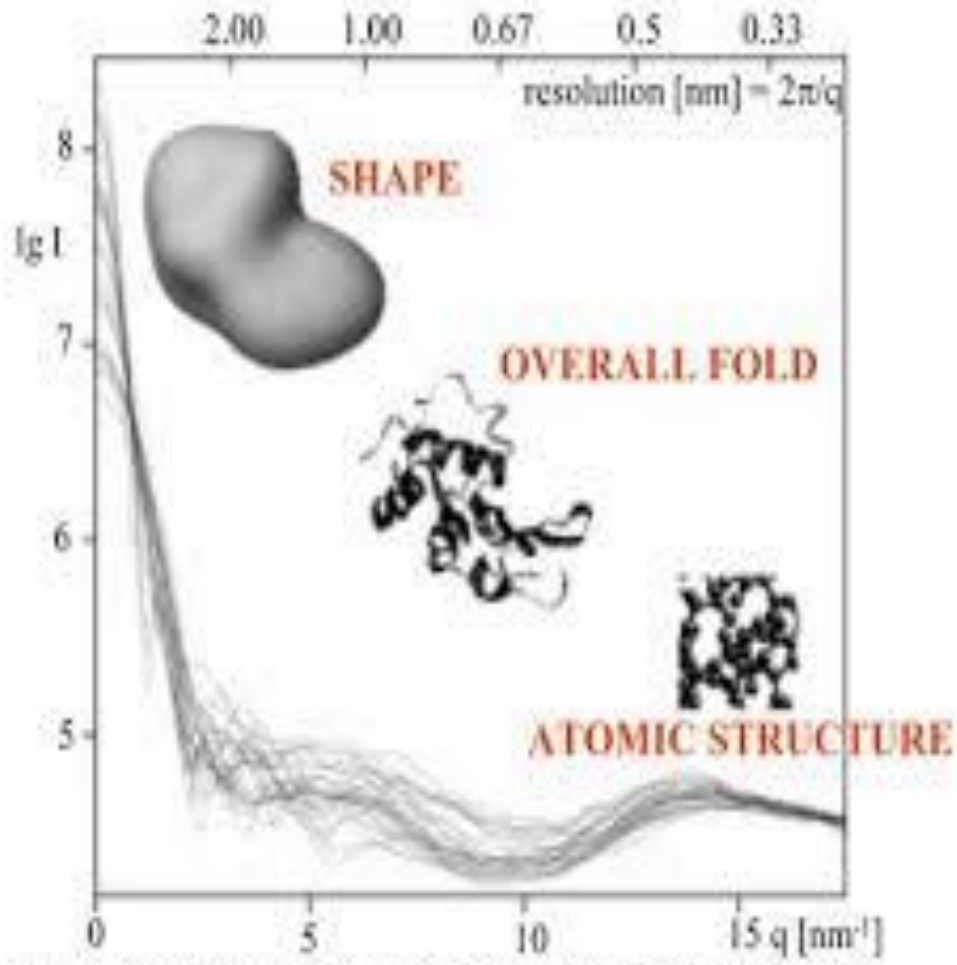


$$q = \frac{4\pi \sin(\theta)}{\lambda}$$



http://iramis.cea.fr/scm/lions/techniques/saxs/

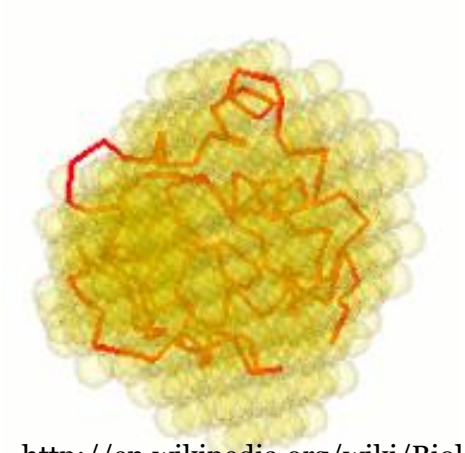
Modeling Methods in SAXS/SANS



from D. Svergun and M. Koch, Biophysical methods (2002) 654-660

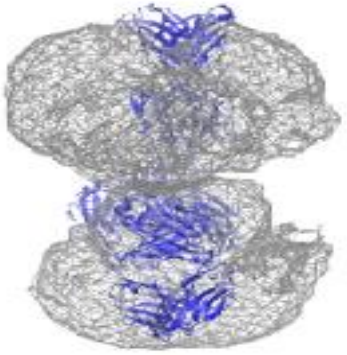
- Ensemble methods
  - Approaches
    - Dummy atom
    - Atomistic modeling

