Visualizing Protein Dynamics: a combined crystallography, SAXS and computational approach

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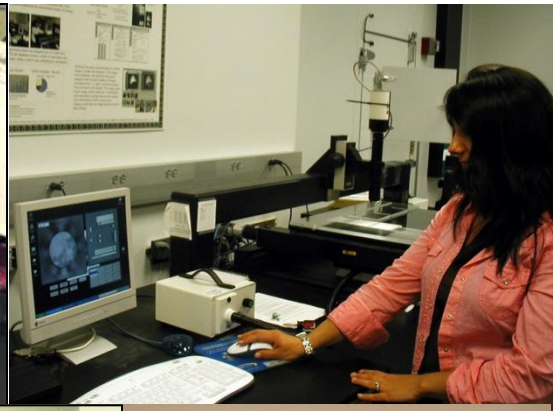
And the people who really matter


Rochester

Erin Quartley

Stephanie Corretore

Buffalo

Tom Grant

Jen Wolfley

Elizabeth Snell

Tina Veatch

Angela Lauricella

Eleanor Cook

Interests of the Snell and Luft Laboratories

- **Dynamics in systems: biological and physical**
  - X-ray induced structural perturbation
  - Correlated motion
  - Natural dynamics
- **Complexes**
  - Structural studies on proteins with multiple partners
- **Viruses**
  - Enabling high-throughput virus studies
- **Crystal growth and crystallography**
  - High-throughput technology applied to fundamental studies
  - Information extraction
  - Automating the whole pipeline
- **Developing Small Angle X-ray Scattering**
  - Characterization of samples
  - Identification of dynamic systems
  - Resolving validation in SAXS
- **Computational techniques**

In this talk ….

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Outline

- **Biological dynamics**
- **Crystallography**
- **Small Angle X-ray Scattering**
- **A biological case**
- **A structure, a puzzle, a question?**
- **A solution**
- **Computational analysis**
- **An answer, more questions, more solutions**
- **Where are we going next?**

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Dynamics in systems: Biological

- Dynamic motions of biological systems are almost universal in biology encompassing processes as diverse as mitosis, signal transduction, regulatory pathways, immune response, protein folding and enzymatic pathways.
- Or, greatly simplified by a previous talk, “If it doesn’t wriggle then it’s not biology”
- How common are wriggles?
- It is estimated that conformational flexibility results in unstructured regions of 40 amino acids or more in 50% of eukaryotic proteins
  Vucetic *et al.,* Proteins 52, 573-584 (2003)
- How many of these are functional wriggles?
- We don’t know yet ….

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Crystallography

- X-ray crystallography accounts for 86% of our structural knowledge in the Protein Data Bank, structural knowledge on an atomic scale. NMR and Cryo-EM provide 14% and 0.4% respectively.
- Twenty three Nobel prizes have been associated with X-ray crystallography – it is a key tool in biomedical science.
- However, where failure as well as success is tracked in the case of the structural genomics community over 80% of targets that produce soluble purified protein (28,000) fail to produce structures.
- To put this in perspective, this is equivalent ~50% of the total current PDB content.
- This is especially alarming given the effort and resources required for cloning, expression and purification of a target ORF sample.
- Crystallography gets an A+ for effort and results but a C- for overall success (but it's the best technique we have)

Crystallography and dynamics

- A crystal provides an ordered array of macromolecules.
- This ordering is required to see detail at atomic resolution.
- If dynamic disorder exists, unless this occupies a few defined positions, it is hard to see in the X-ray data.
- Ensemble techniques show where this disorder might be and, for small scale disorder, they can indicate it's extent. However they cannot provide long-range correlation of motions.
- Laue methods allow for visualization of dynamic motion in a time-resolved manner but require a trigger to ensure that the motion is identical in as many molecules in the crystal as possible.
- Cryotrapping can also capture stills within a dynamic pathway but also requires that the motion is captured at an identical state in each molecule.
- Crystallography is powerful for 'static' systems, but challenging for systems involving dynamics.

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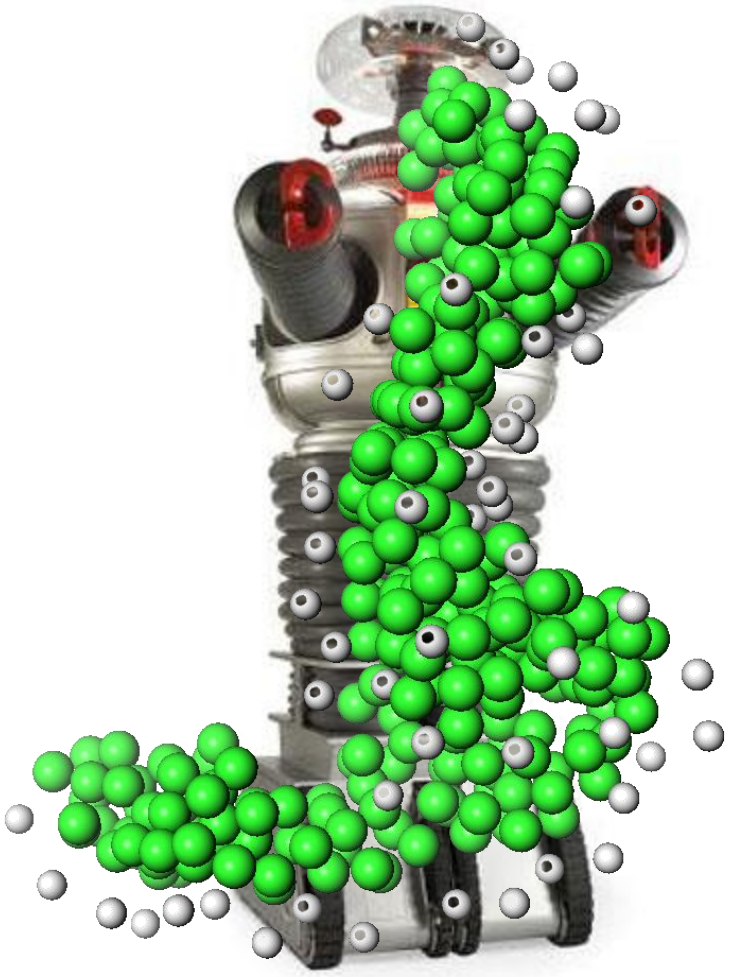
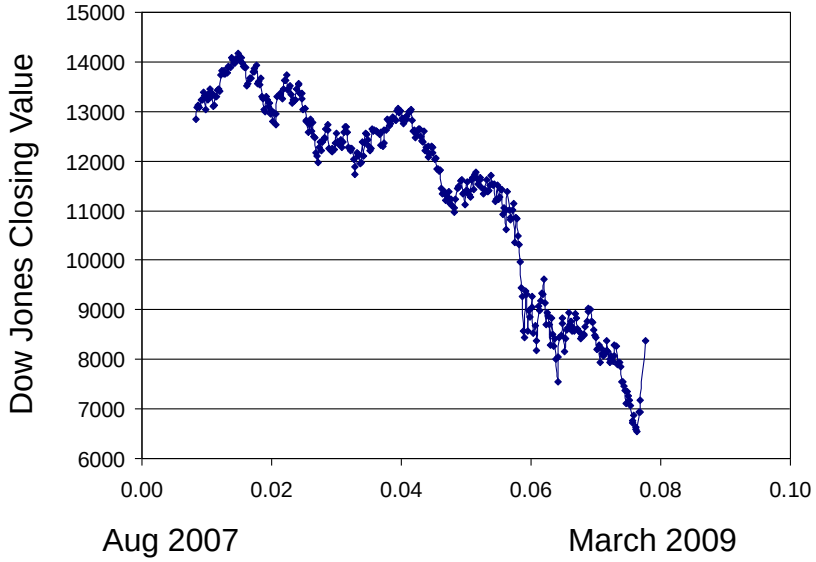
Danger, Danger ….





The scattering data from SAXS provides a 1D Fourier transform of the envelope of the particle.

It's possible to fit multiple envelopes to the data.

You will always get an envelope despite the data!

Our initial SAXS studies

- In an effort to develop a high-throughput SAXS based structural characterization pipeline to supplement crystallographic analysis we have used SAXS to investigate 27 structurally unknown samples provided by our collaborators at the NESG.
- These encompass a broad range of molecular weights from 9 to 170 kDa.
- Analysis of the scattering data showed that 18 samples were globular, 5 were aggregated and 4 natively unfolded.
- For 22/27 of these targets we were able to calculate the molecular weight from the SAXS data and use this to derive the oligomeric state.
- The molecular weight of the oligomer is necessarily an integer multiple (including unity) of the calculated molecular weight, providing a control.
- Three of these recently gave X-ray structures and for another a homology structure is available.
- For fourteen we have molecular envelopes but no validation that they are correct.

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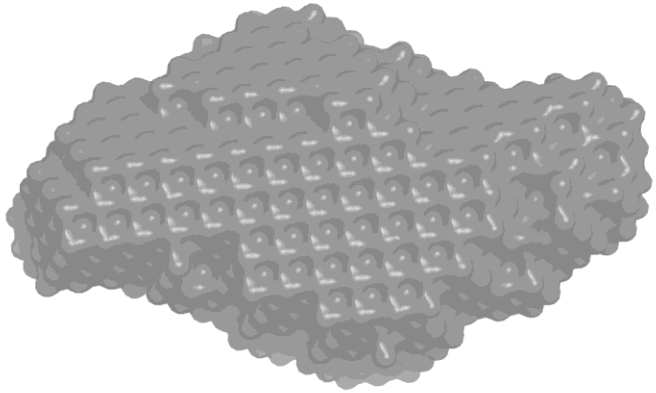
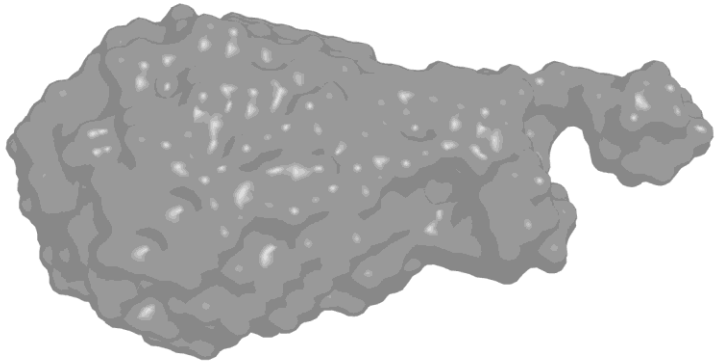
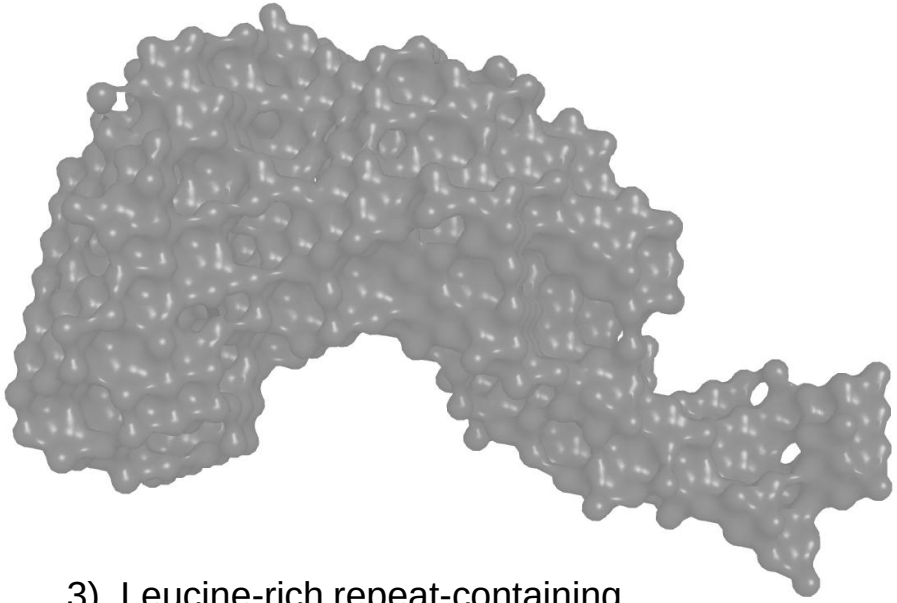
Ab intio envelopes



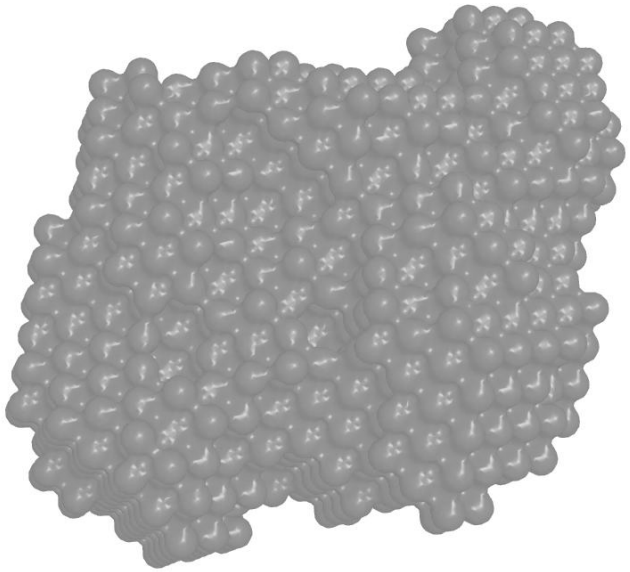
1). alr0221 protein from Nostoc (18.6 kDa)



2). C-terminal domain of a chitobiase (17.9 kDa)

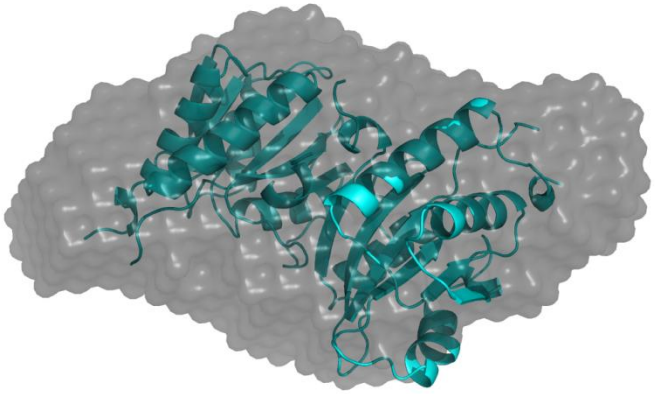


3). Leucine-rich repeat-containing protein LegL7 (39 kDa)



4). *E. Coli.* Cystine desulfurase activator complex (170 kDa)

Overlaid with subsequent X-ray structures



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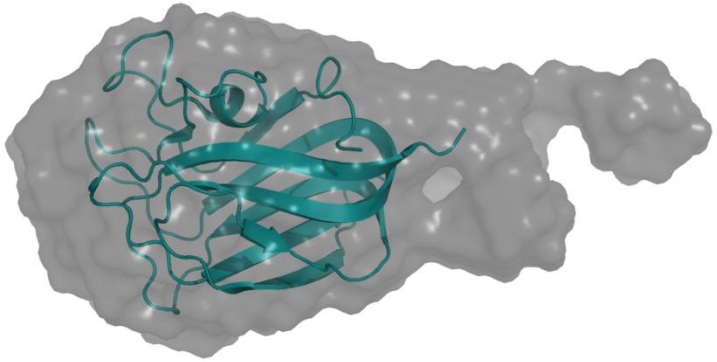

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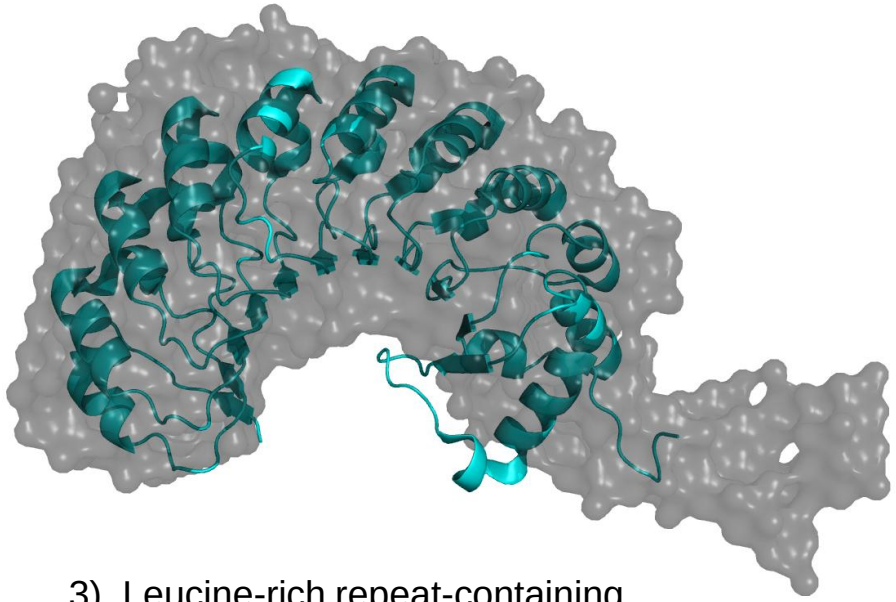

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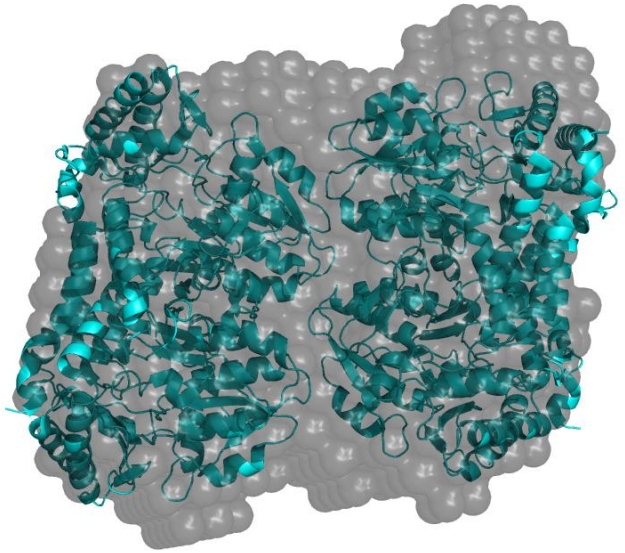

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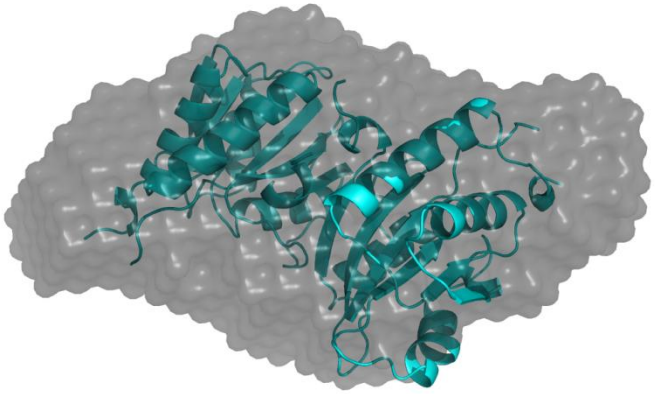
And data on what was missing …



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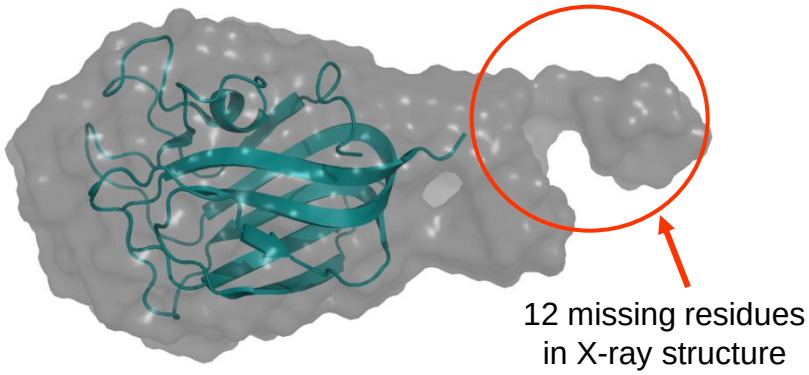