Dummy atom model



http://en.wikipedia.org/wiki/Biological_small-angle_scattering

Atomistic model

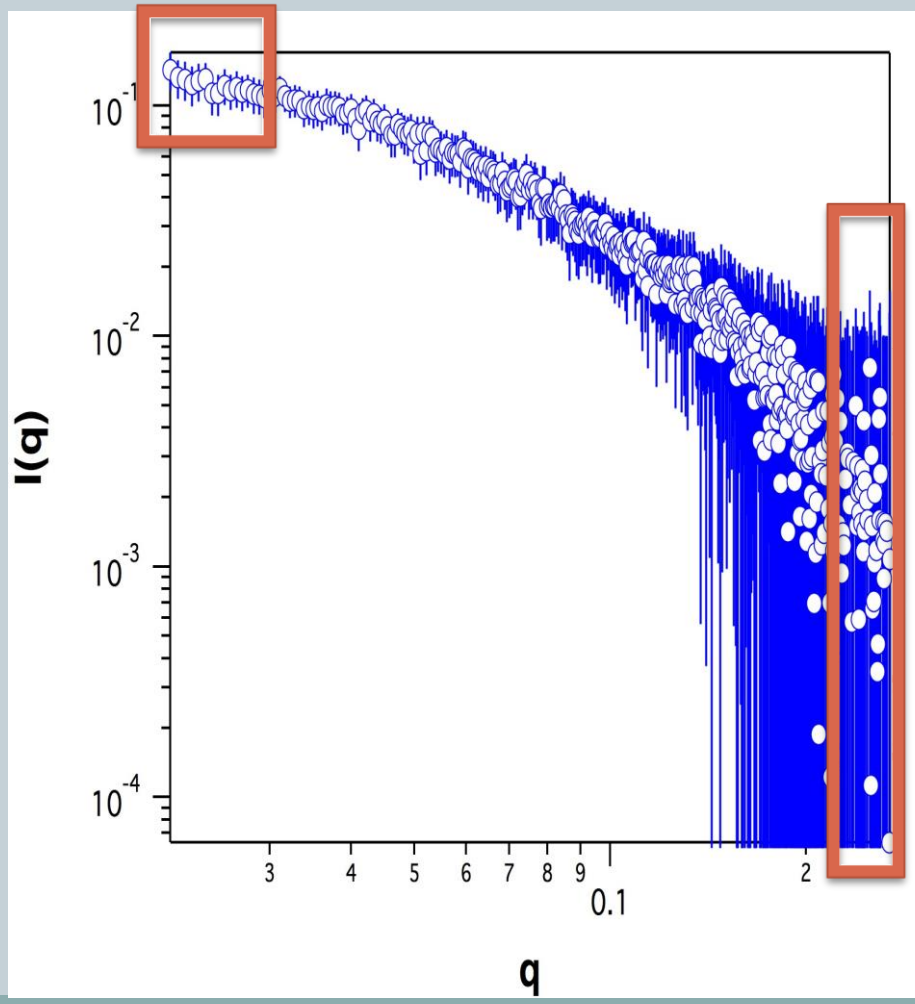


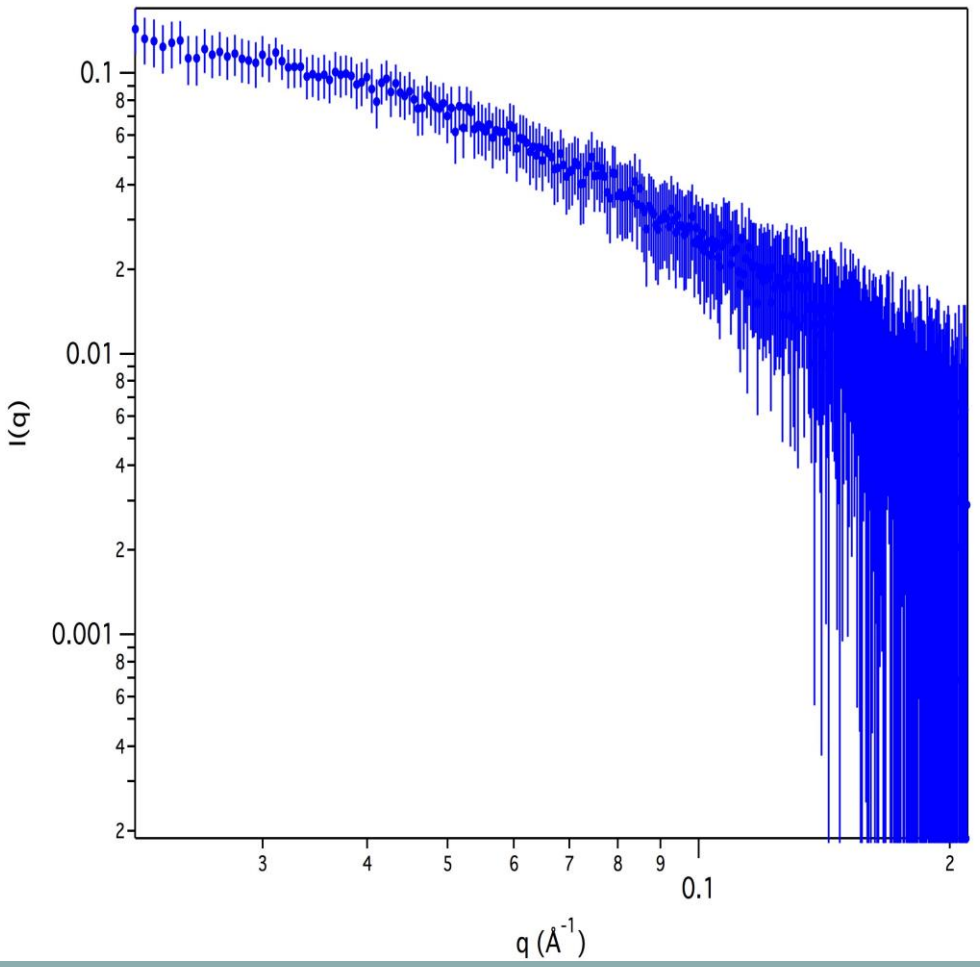
Adapted from Clark et al. (2013)

Data reduction of Ig58/59

Processed Scattering Data

Truncated Scattering Data

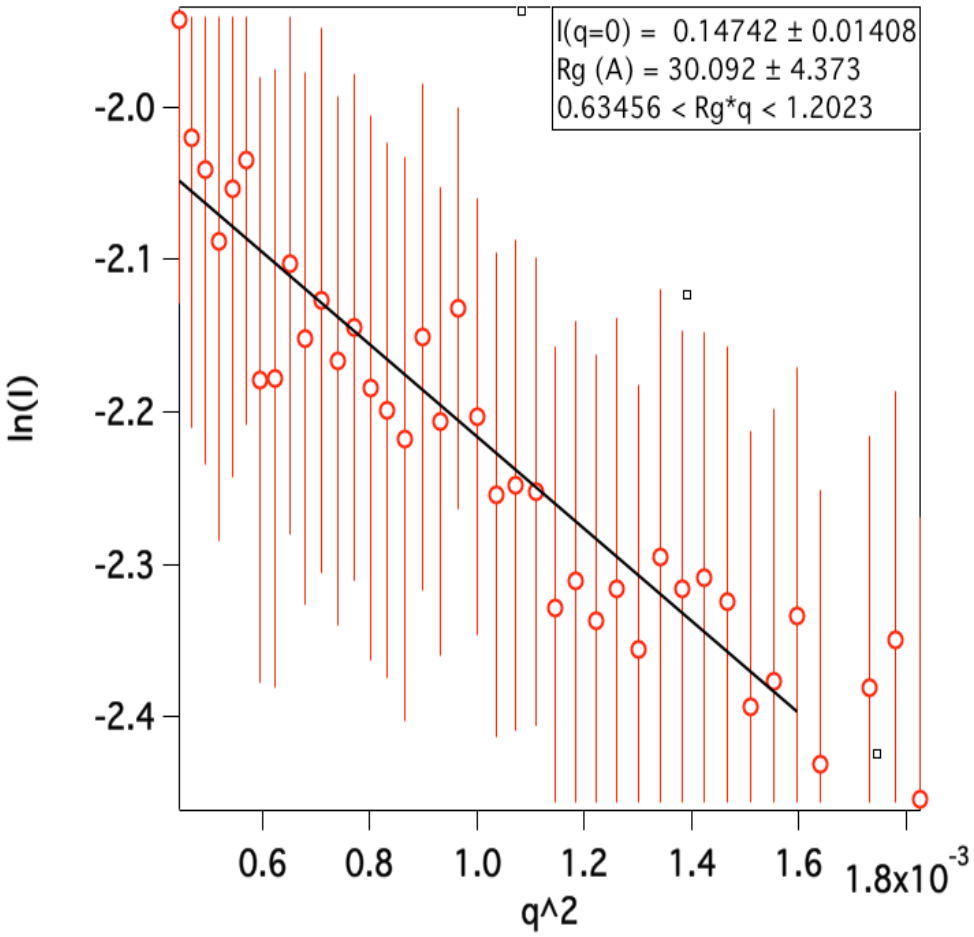




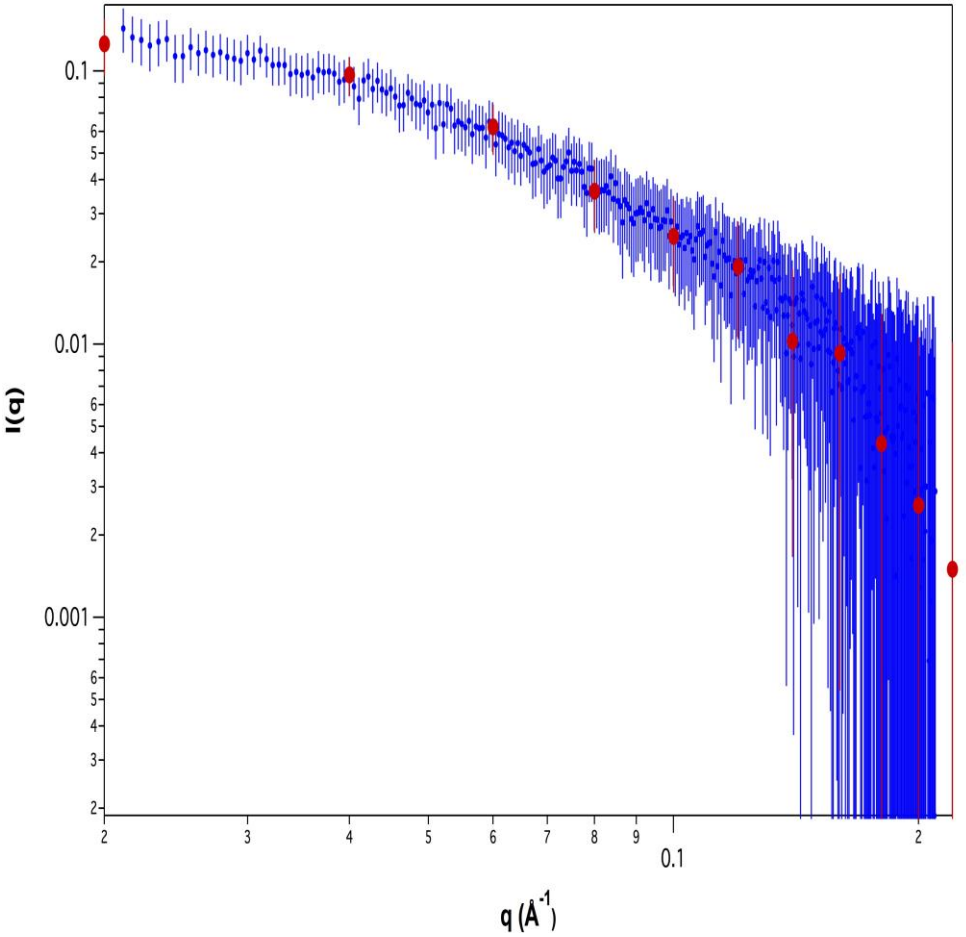
Potential non-ideality

Ig58/59 Analysis