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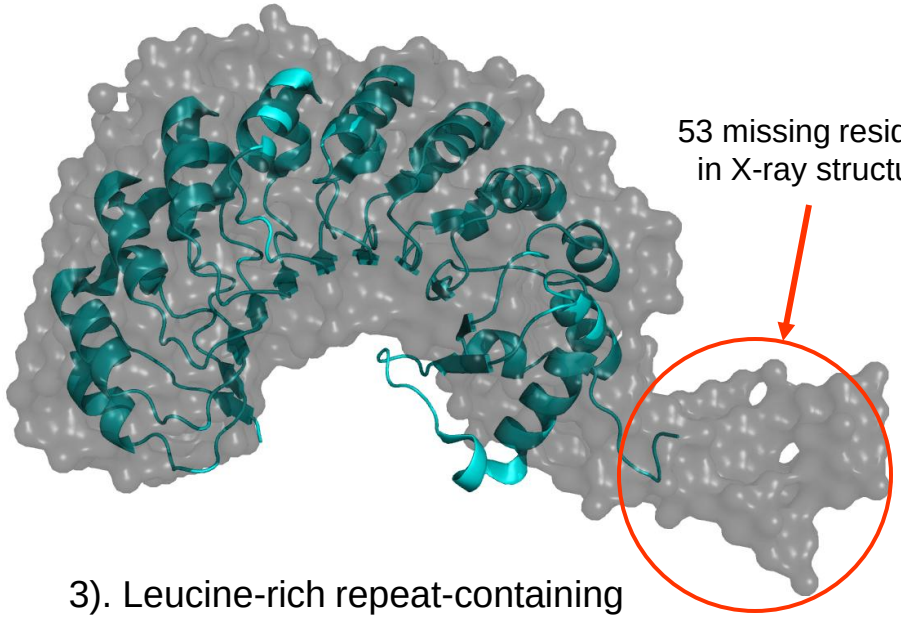

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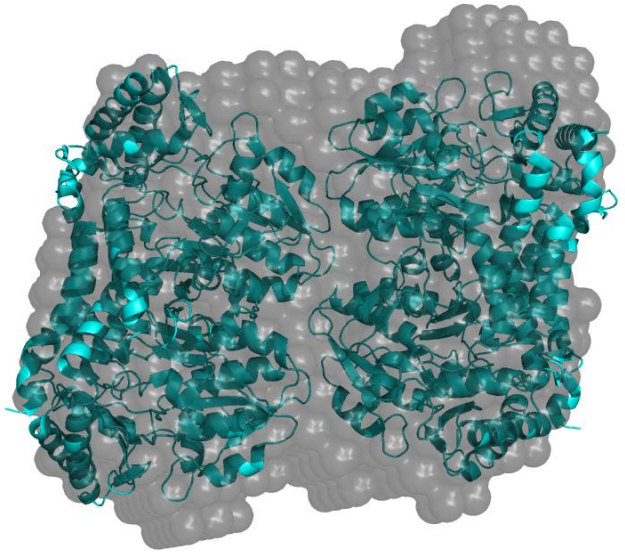

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SAXS and dynamics

- The SAXS solution can be validated when other information is present.
- In a non-symmetrical case (most examples) the X-ray derived structure can be fitted to the envelope.
- The scattering curve from SAXS is derived from a summation of all the particles in solution.
- It is radially averaged over all these particles (losing 3D information) but samples all positions of the particle and all conformations (sampling dynamic information).

- SAXS is sensitive to information that crystallography does not see.
- SAXS is sensitive to dynamics.
- SAXS is a low-resolution technique - Crystallography is sensitive to information SAXS cannot see.
- **Crystallography and SAXS are complementary.**

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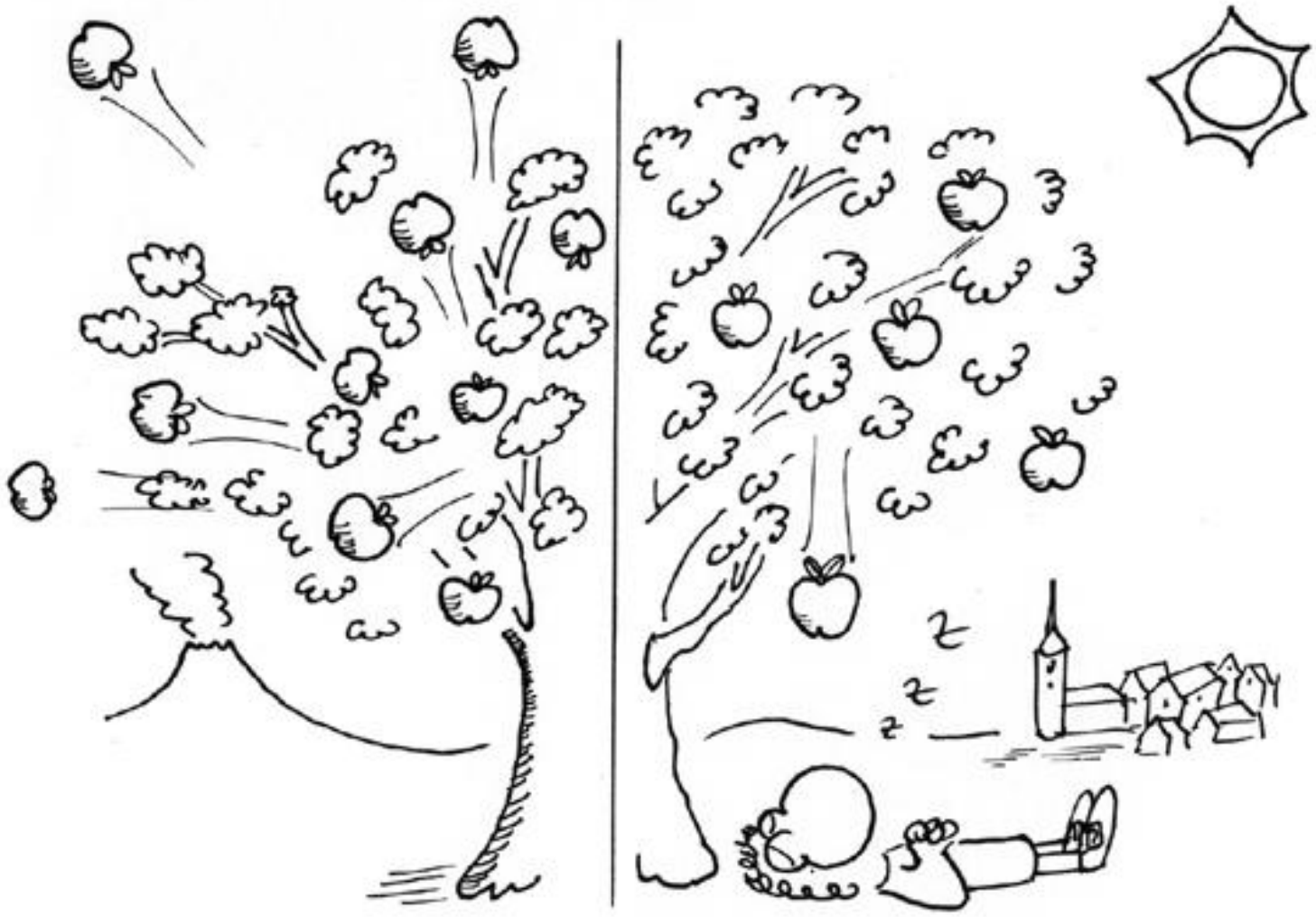
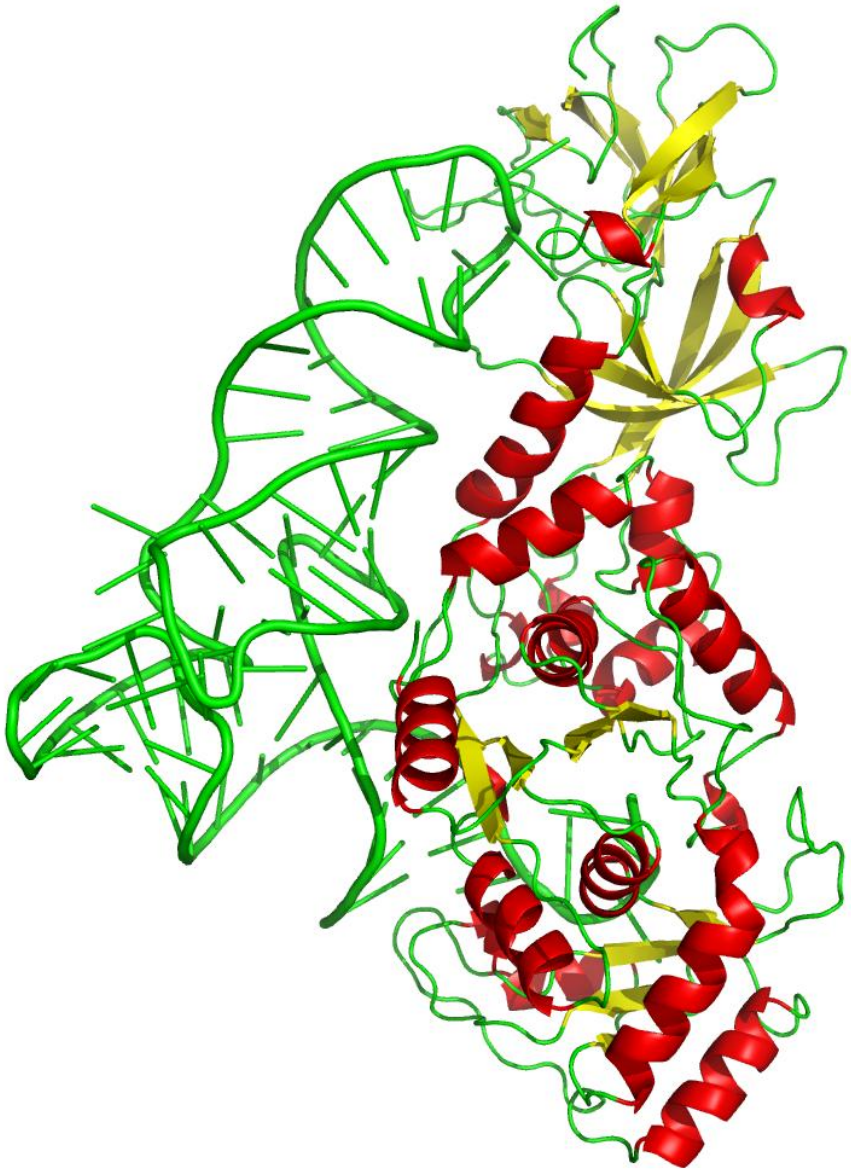
PHYSICS FOR BIOLOGISTS



A long time ago the apple trees used to shoot the apples in all directions. Only those that did it downward got reproduced. Then, after millions years of natural selection and evolution, gravity was finally discovered.

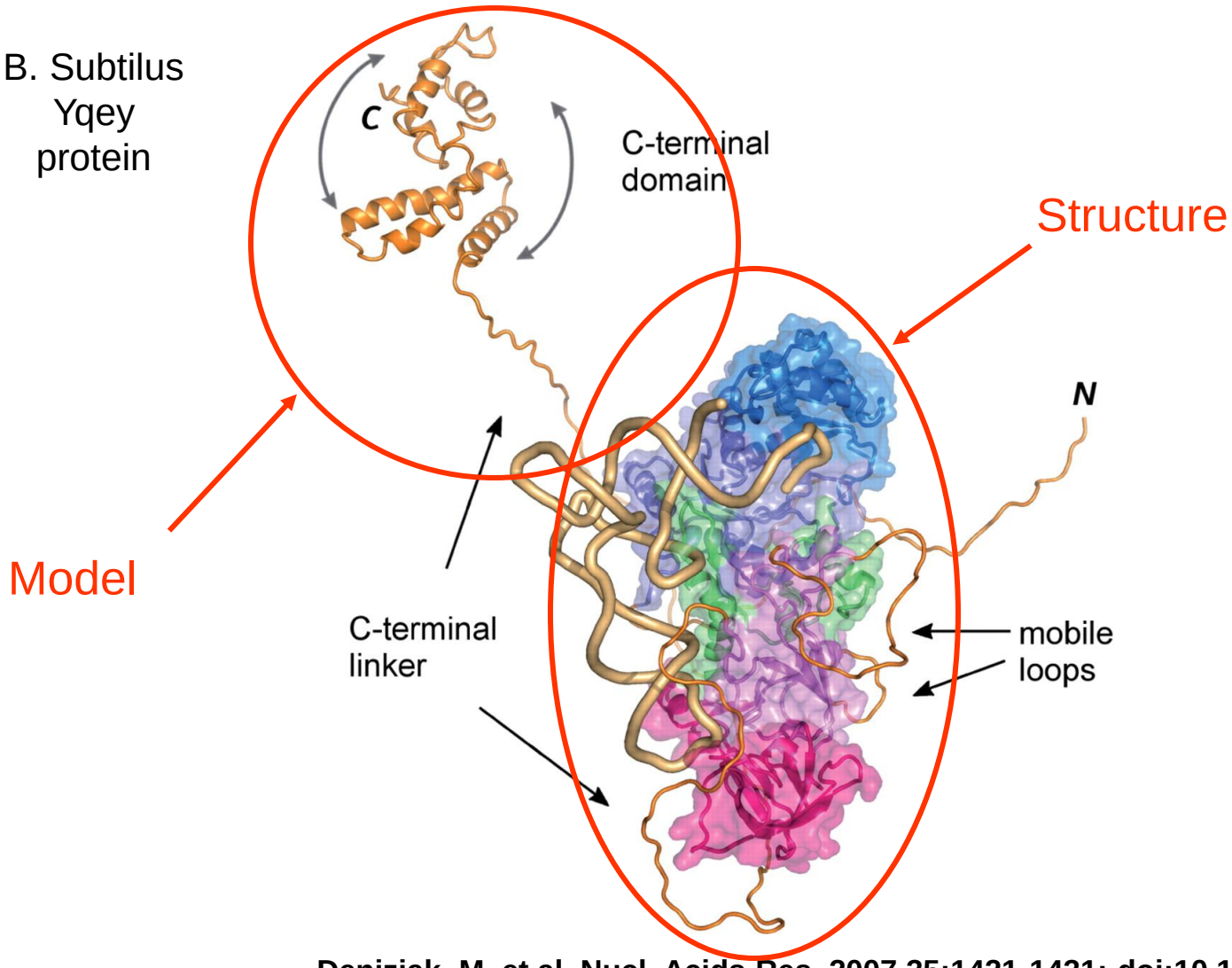


E. coli glutaminyl-tRNA synthetase with its tRNA (entry 1gtr)

These enzymes are not gentle with tRNA molecules. The enzyme firmly grips the anticodon, spreading the three bases widely apart for better recognition. At the other end, the enzyme unpairs one base at the beginning of the chain, seen curving upward here, and kinks the long acceptor end of the chain into a tight hairpin, seen here curving downward. This places the 2' hydroxyl on the last nucleotide in the active site, where ATP and the amino acid (not present in this structure) are bound.

Structures only known from *E.coli* and *D. radiodurans*

Model of D. radiodurans GlnRStRNA^{Gln} complex



Deniziak, M. et al. Nucl. Acids Res. 2007 35:1421-1431; doi:10.1093/nar/gkl1164

Nucleic Acids Research

Biology

- Yeast *Saccharomyces cerevisiae is* a well-established model system for understanding fundamental cellular processes of higher eukaryotic organisms.
- Our target today is Glutaminyl tRNA synthetase (Gln4) from yeast *Saccharomyces cerevisiae*
- Many eukaryotic tRNA synthetases like Gln4 differ from their prokaryotic homologs by the attachment of an additional domain appended to their N or C-terminus, but it is unknown how these domains contribute to tRNA synthetase function, and why they are not found in prokaryotes
- The 228 amino acid N-terminal domain of Gln4 is among the best studied of these domains, but is structurally uncharacterized.
- The N-terminal domain appears to have non specific RNA binding.
- The role of a nonspecific RNA binding domain in the function of a highly specific RNA binding enzyme is baffling, but clearly crucial given its prevalence among tRNA

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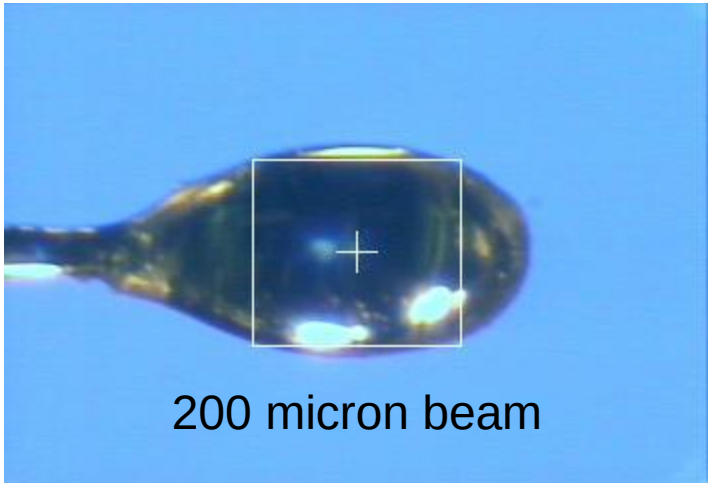
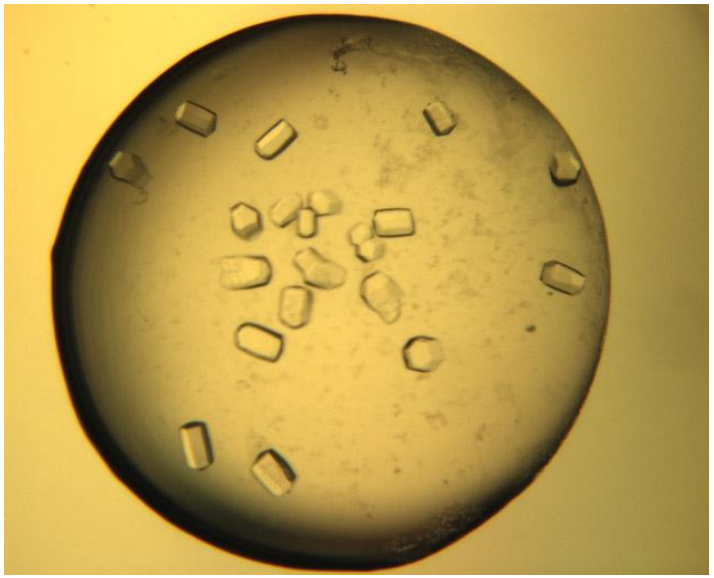
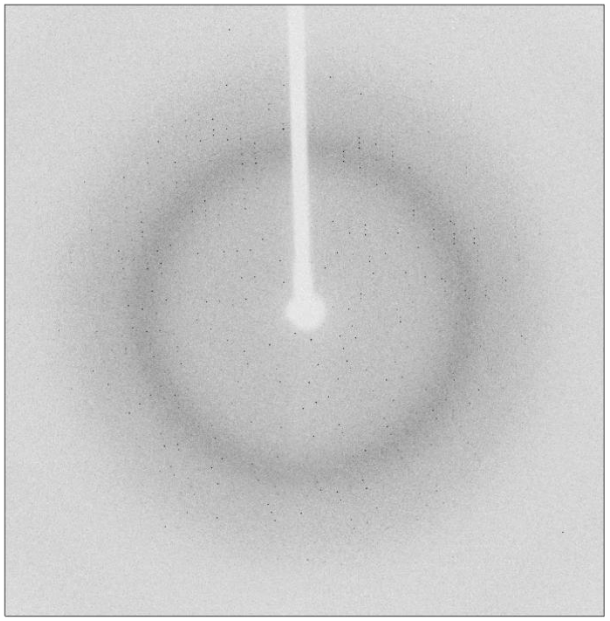
Crystallization/Data collection

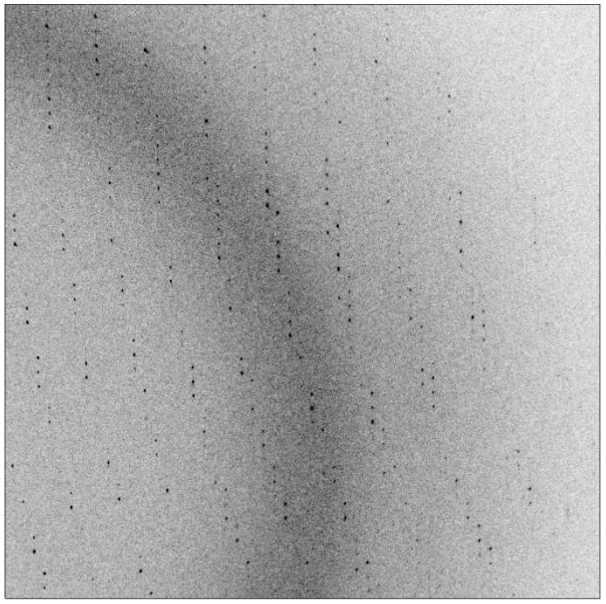
- Gln4 Screened against 1536 different biochemical conditions, ~1000 forming an incomplete factorial of chemical space and ~500 representing commercially available screens.
- Crystal leads seen, several were chosen based on ease of cryoprotection of the native hit.
- Crystals were optimized with a Drop Volume Ratio versus Temperature (DVR/T) technique.
- Cryoprotected and ‘drop’ shipped to SSRL by FedEx.
- Only 2 structures for related glutaminyl tRNA synthetases are available (~40% sequence homology), we had 228 extra residues (almost 40% more residues) therefore we expected problems in molecular replacement and didn’t have a SeMet example.
- EXAFS data indicate Zinc present in the *E. coli.* Case (not seen in the X-ray structure). The zinc acts to stabilize the structure in a pseudo zinc finger motif.
- We collected data remotely with an excitation scan to determine if Zinc was present.
- It was!