Guinier Plot



Truncated Scattering Data



Structure Generation

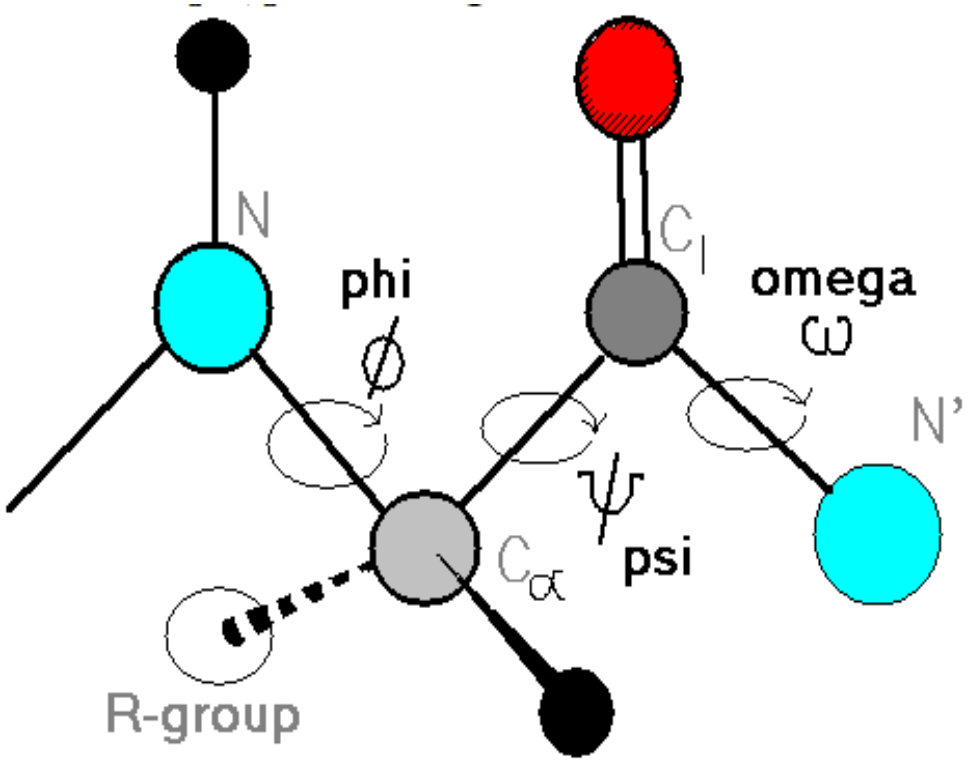
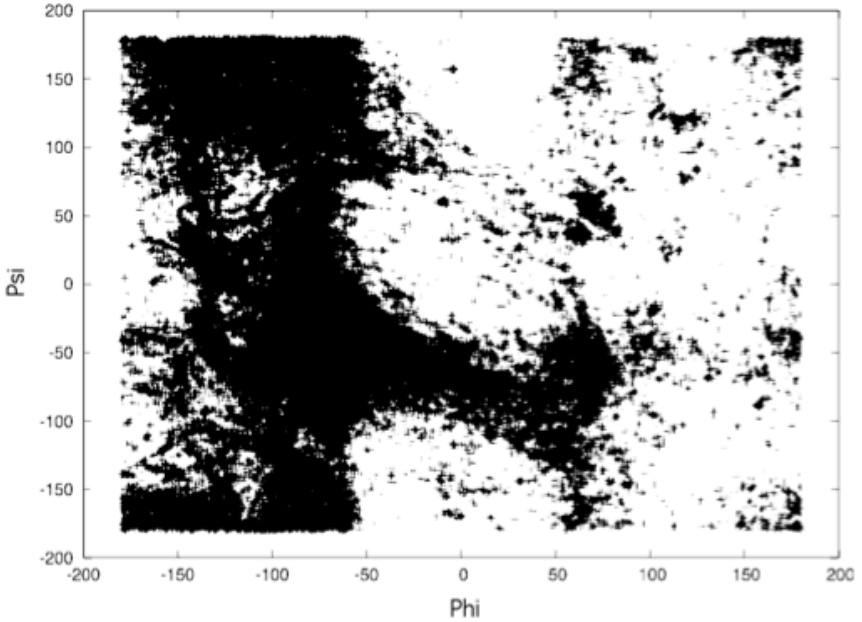
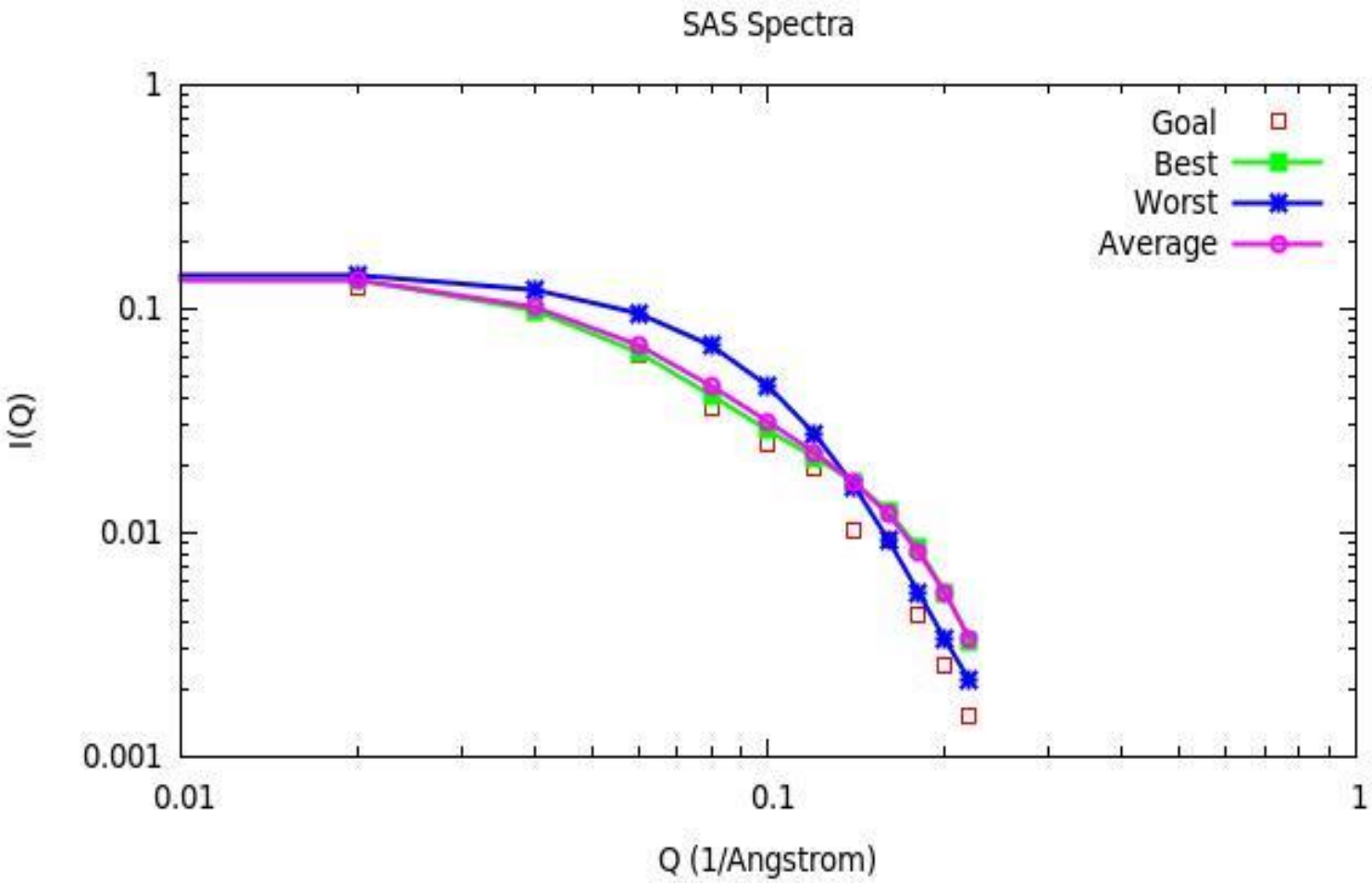


Figure adapted from http://www.bmb.uga.edu/wampler/tutorial/prot2.html



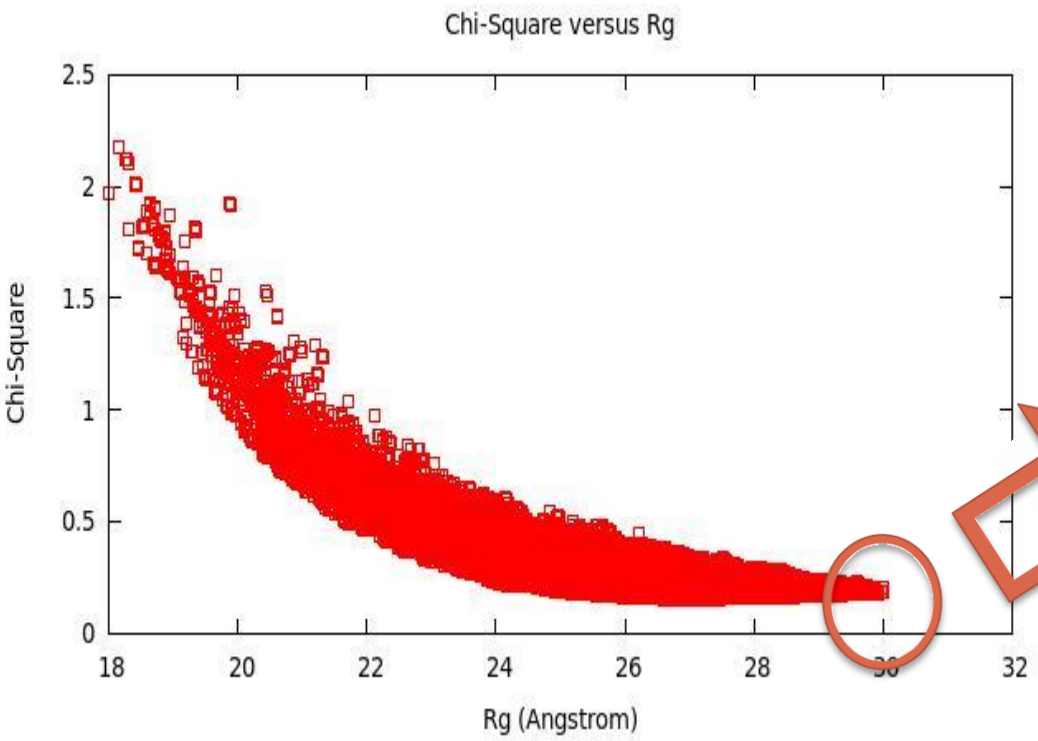
Figures adapted from Computer Physics Communication 183 (2012) 382-389