80% PEG 400 in the crystallization cocktail

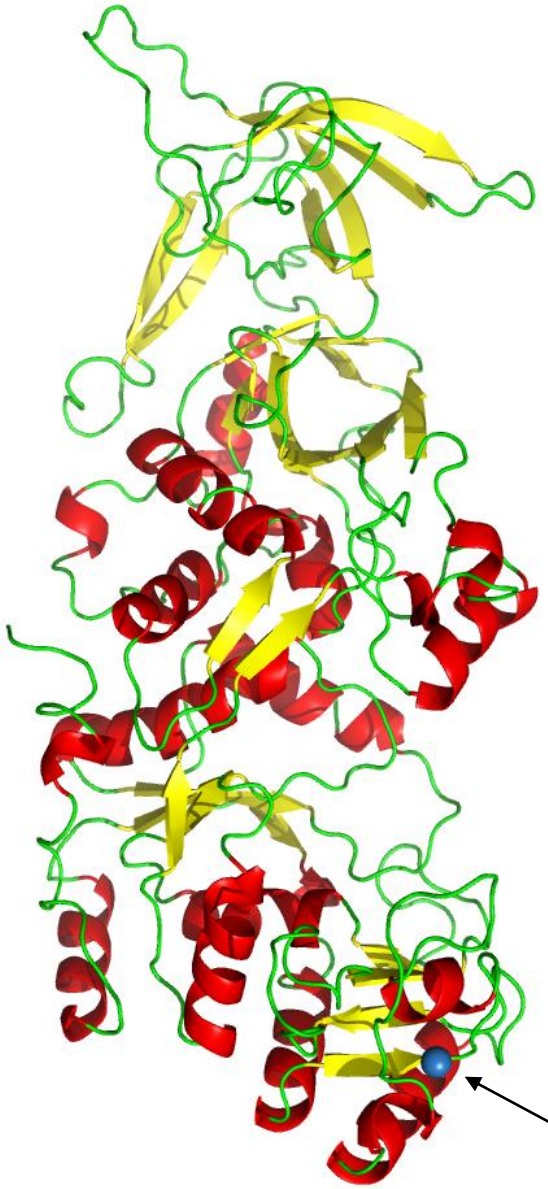




Data collection/Processing

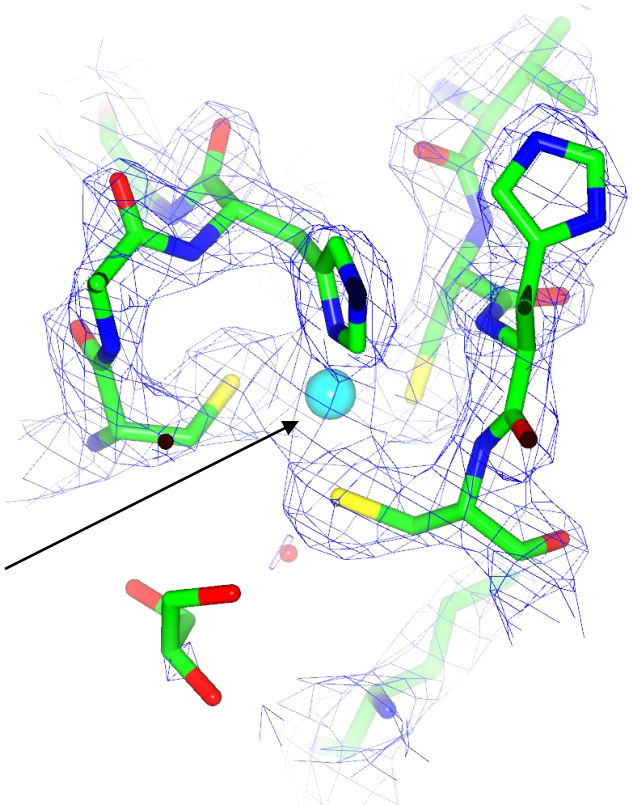
- We used beamline 11-1 at SSRL with a Mar 325 CCD detector, 340 mm crystal to detector distance.
- We collected 200˚ of data, 0.4˚ per frame, 500 images, 3.7s per frame, wavelength 1.169 Å (as close as we could get to Zinc on the beamline used) (deliberately high redundancy for the anomalous signal).
- We indexed in P3121, a=b=176.75 Å, c=72.22 Å, α=β=90, gamma=120˚

	Overall	Inner Shell	Outer Shell
Low resolution limit (Å)	40.00	40.00	2.64
High resolution limit (Å)	2.5	7.91	2.5
R_{merge}	0.104	0.036	0.743
R_{pim}	0.032	0.011	0.273
	3.2%	1.1%	27.3%
Total number of observations	508484	17694	51511
Total number unique	44752	1523	6332
Mean((I)/sd(I))	24.6	86.6	2.2
Completeness (%)	99.7	99.9	97.9
Multiplicity	11.4	11.6	8.1

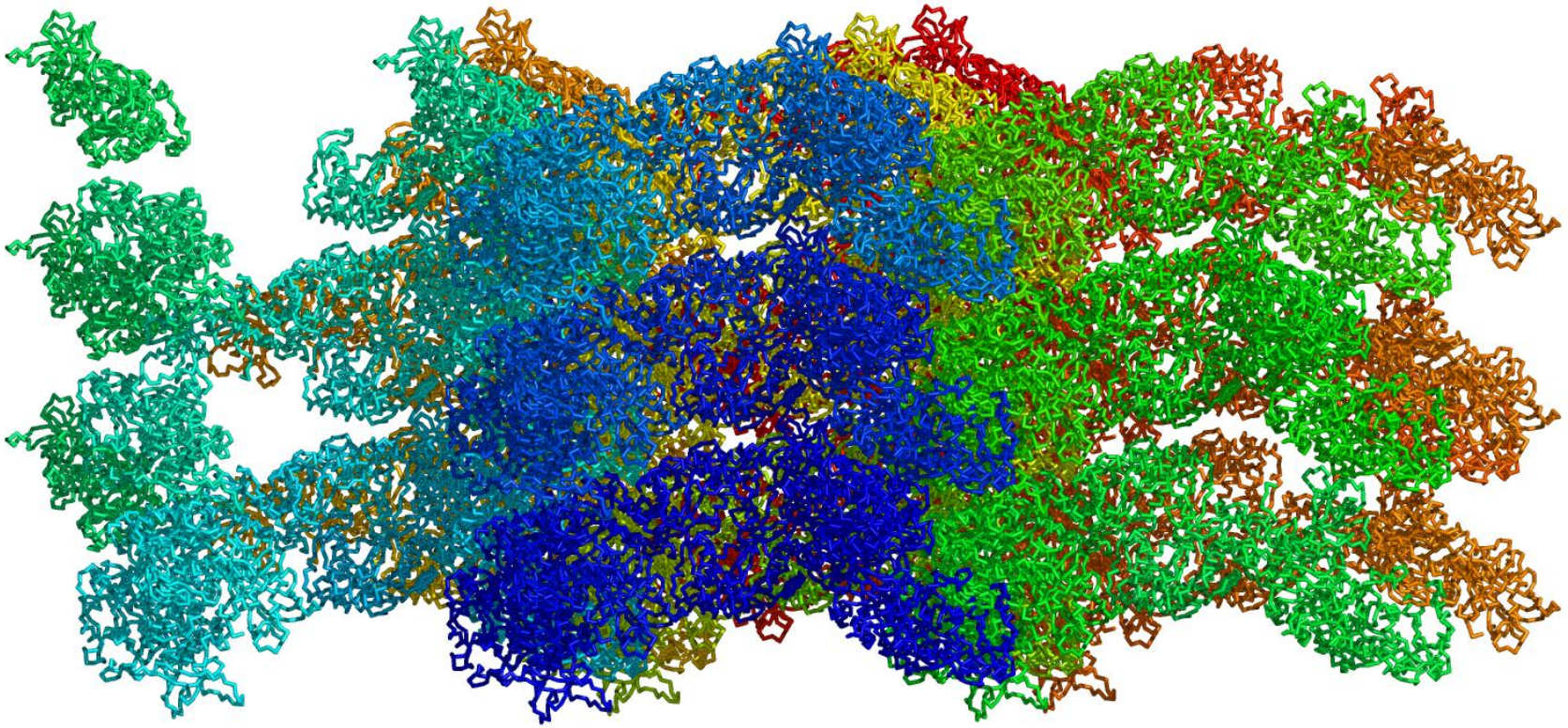


Structure solved (with help of the zinc signal) and refined with Phenix.

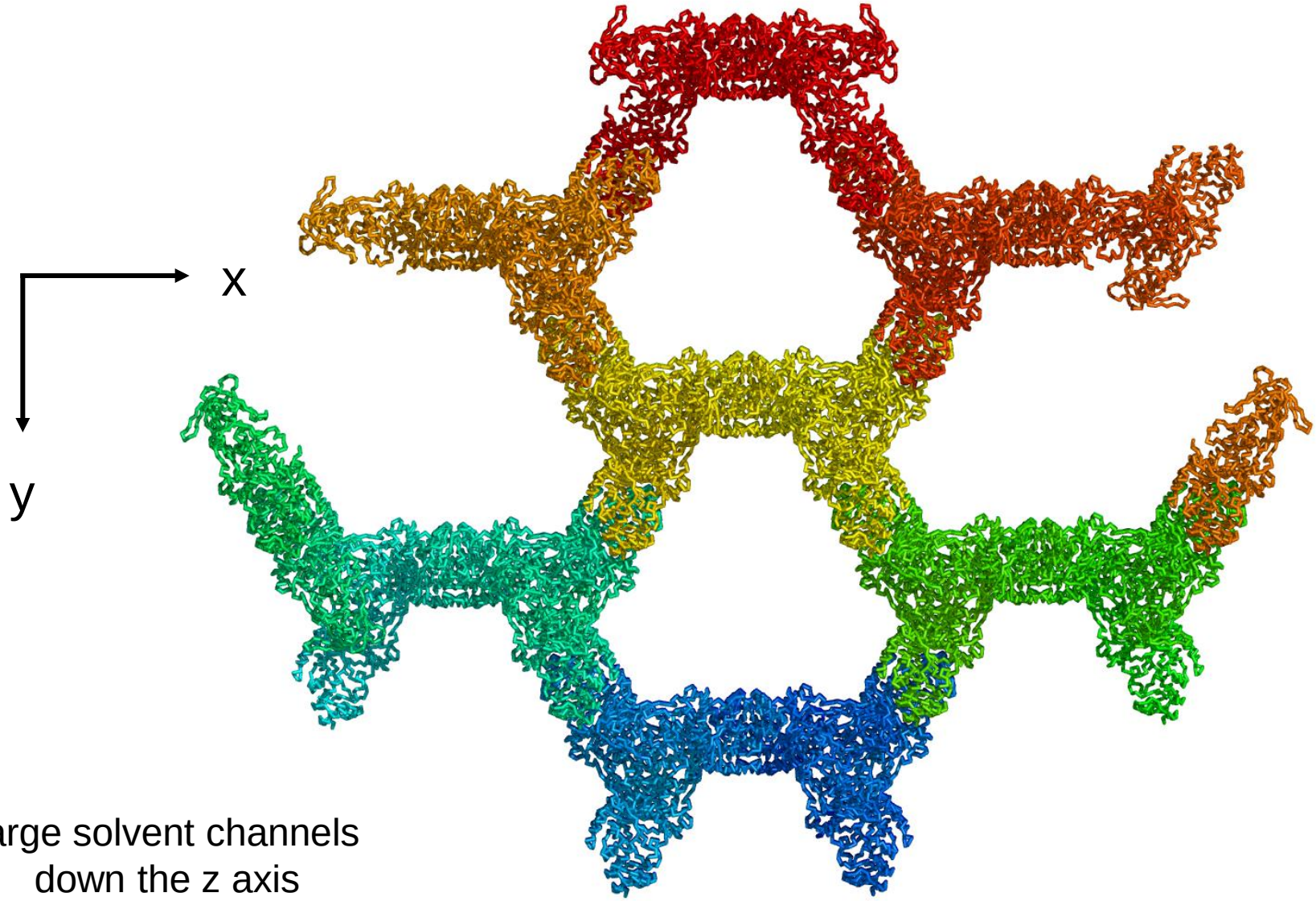
Overall R and R_{free} are 15.9 and 21.1% respectively.