Ig58/59 Analysis



Ig58/59 Analysis

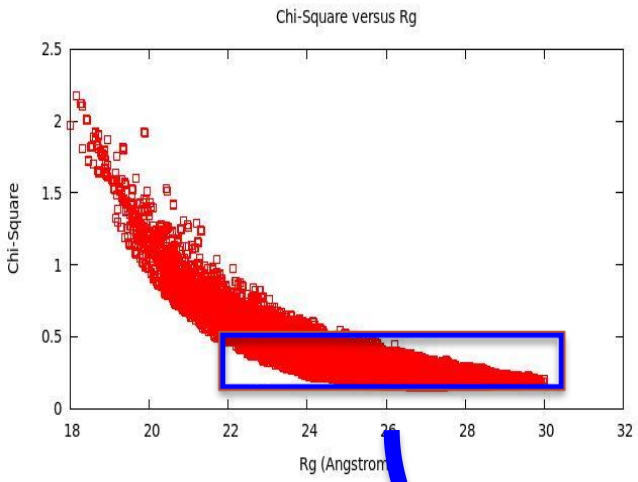
Chi-squared Analysis

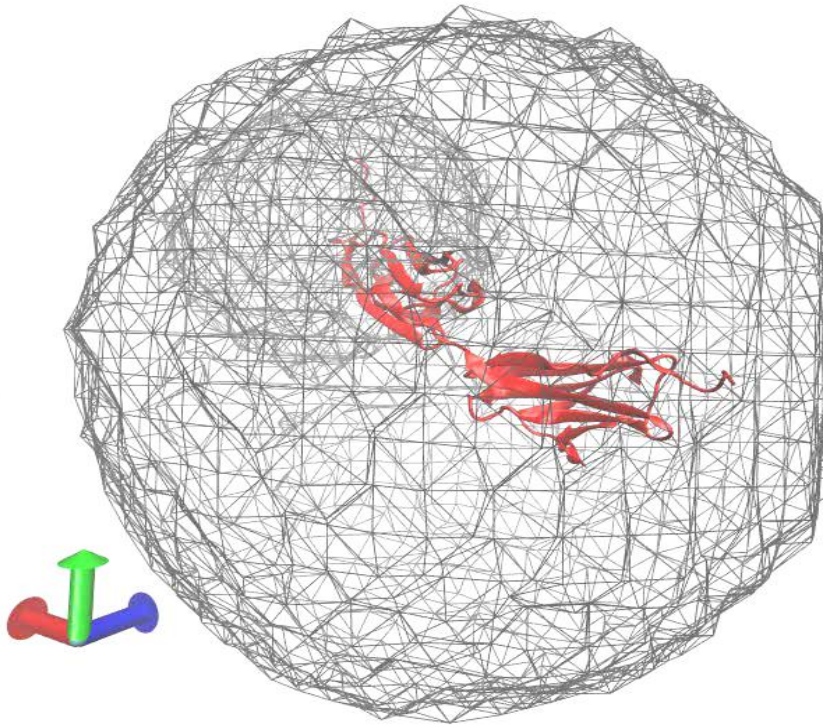


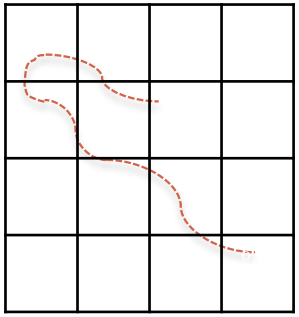
30.36Å

Rg (A) = 30.092 ± 4.373

Ig58/59 Density Map



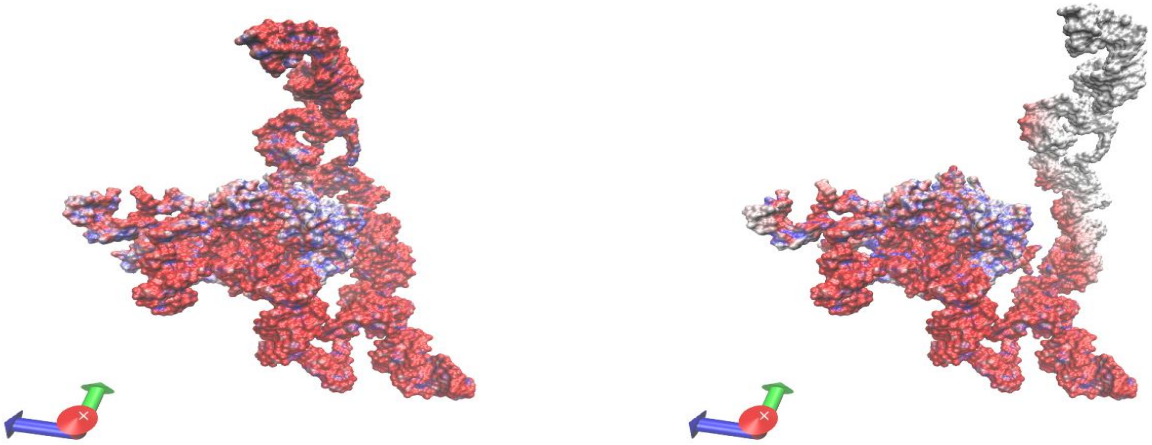




2-Dimensional Example

Free Energy Calculations

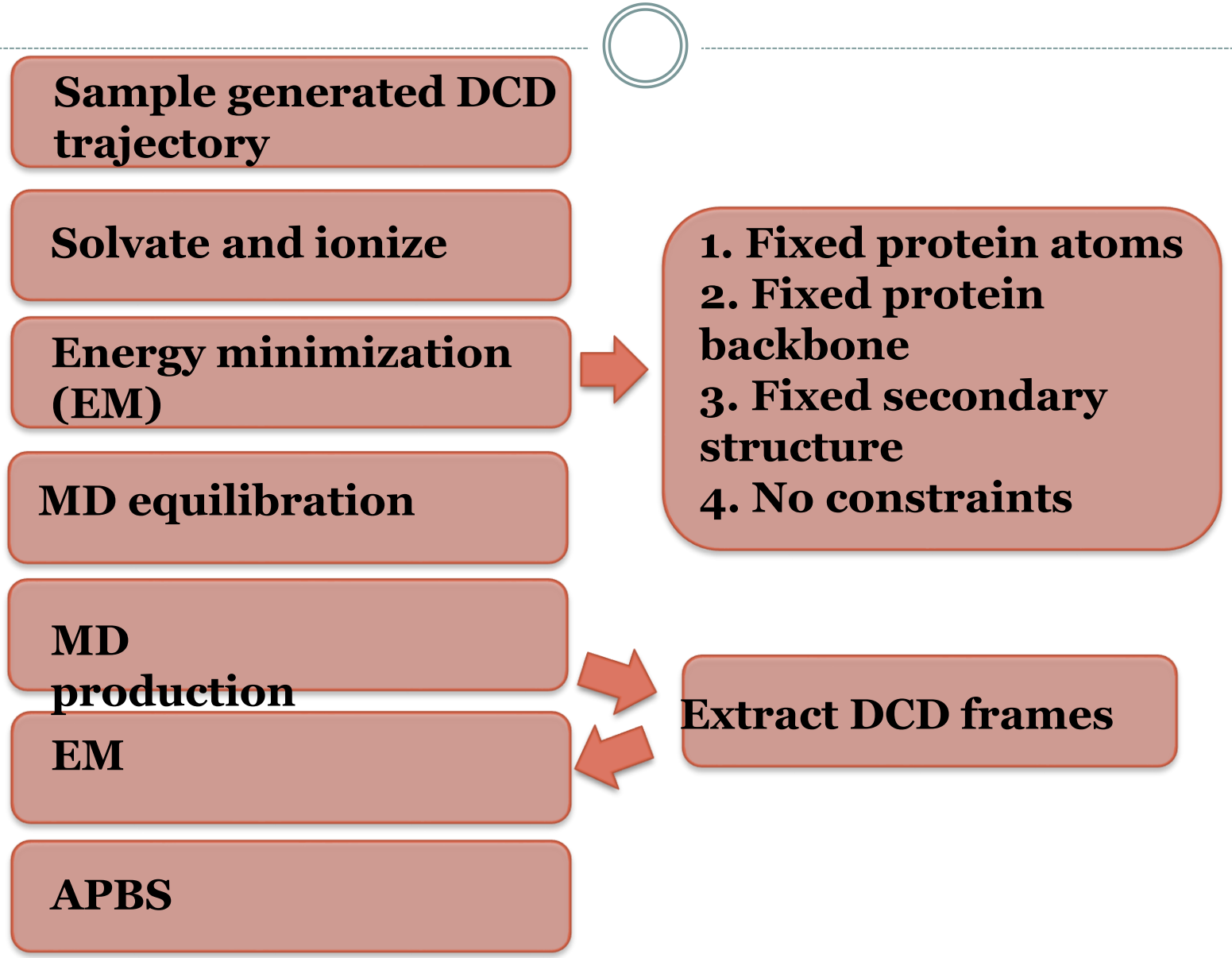
Problem : Computationally intensive to calculate free-energy in MMC



Solution :

(ΔG) = molecular mechanical + solvation free energy

Free Energy Calculations



Conclusions

- SAXS data suggests extended structure
  - Contamination in sample
  - Intrinsic motion within globular domains
- Future directions
  - SANS (8/21)
- Free energy calculations
  - Study ongoing

Acknowledgements

- Dr. Joseph Curtis
- Dr. Julie Borchers
- Dr. Susan Krueger
- Dr. Nicholas Clark
- Dr. Nathan Wright
- James Madison University

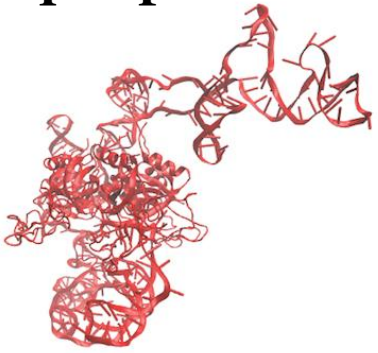
Free Energy Systems

- Ig58/59
- Chd1 (Wt) + Chd1 (mutant)
  - ATP-dependent chromatin remode
  - Disrupt DNA binding
- Hfq + rpoS RNA leader
  - Accelerates base pairing between sRNA & mRNA
  - Provides translational control

Chd1 (wt)