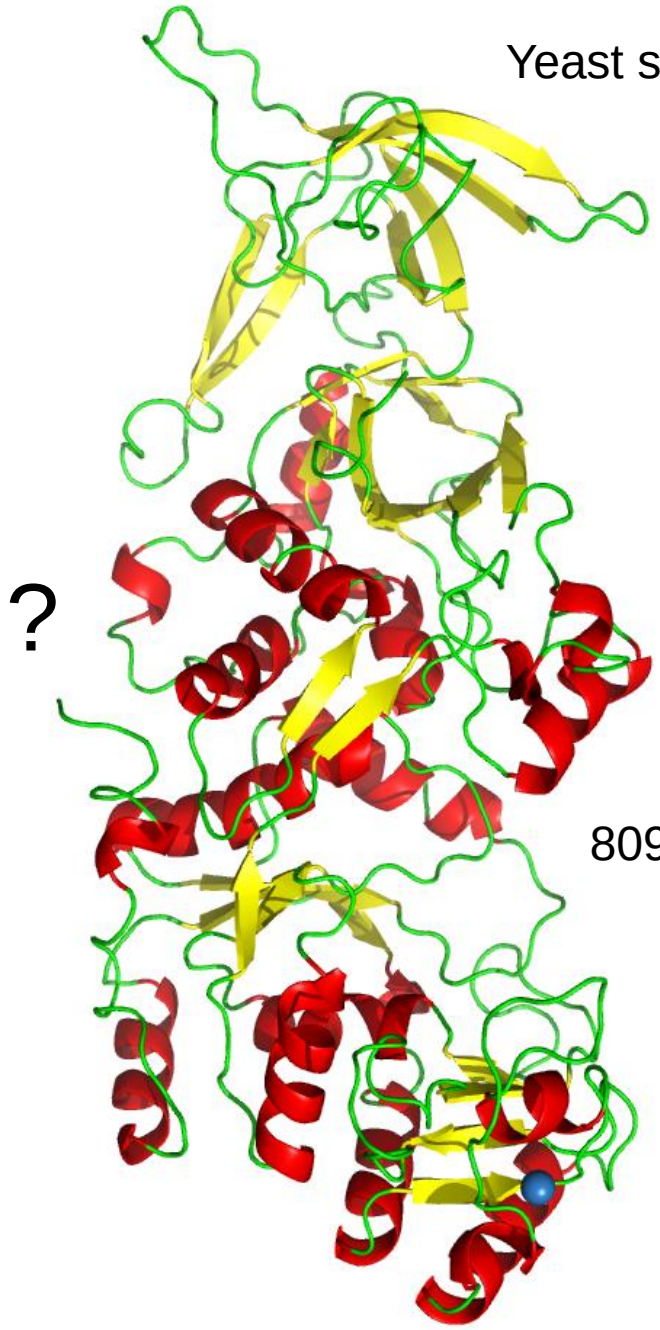
Zinc



Tight packing in z and y



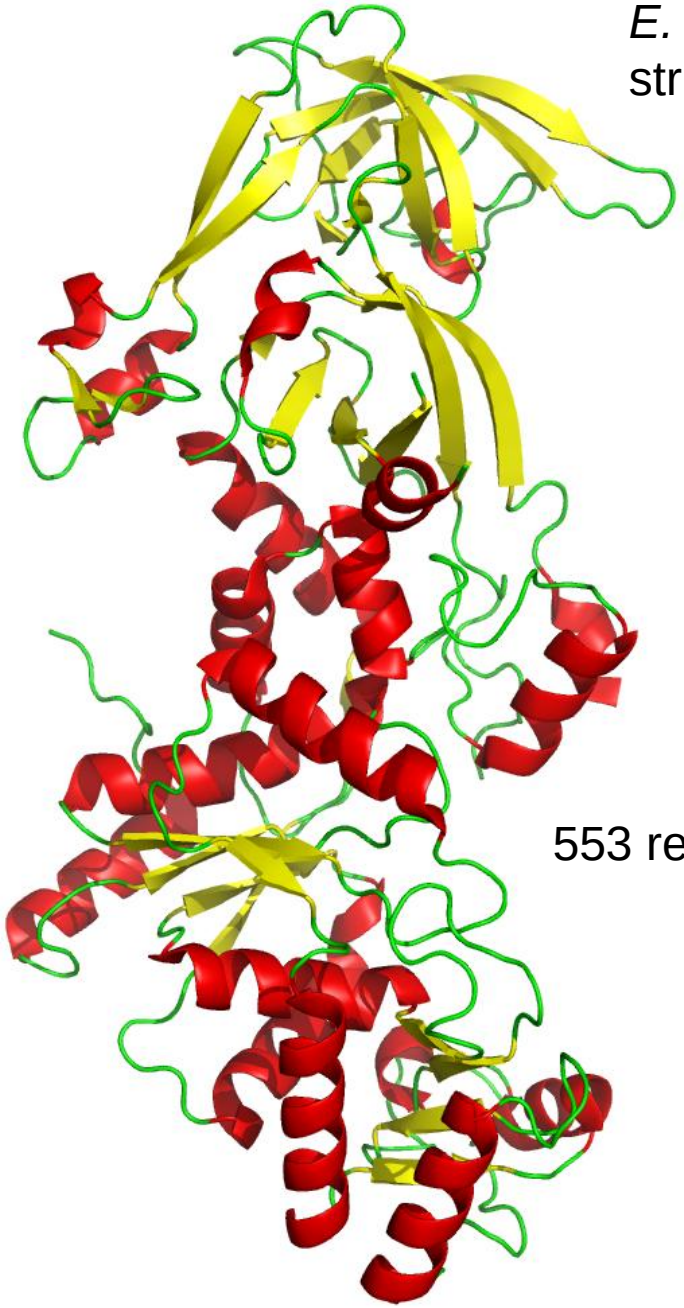
Large solvent channels
down the z axis



Yeast structure

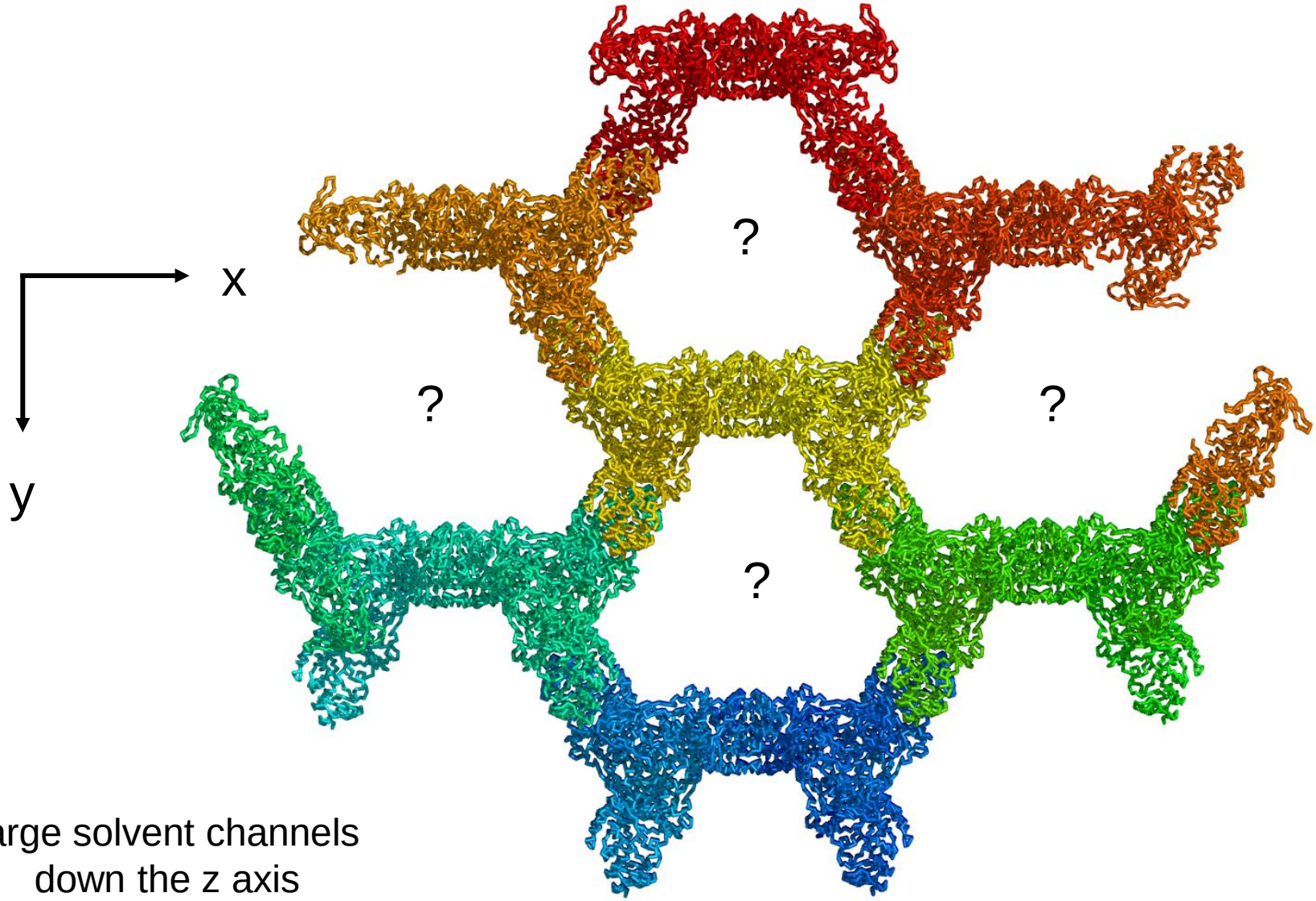
?

809 residues



E. coli. structure

553 residues



Large solvent channels
down the z axis

Missing residues

- There were 216 missing residues from the structure, 95% of the N-terminal domain.
- Where they in the mix to start with?.
- SDS PAGE gel on the remaining crystals indicated that the full length protein was present.
- For a more concrete answer the protein was re-expressed with a His tag attached to the N-terminal domain.
  - It was purified with a nickel affinity column.
  - It was crystallized and the structure solved, again with missing residues.
  - A western blot on the dissolved crystals confirmed the presence of the N-terminal domain His tag.
  - No protein degradation had taken place during crystallization.
- For the re-expressed protein the full N-terminal domain was present in the protein but not seen in the crystallographic structure.

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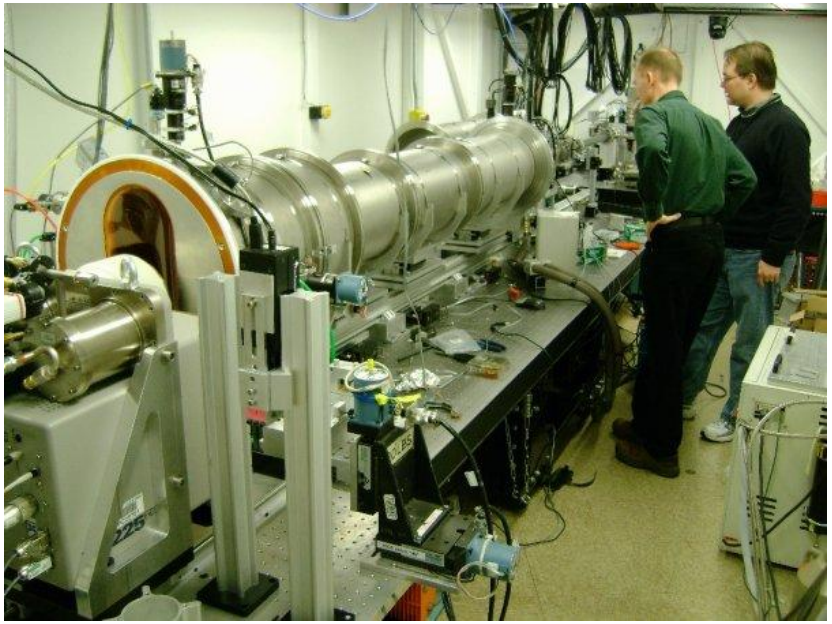
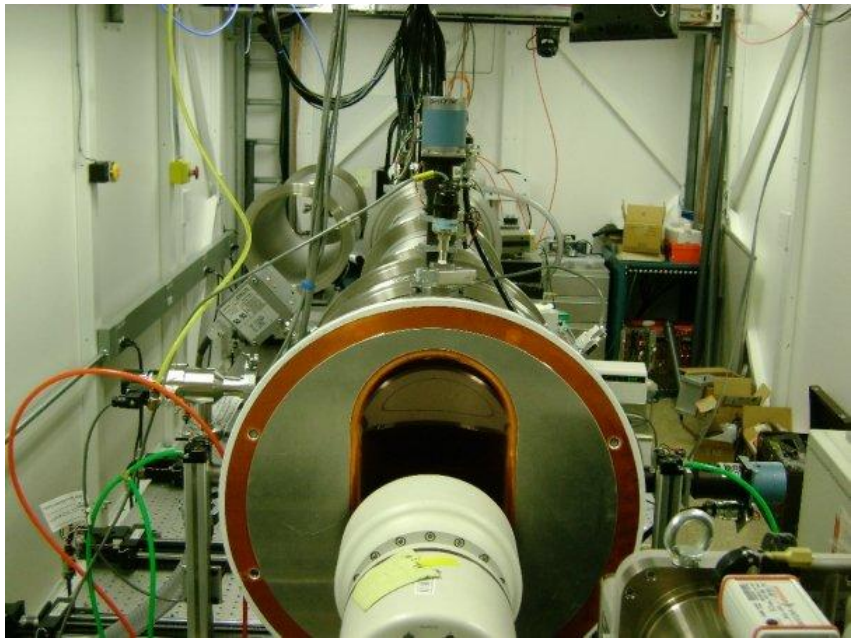
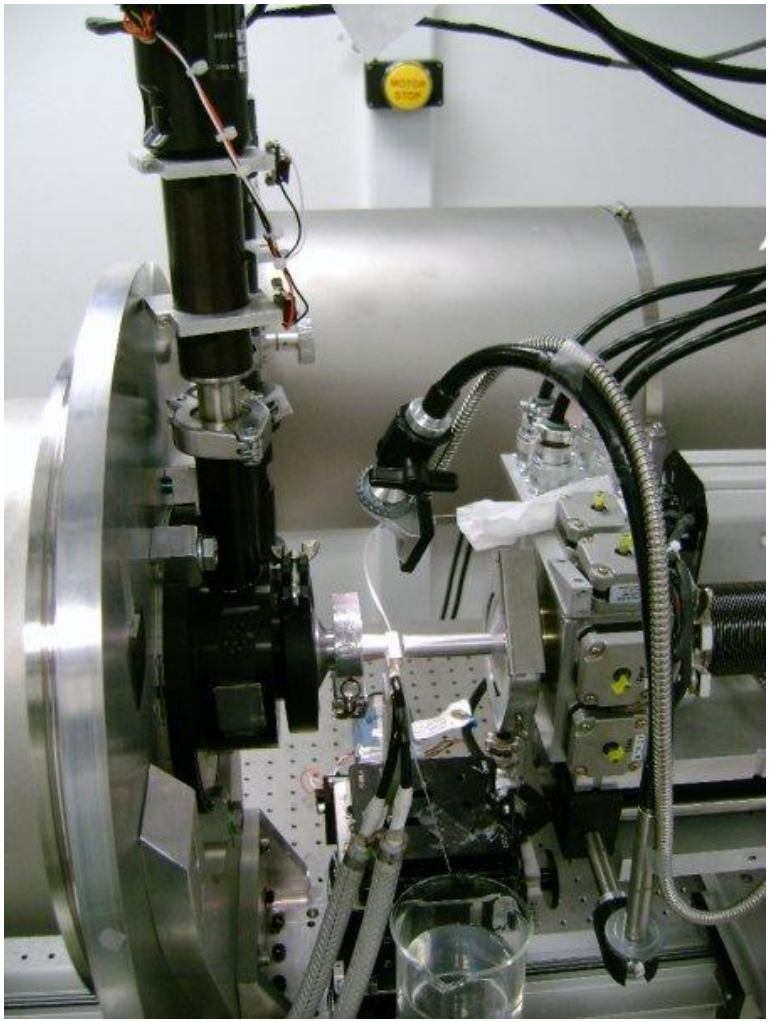
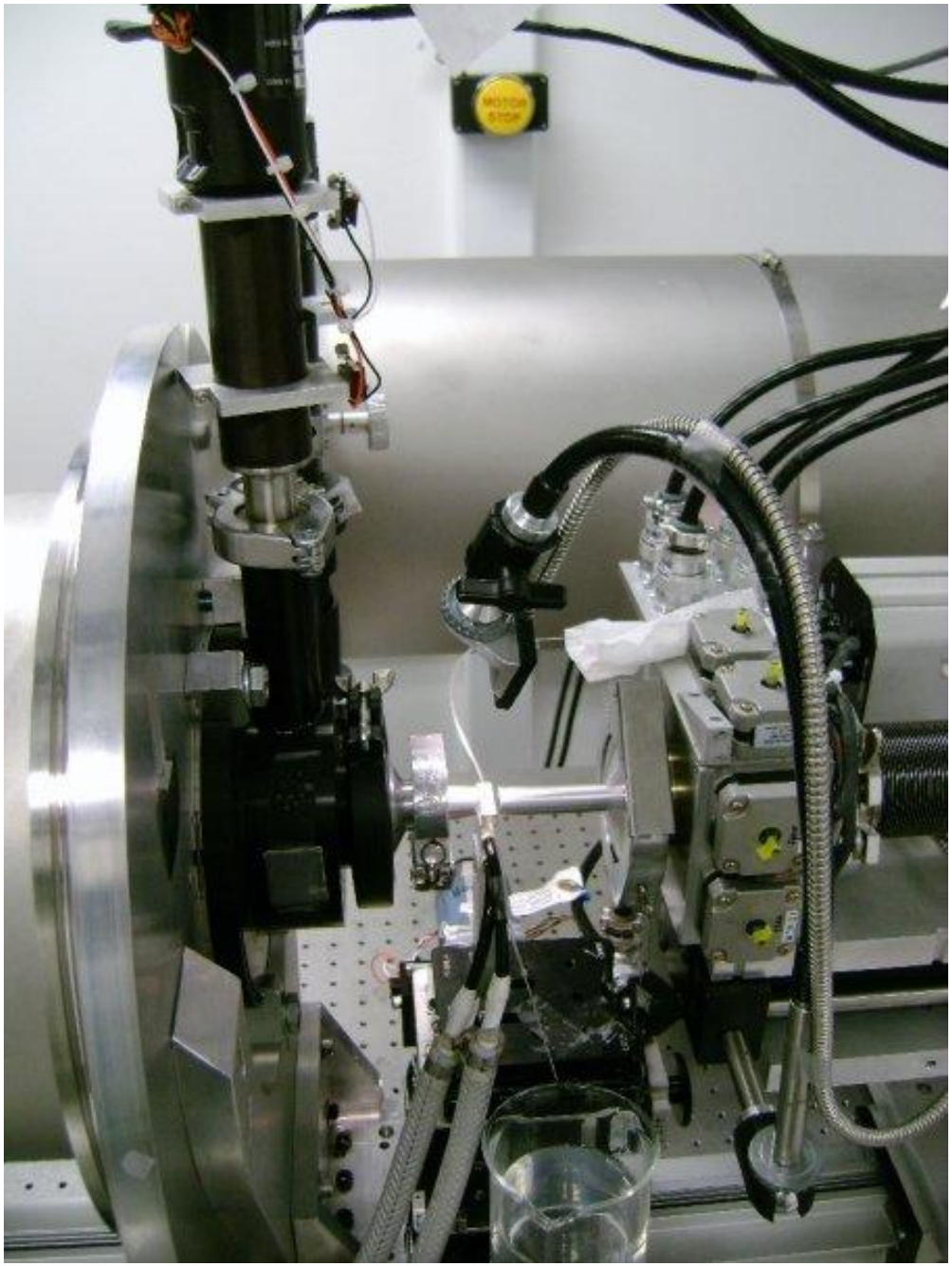






SSRL Beamline 4-2 (nice restaurants, San Francisco, Yosemite, wine, the Jelly Belly factory etc.)



Initial protocol

5 concentrations

Start with buffer then lowest concentration first

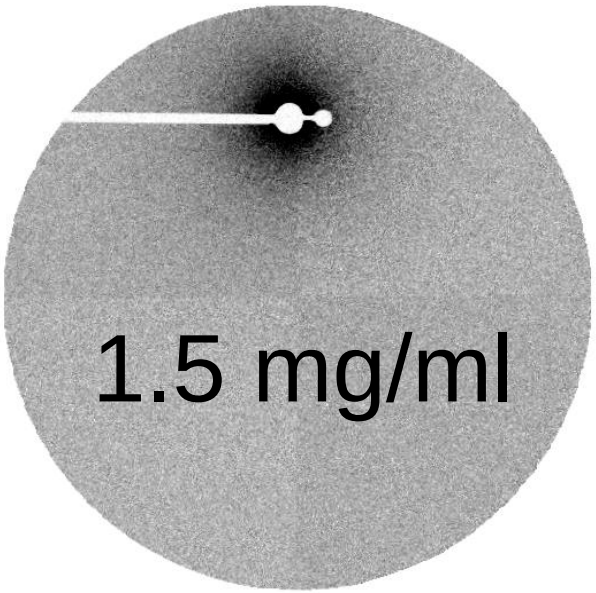
Using the lowest first means that residual protein in the capillary would not alter the assumed concentration greatly.

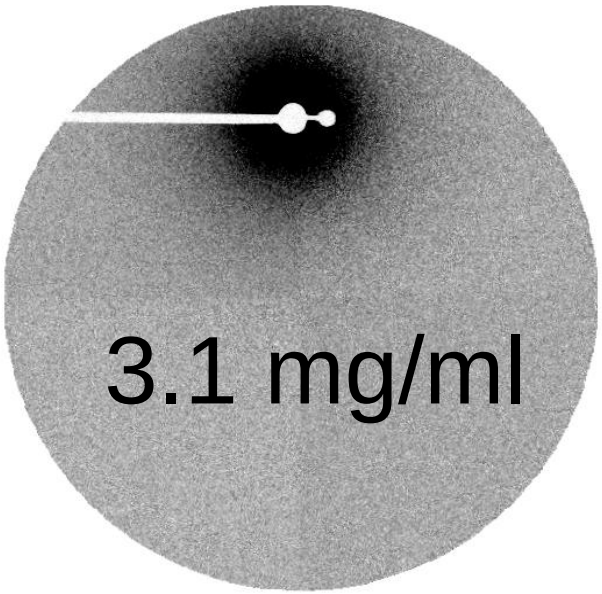
Up to 12 exposures, 1.5s each.

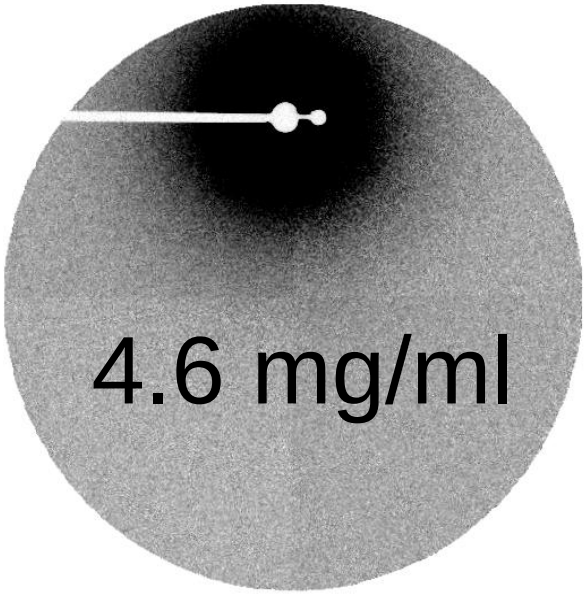
Load next concentrations and repeat.

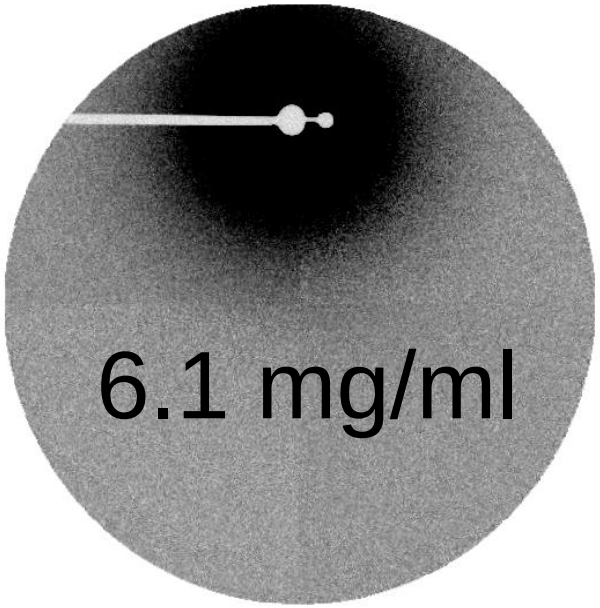
Repeat the buffer.

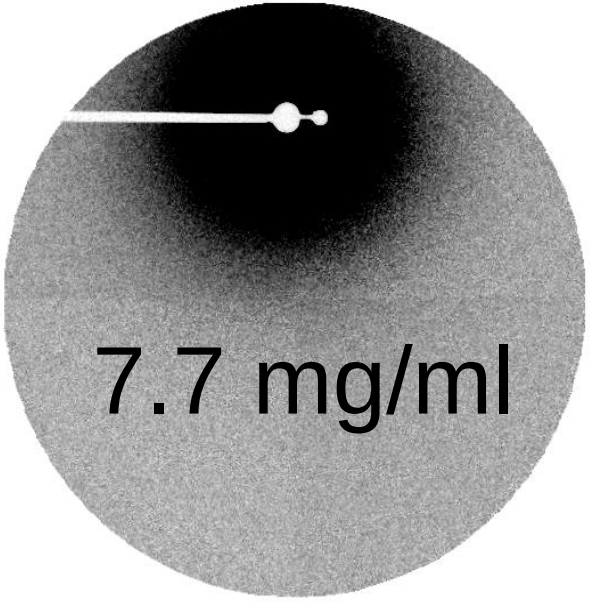
Clean the capillary with bleach followed by water.

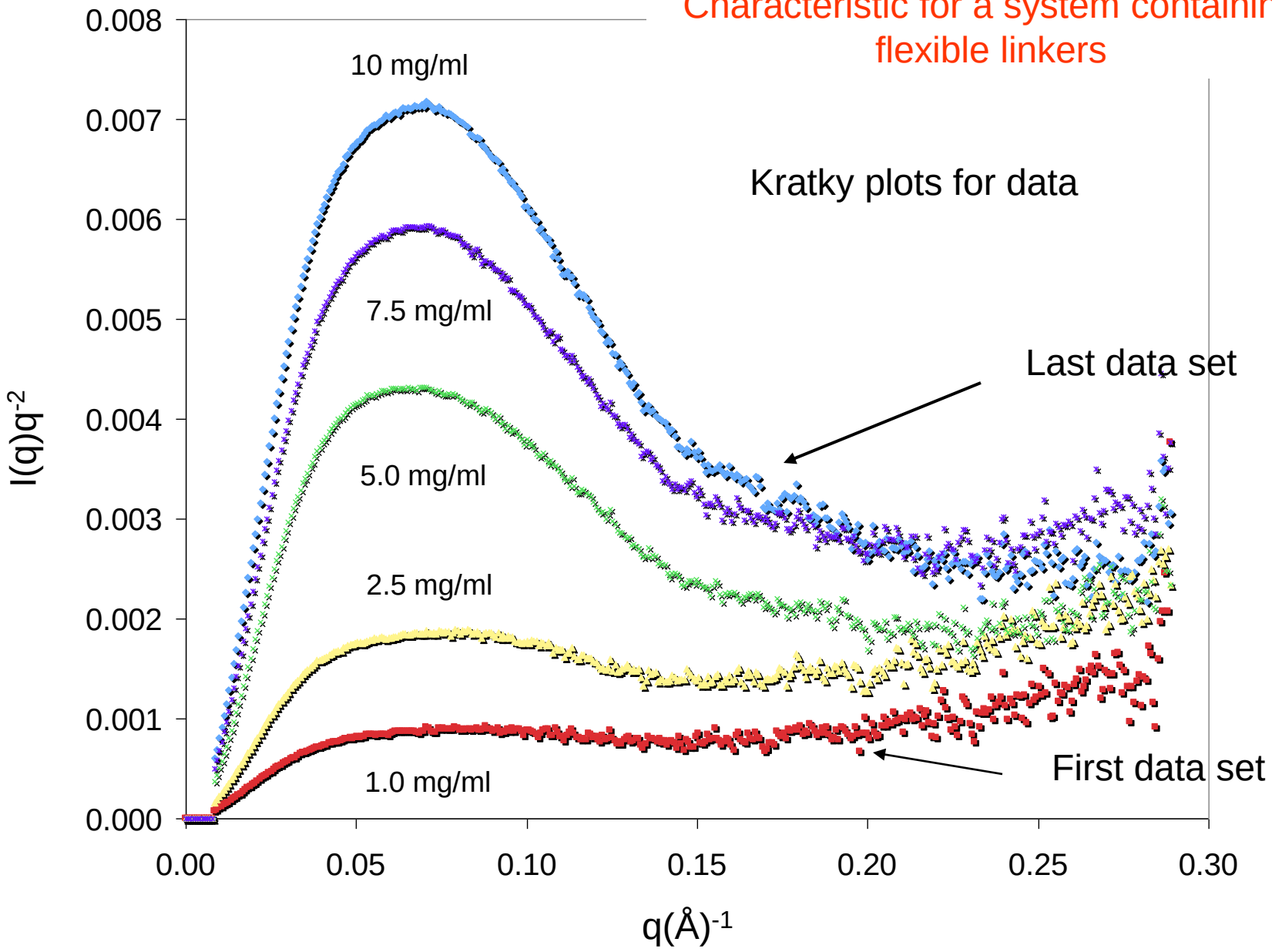

1.5 mg/ml


3.1 mg/ml

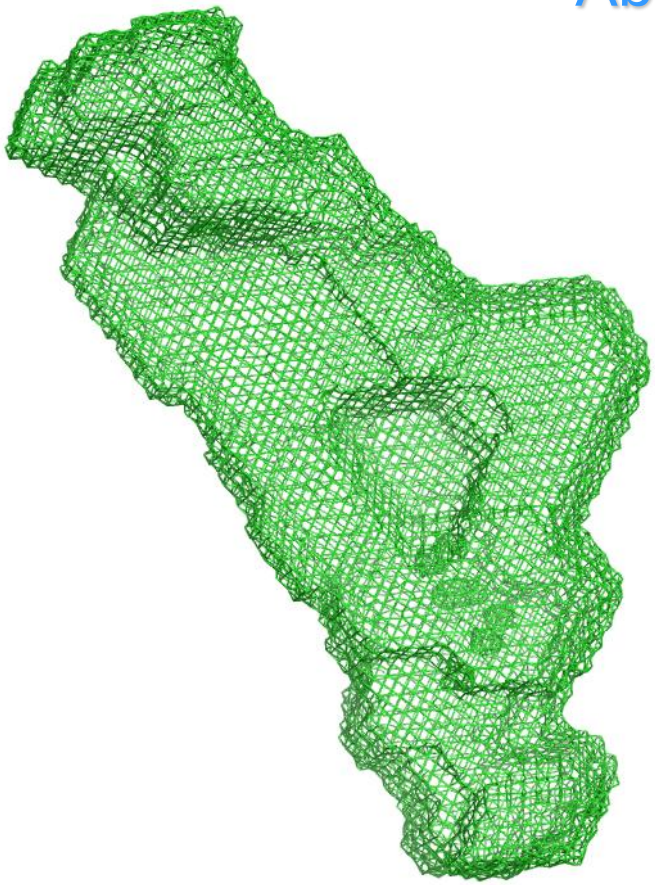

4.6 mg/ml


6.1 mg/ml

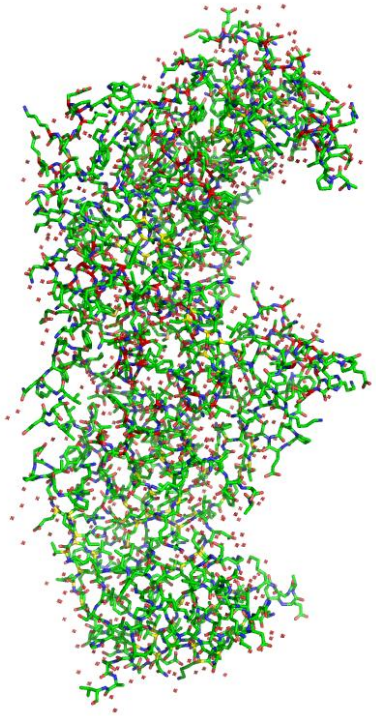

7.7 mg/ml


Characteristic for a system containing flexible linkers
Kratky plots for data
10 mg/ml
7.5 mg/ml
5.0 mg/ml
2.5 mg/ml
1.0 mg/ml
Last data set
First data set
I(q)q-2
q(Å)-1
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.00
0.05
0.10
0.15
0.20
0.25
0.30