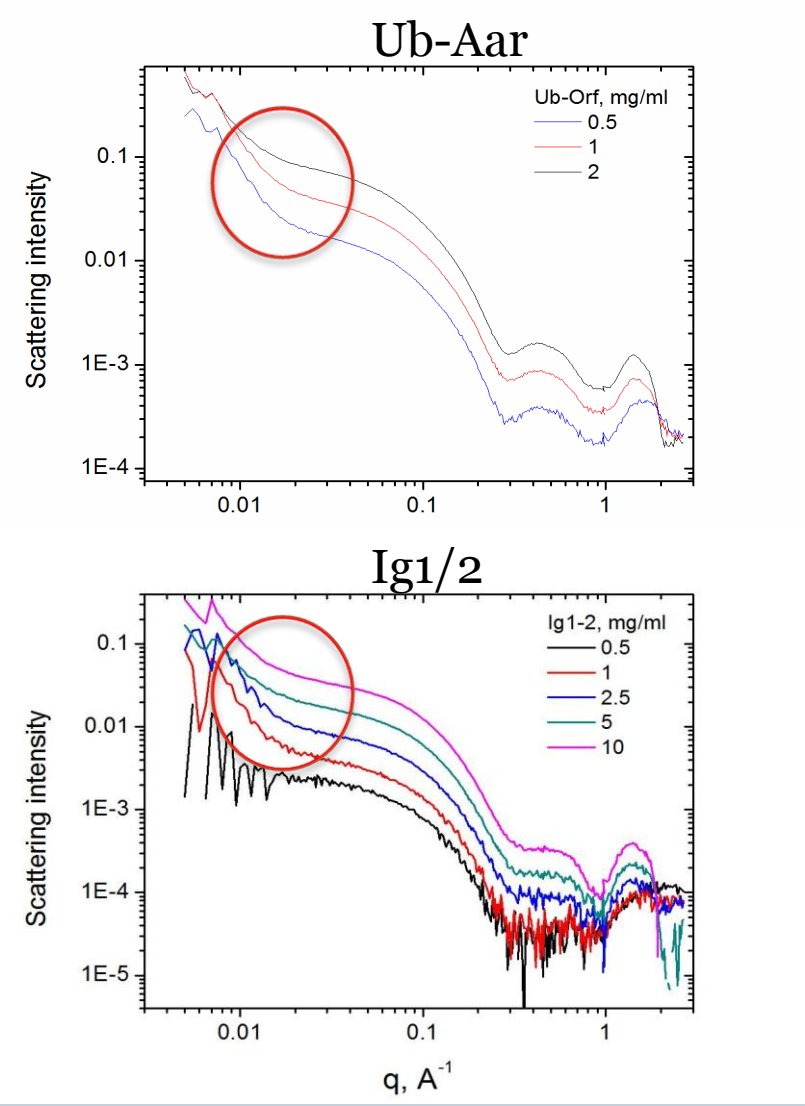
Chd1 (mut)

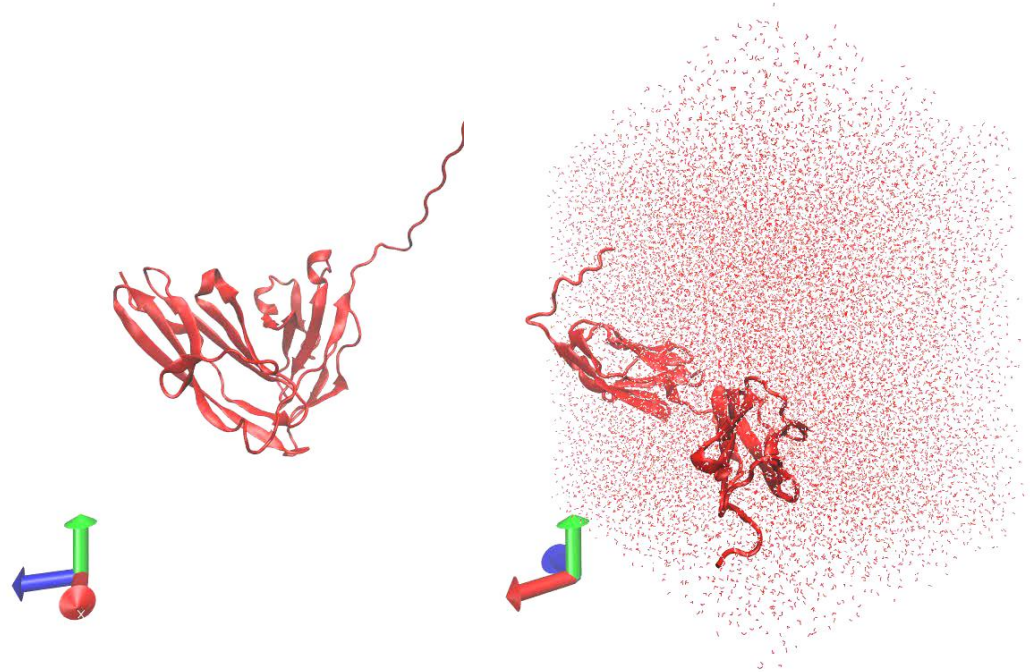
Hfq + rpoS RNA



Overview

- Small angle X-ray scattering (SAXS) data sets
  - Ubiquitin-Aar
  - Ig1/2
  - Ig58/59
- SAXS analysis
  - Ig58/59
- Free energy calculations
  - Ig58/59
  - CHD1
  - rpoS RNA/HFQ



Ig58/59 Structure Generation

- Crystal structure
- NAMD vacuum minimization
- VMD solvation box
- VMD ionization
- NAMD minimization
  - Fixed protein atoms
  - Fixed protein backbone
  - Fixed secondary structure
  - No constraints
    - NAMD/CHARMM molecular dynamics