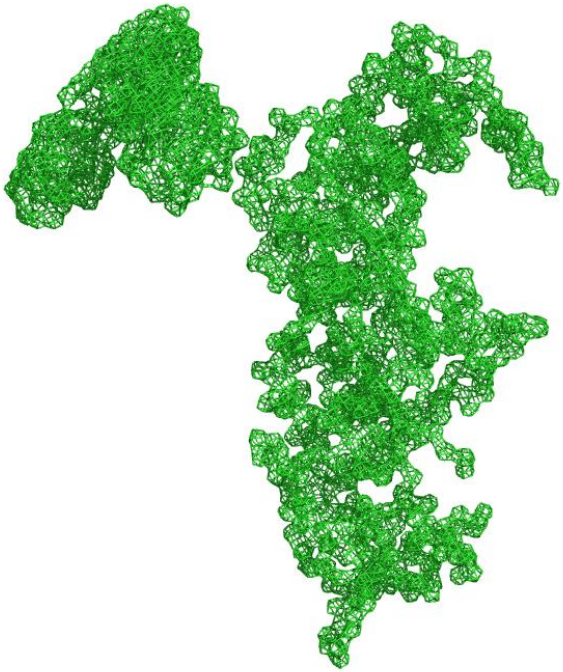
Ab initio reconstruction



Completely *ab initio*

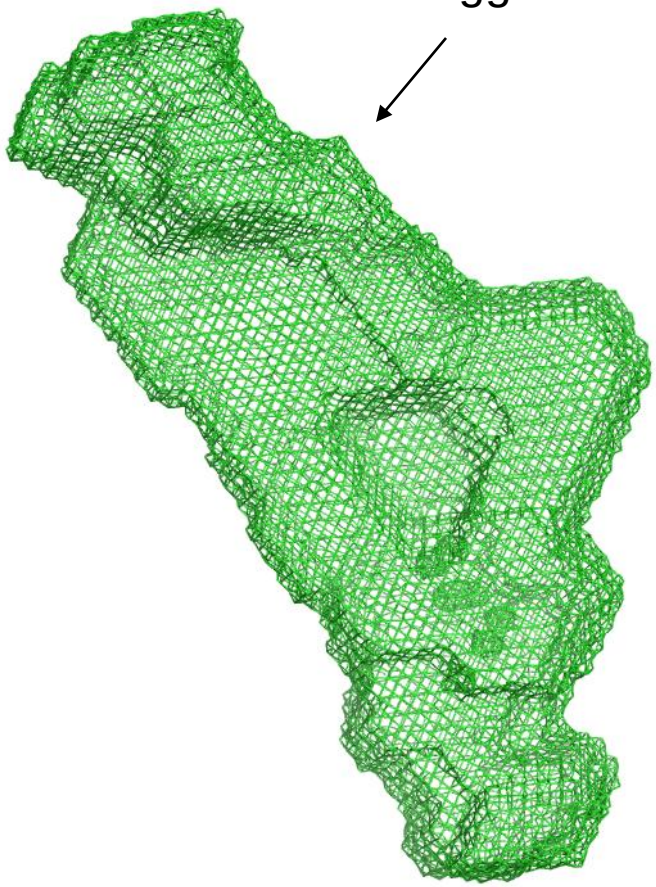


The crystal structure

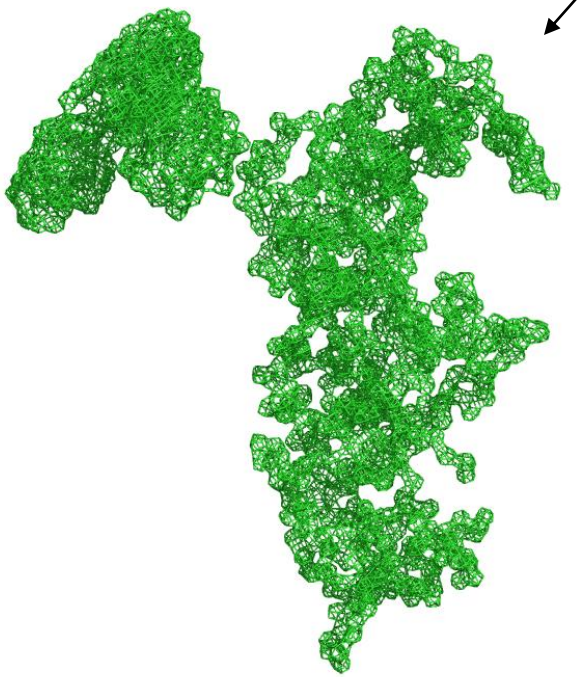


SAXS with the crystal structure

Suggests multiple conformations



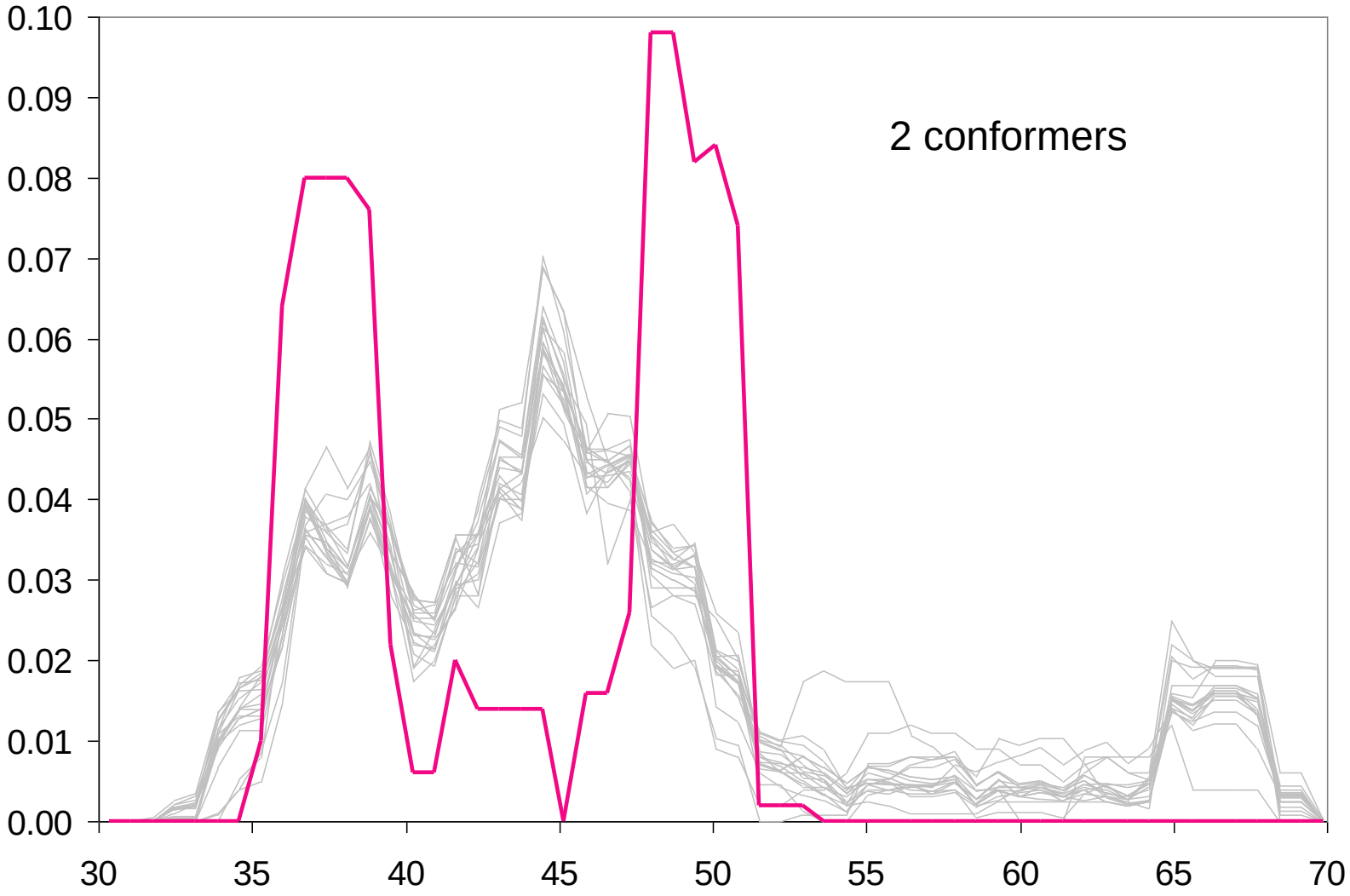
With this being the average

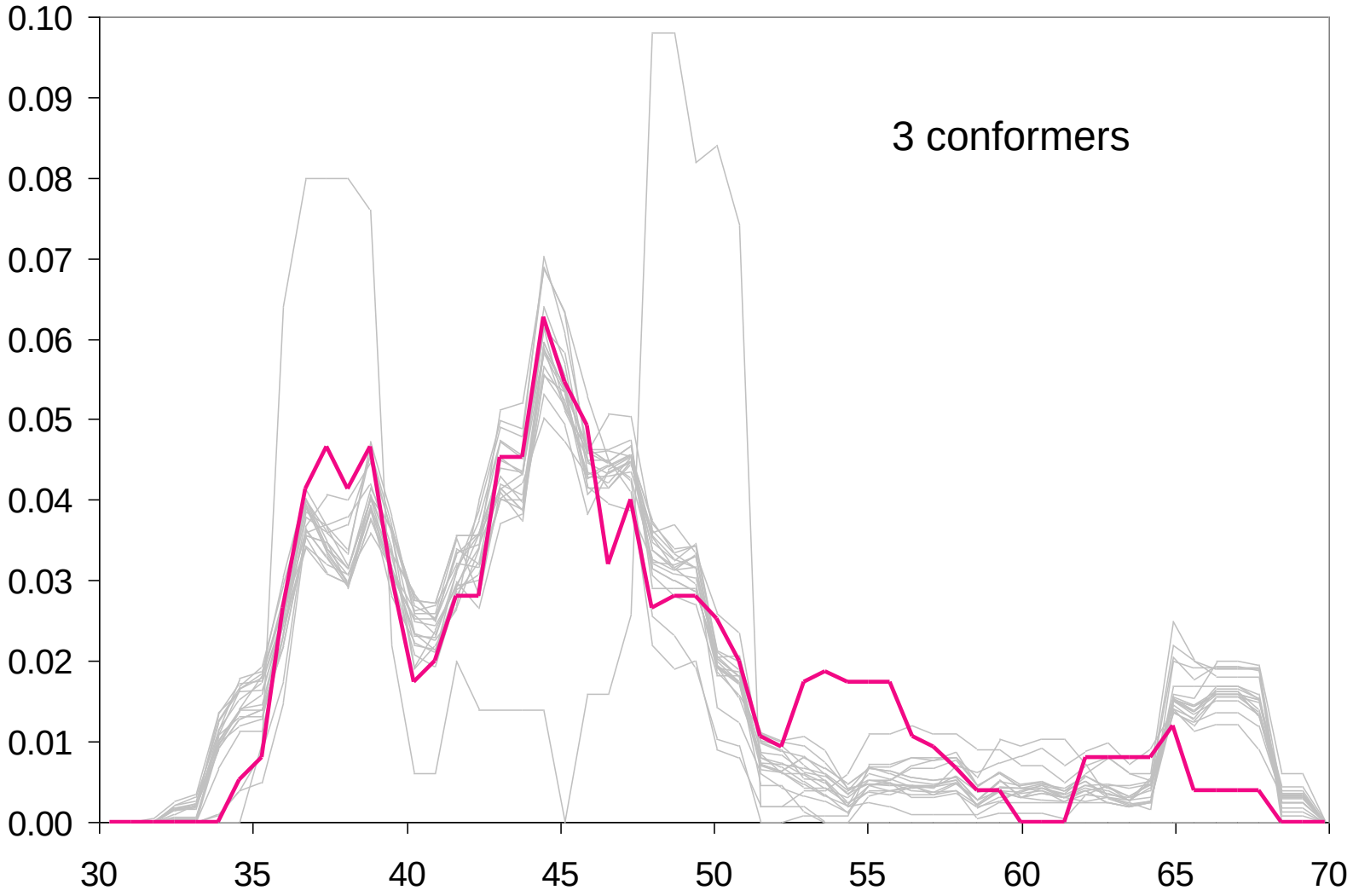


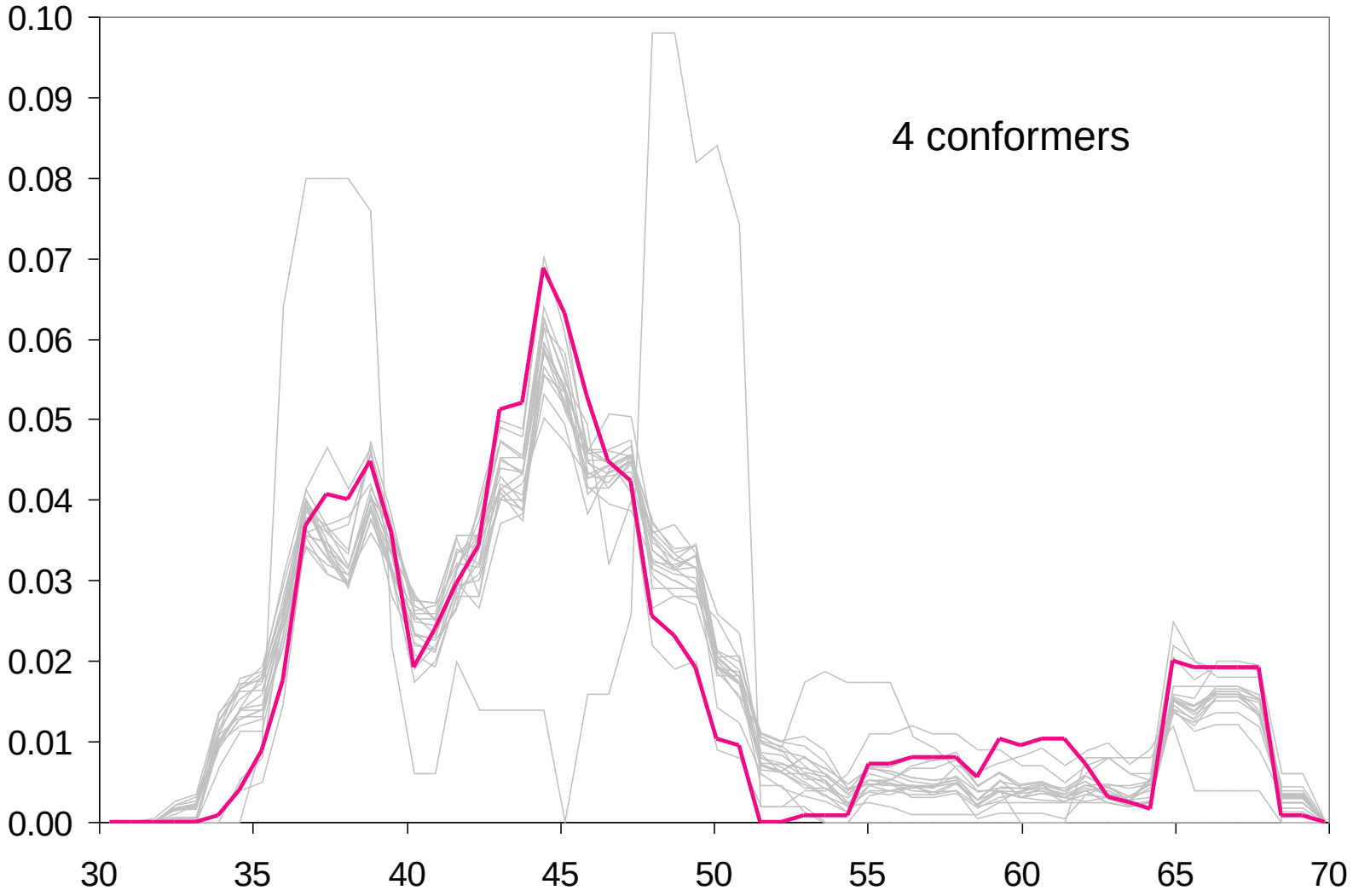
Ensemble optimization

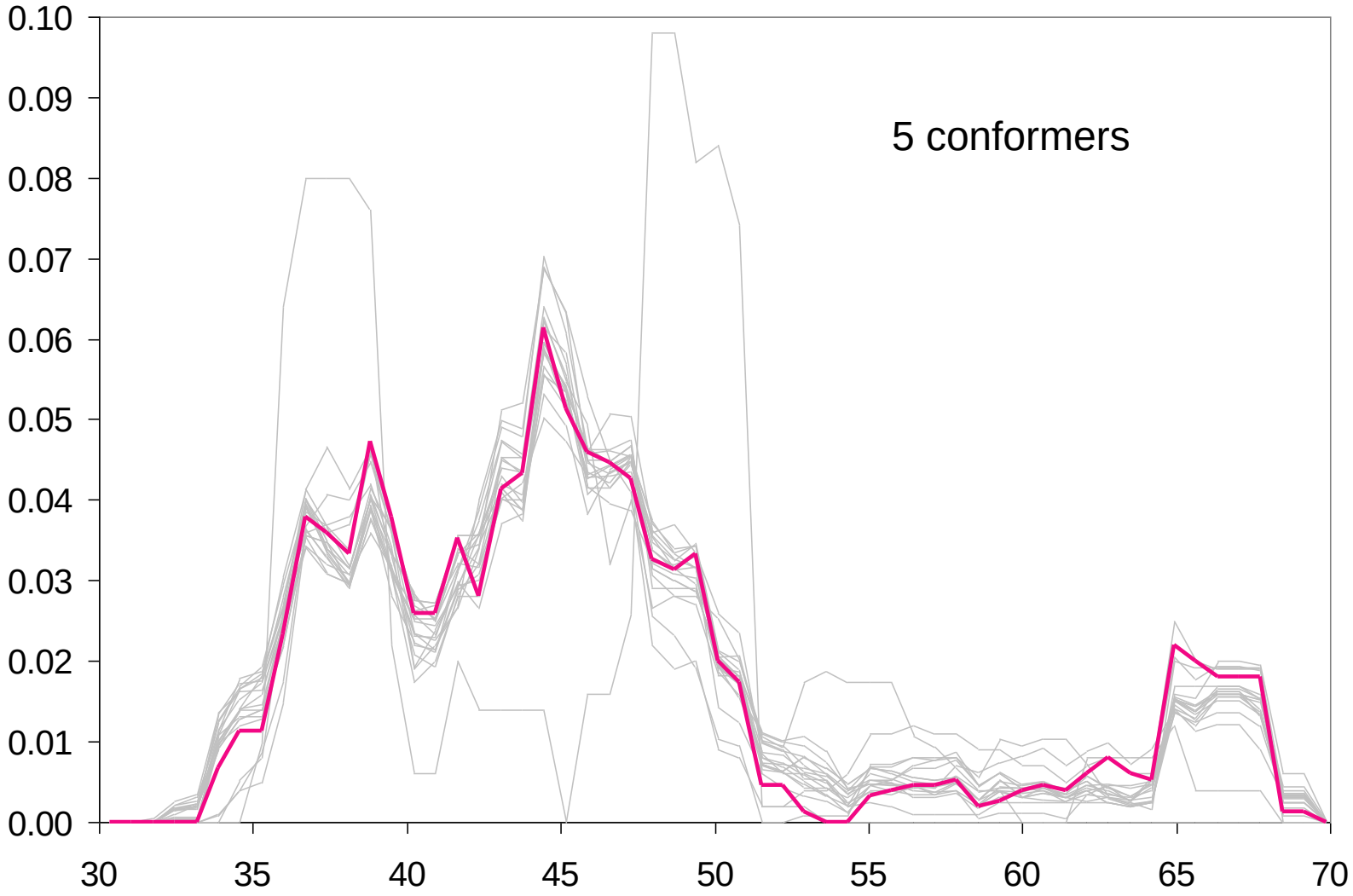
- The Ensemble Optimization Method (EOM) was used to assess the flexibility of the Gln4 N-terminal domain.
- RanCh (**Ran**dom **Ch**ain Generator) generated 10,000 conformers of the N-terminal sequence of Gln4 covering all possible configuration space.
- Sets of these conformers were binned to create ensembles.
- GAJOE (**G**enetic **A**lgorithm **J**udging **O**ptimization of **E**nsembles) optimized the ensembles by comparing the average scattering profile of their conformers to the experimental data.
- Plotting the *Rg* distribution for successive runs, each using an increasing number of conformers per ensemble, allows us to identify the optimal number of conformers that most accurately characterizes the system.
- Analysis of chi (an error indicator) shows an systematic decrease, converging at eight conformers in each ensemble.

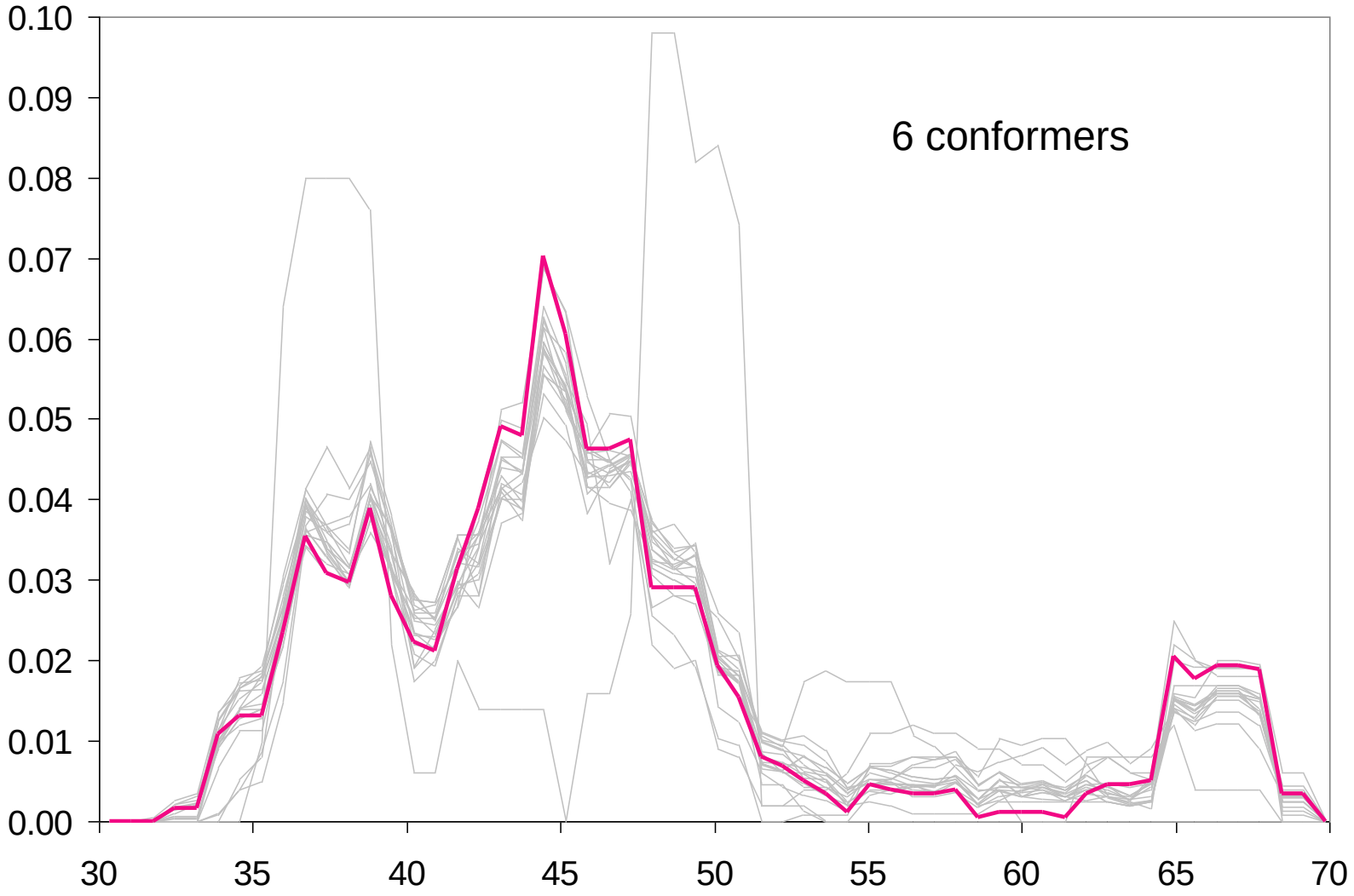
The convergence of the population distribution on distinct populations indicates that dynamic motion or different species are present - when this is not the case the distribution is monomodal (confirmed by similar analyses on static systems).

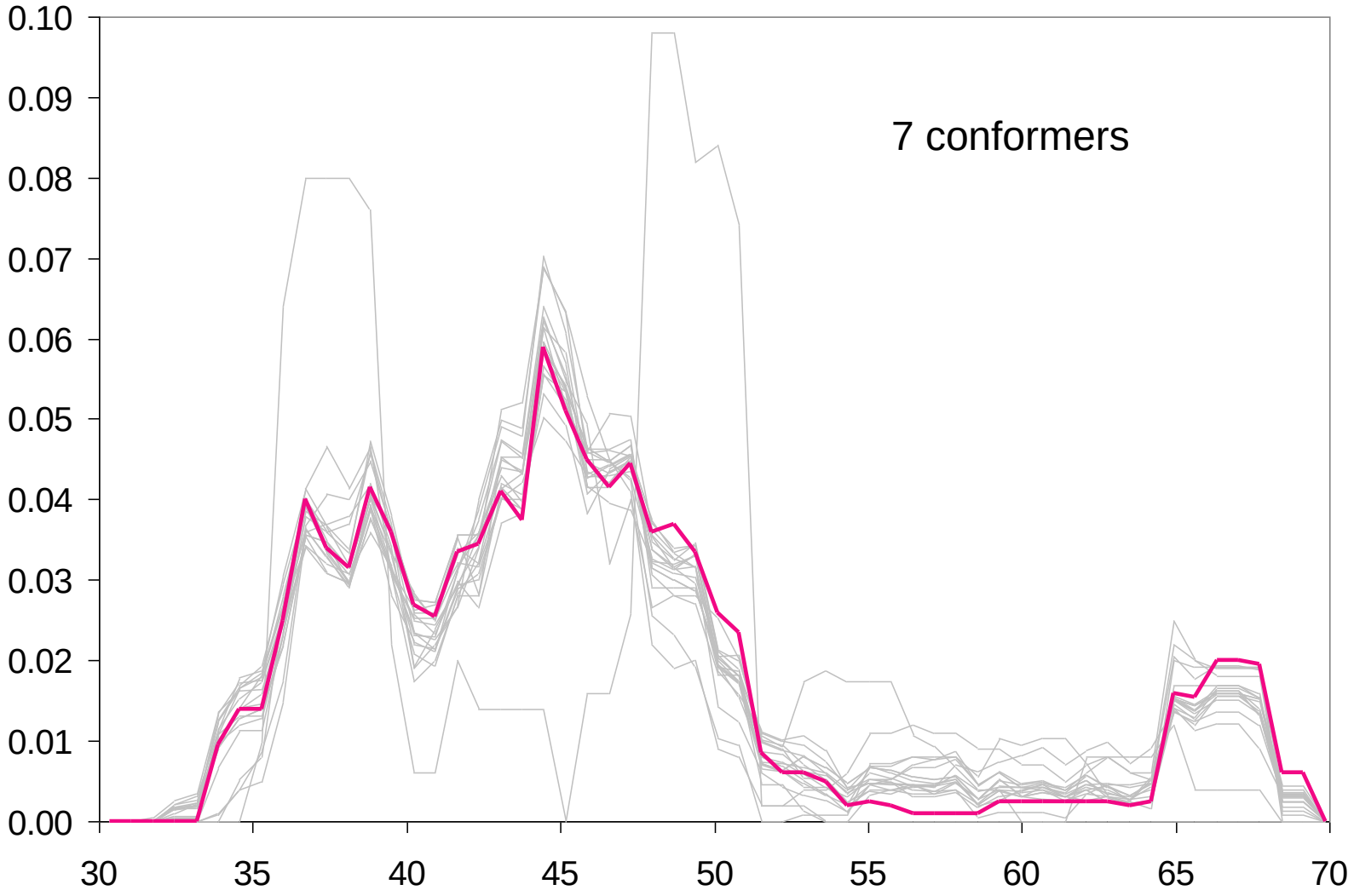

0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70
2 conformers

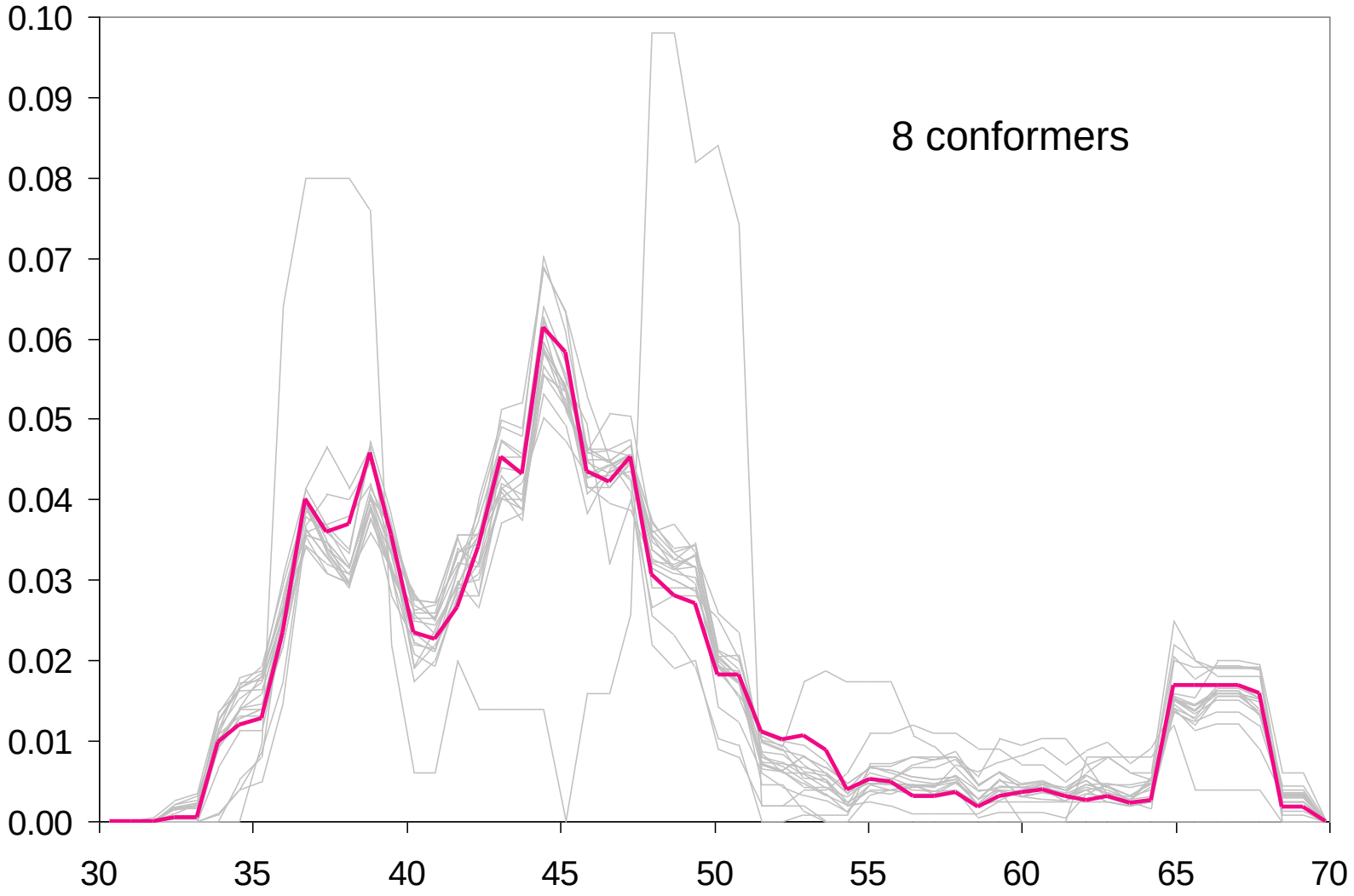

0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70
3 conformers

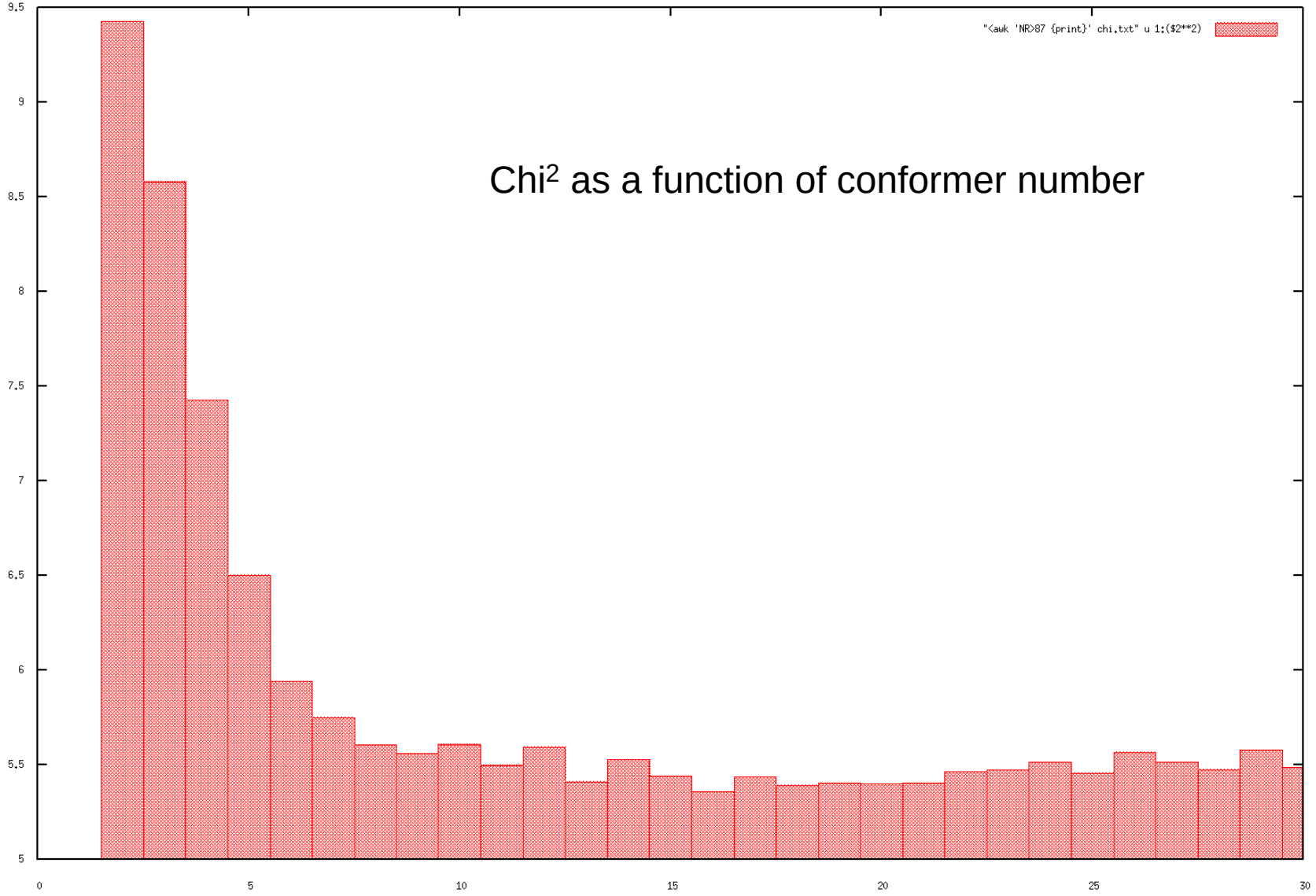

4 conformers
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70


5 conformers
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70


6 conformers
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70

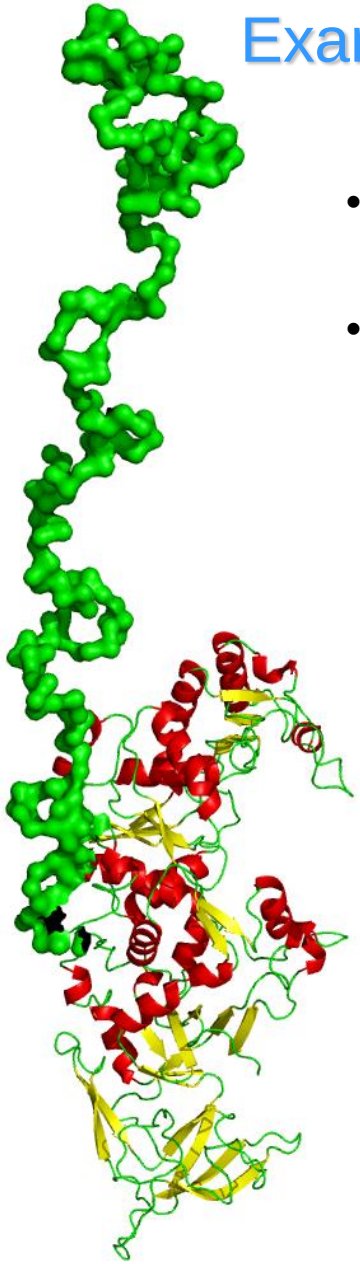

7 conformers
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70

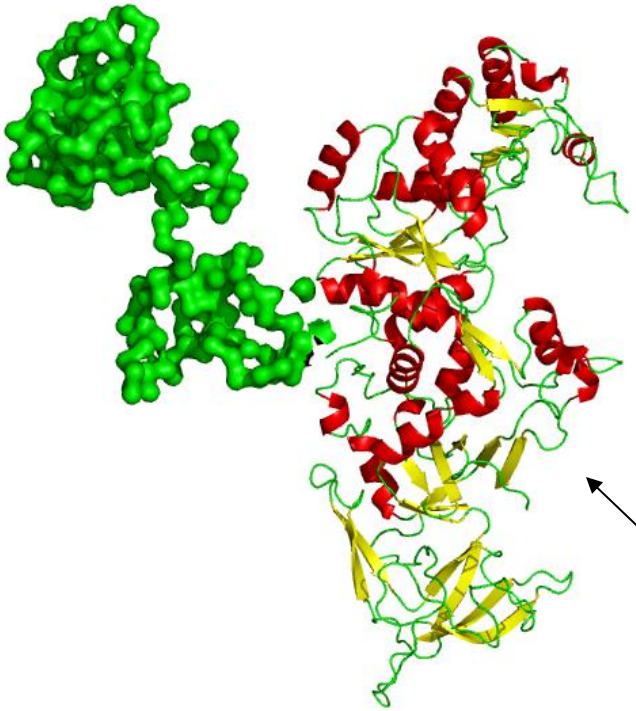

8 conformers
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
30
35
40
45
50
55
60
65
70

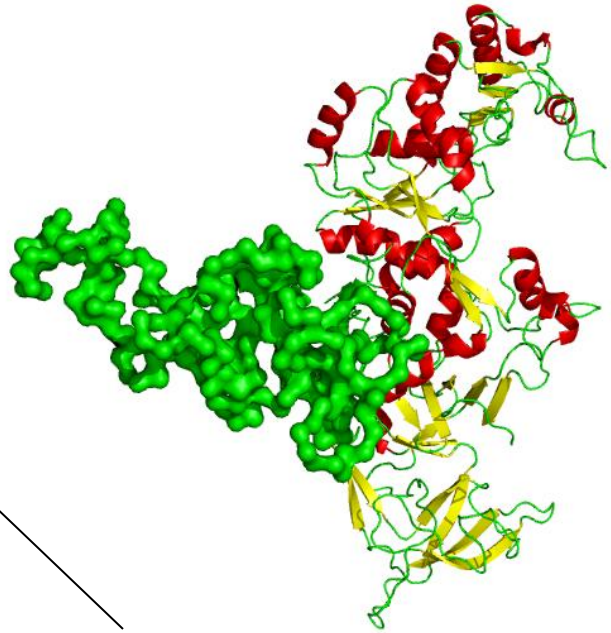

"<awk 'NR>87 {print}' chi.txt" u 1:($2**2)
Chi2 as a function of conformer number
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
0
5
10
15
20
25
30

Example Conformers from the Ensemble optimization

- Ensemble optimization told us that the SAXS data could be best explained with a minimum of 8 different conformers.
- The single *ab initio* model produced by traditional techniques represents the average conformation in solution.







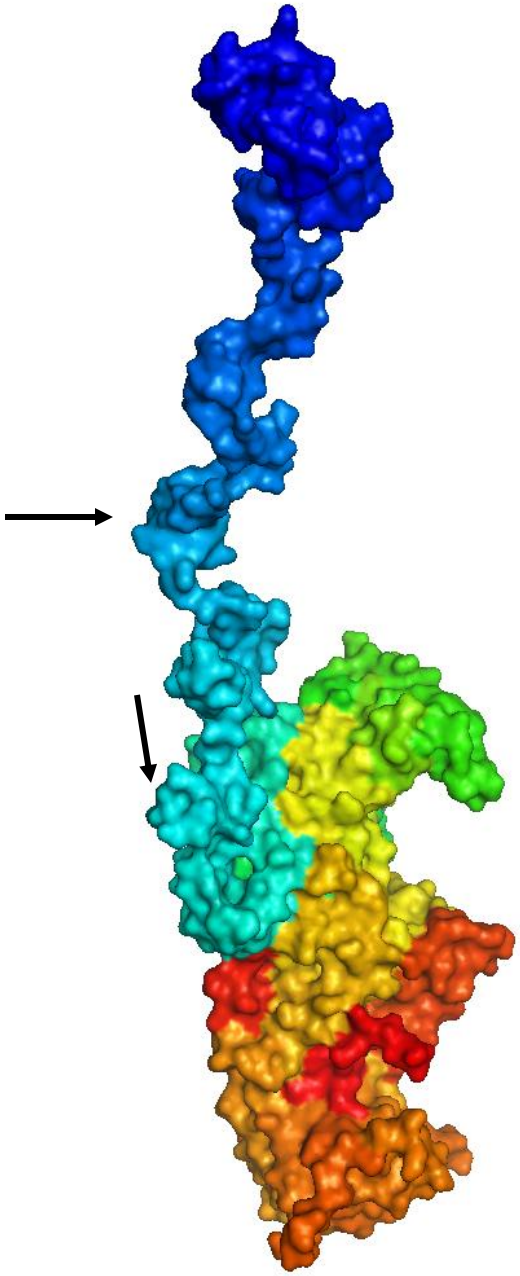
Crystallographic structure used

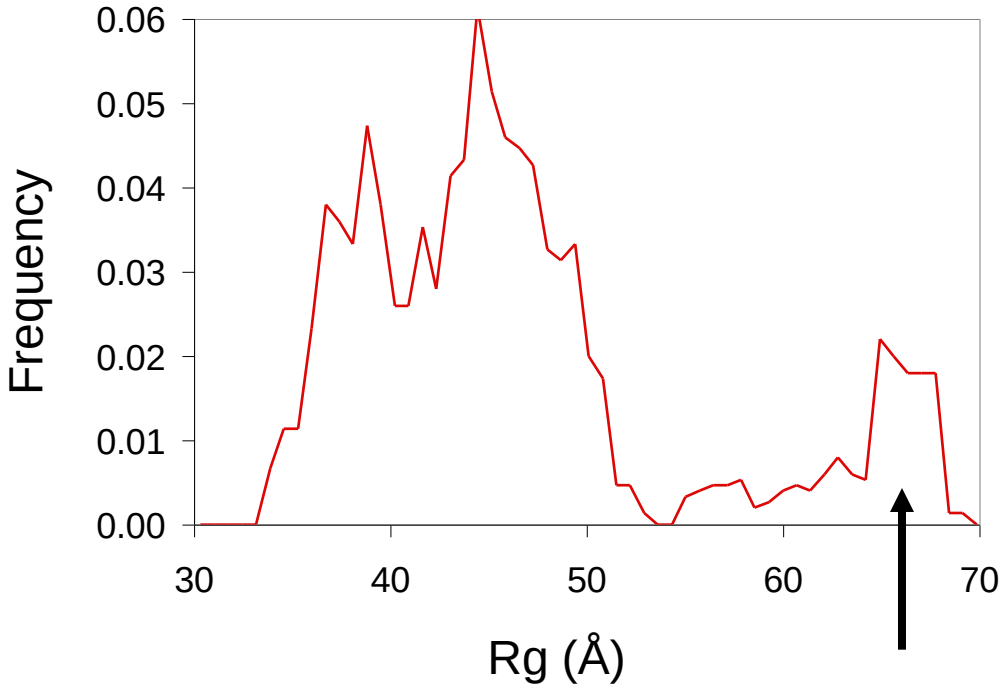
Outline

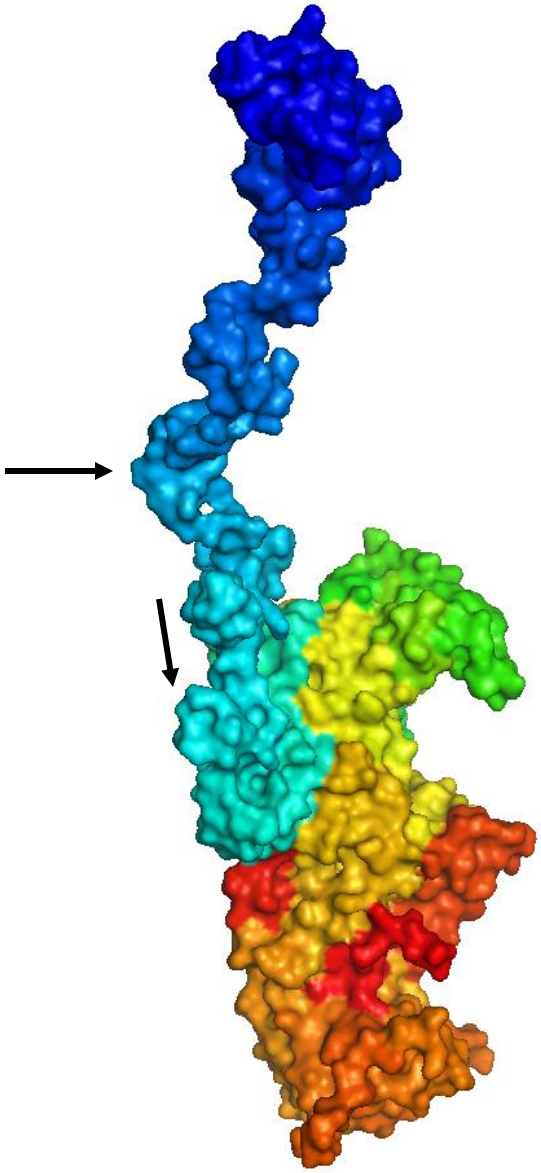
- Biological dynamics
- Crystallography
- Small Angle X-ray Scattering
- A biological case
- A structure, a puzzle, a question?
- A solution
- **Computational analysis**
- An answer, more questions, more solutions
- Where are we going next?

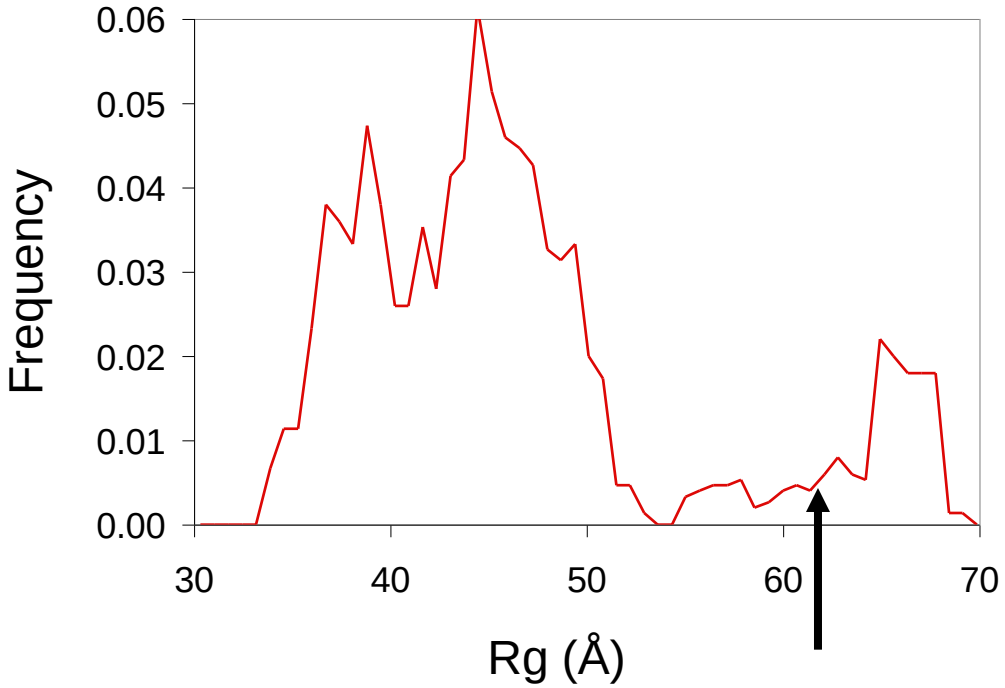
Computationally model the motion

- The motion is too large for us to perform full molecular dynamics simulations with the computing capacity currently available to us (a ~200 processer cluster in-house and shared time on a neighboring 2000+ processer cluster).
- We took the most compact form and the most extended form and using an energy minimization procedure with Morph Server calculated a pathway between the two forms.
- This is a preliminary analysis. A future approach will be to run molecular dynamics simulations on each conformer to evaluate the pathway between nearest neighbors. This appears to be computationally feasible.




0.06
0.05
0.04
0.03
0.02
0.01
0.00
Frequency
30
40
50
60
70
Rg (Å)




0.06
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Frequency
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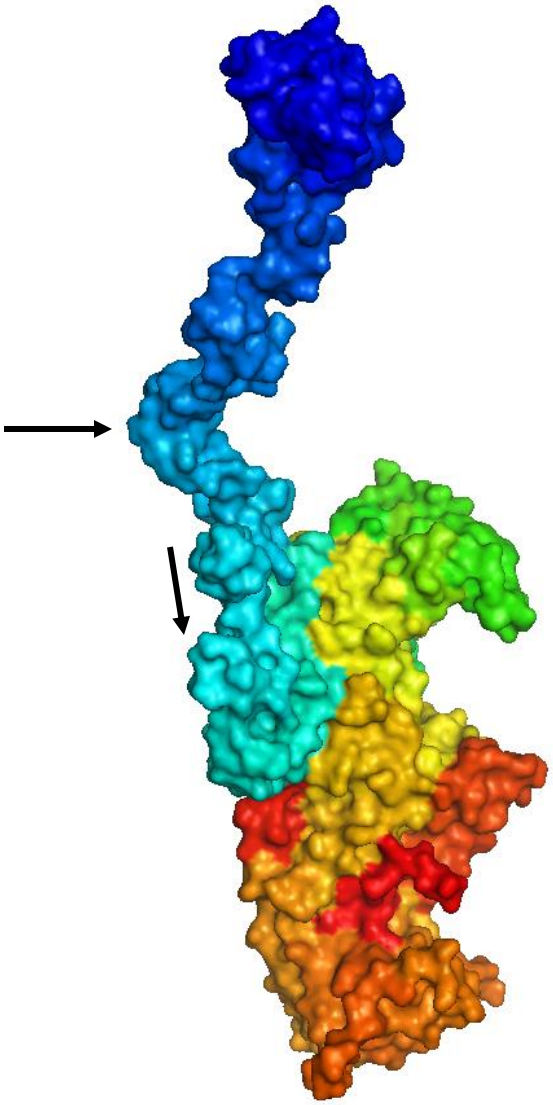
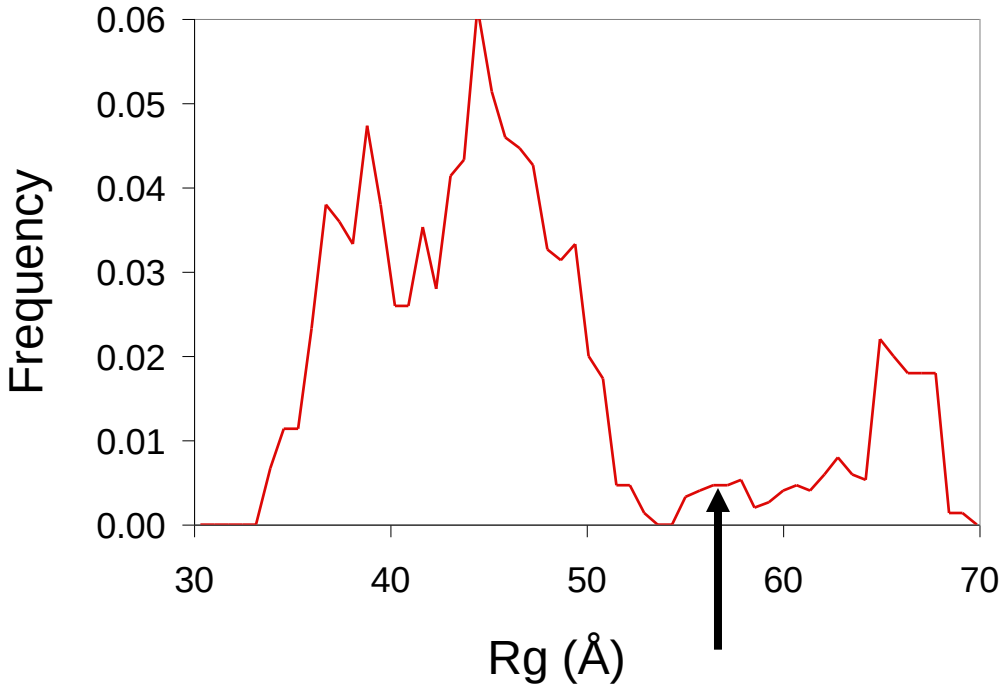
Rg (Å)

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Frequency
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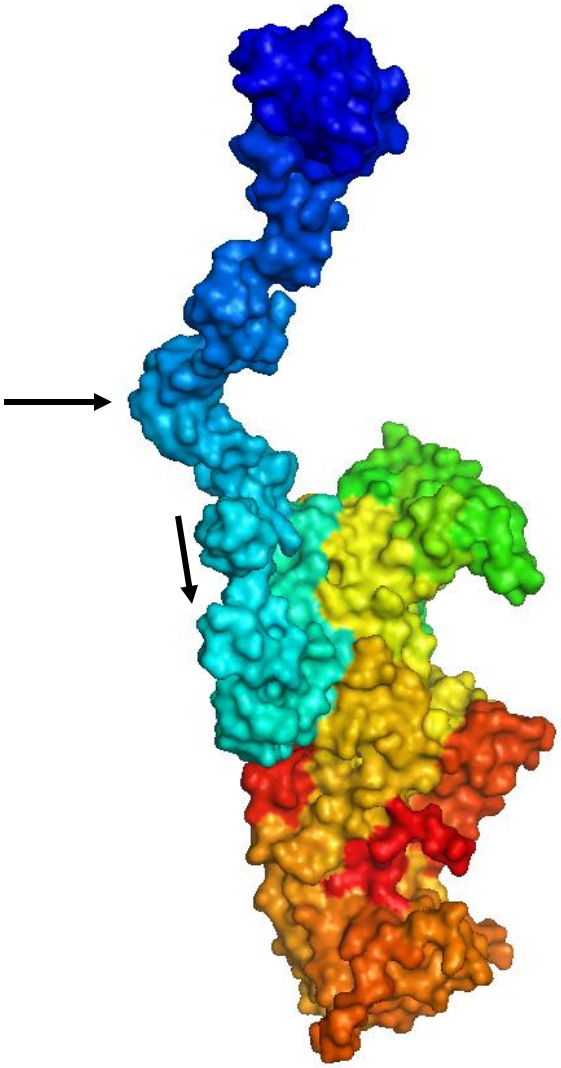
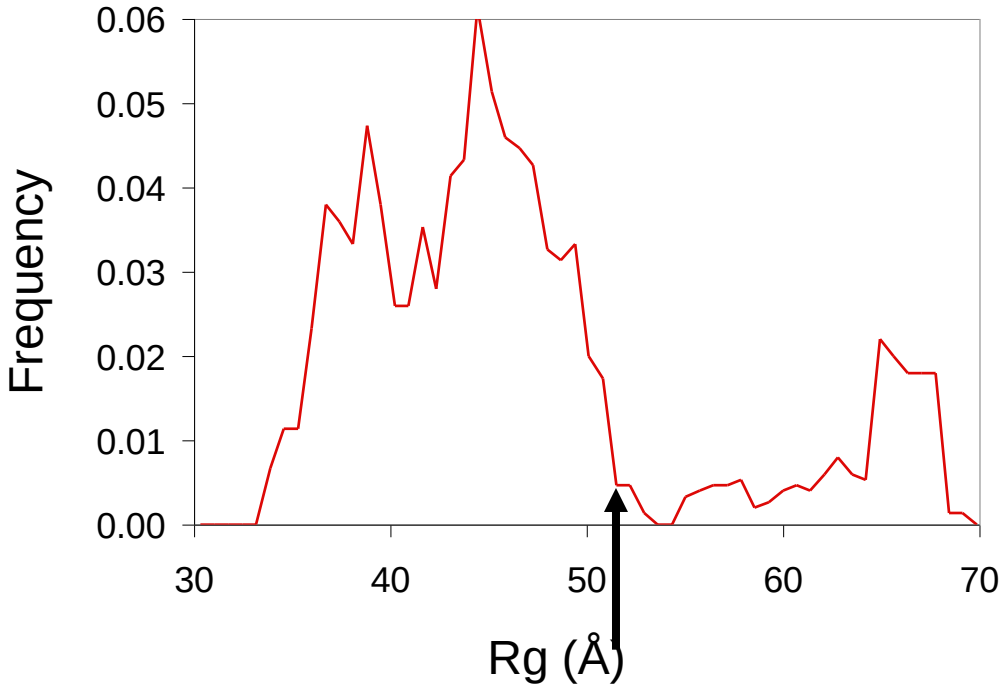
70
Rg (Å)




0.06
0.05
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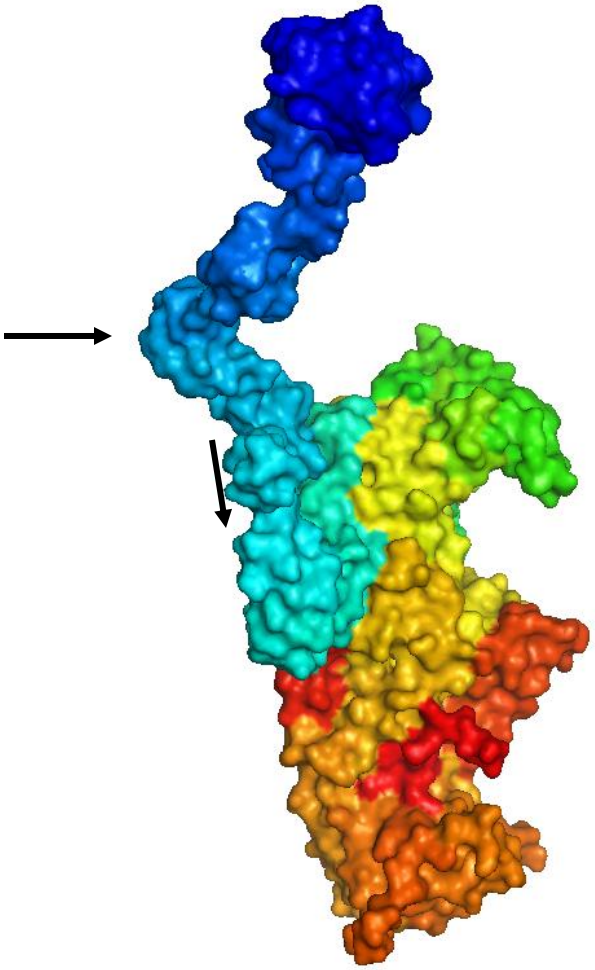
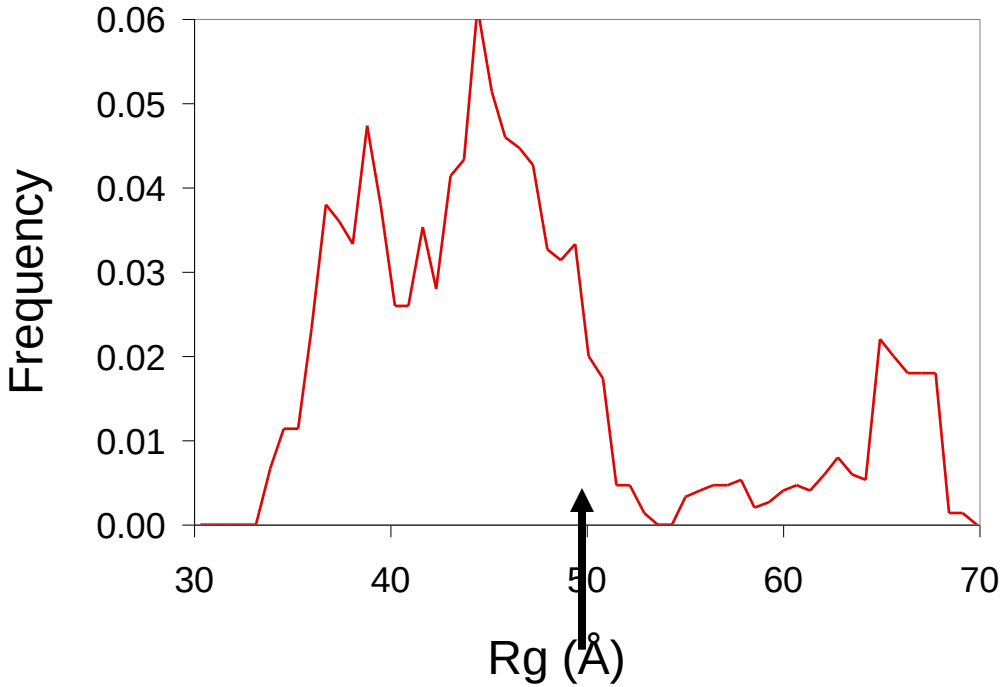
0.00
Frequency
30
40
50
60
70
Rg (Å)




0.06
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Frequency
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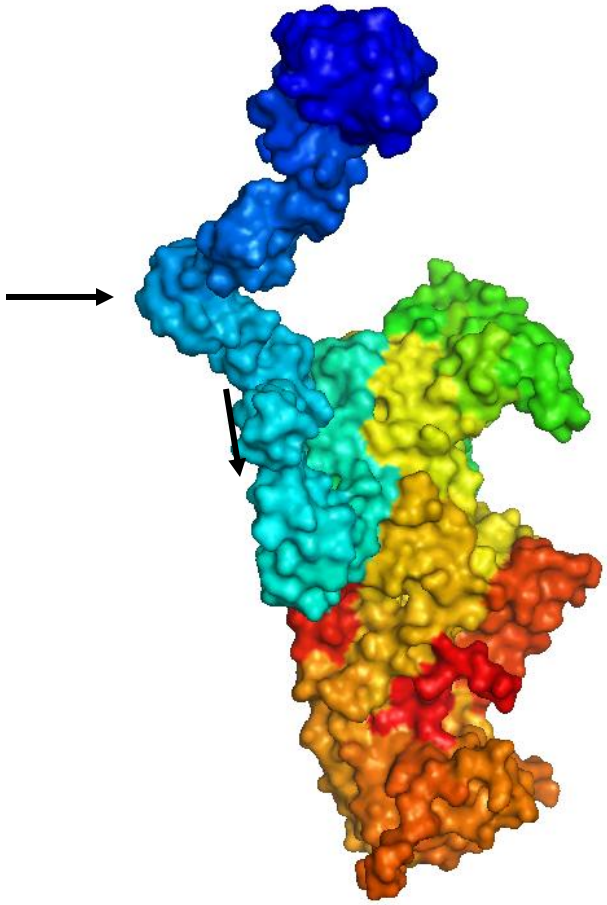
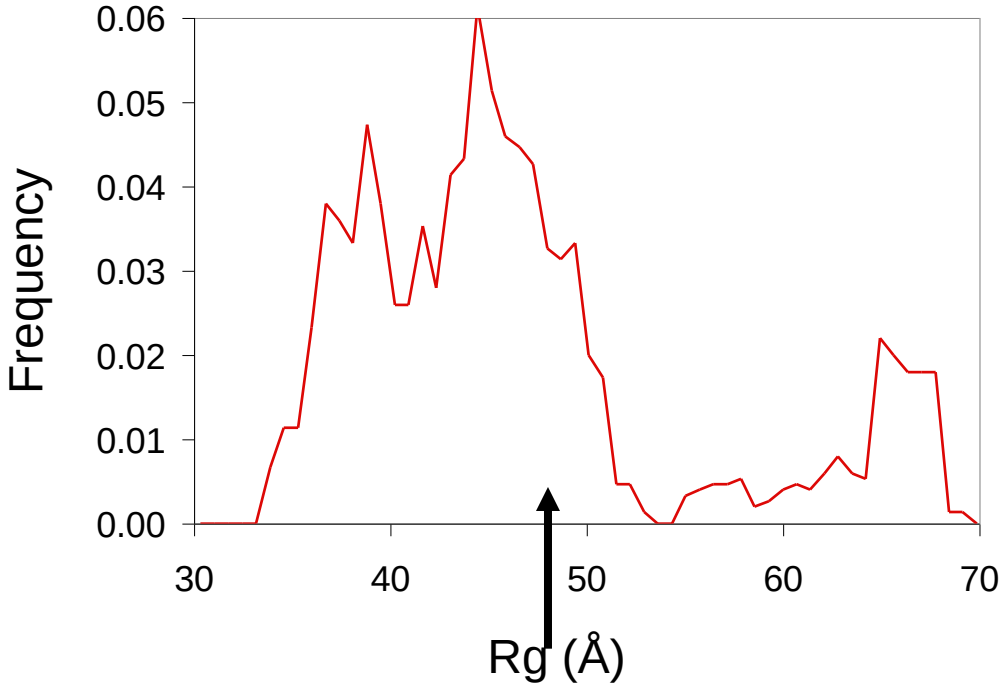
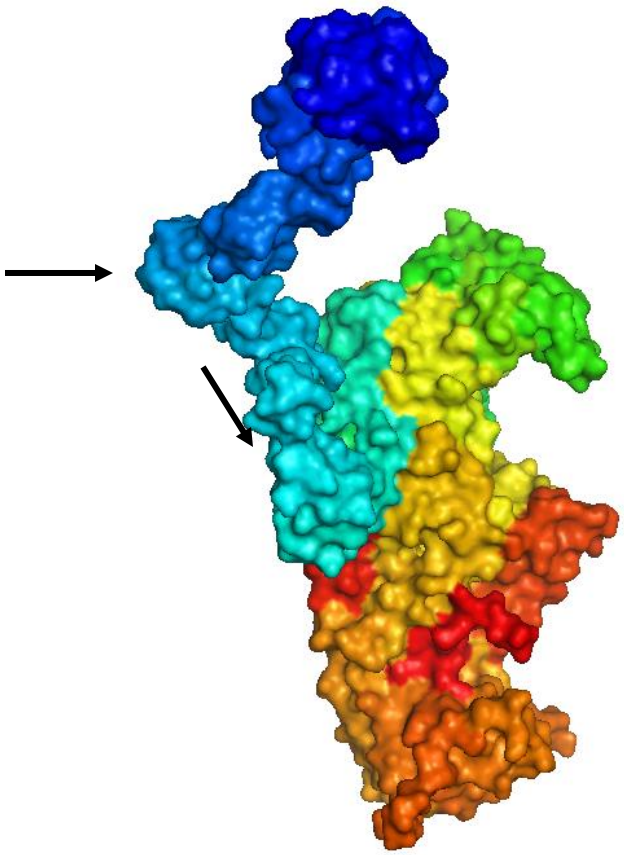
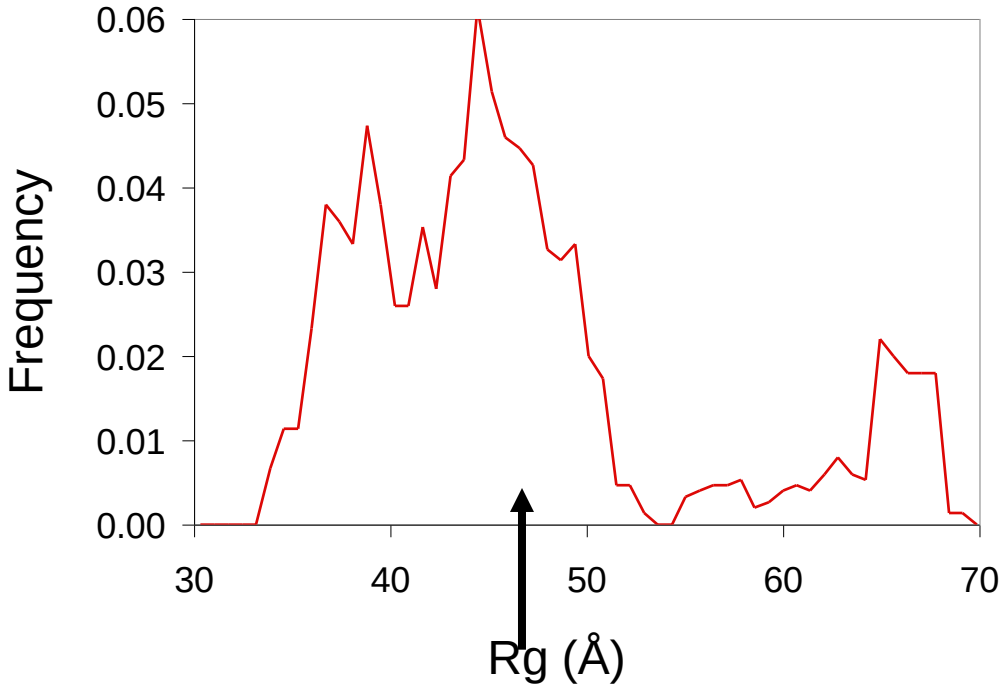
Rg (Å)




0.06
0.05
0.04
0.03
0.02
0.01
0.00
Frequency
30
40
50
60
70
Rg (Å)




0.06
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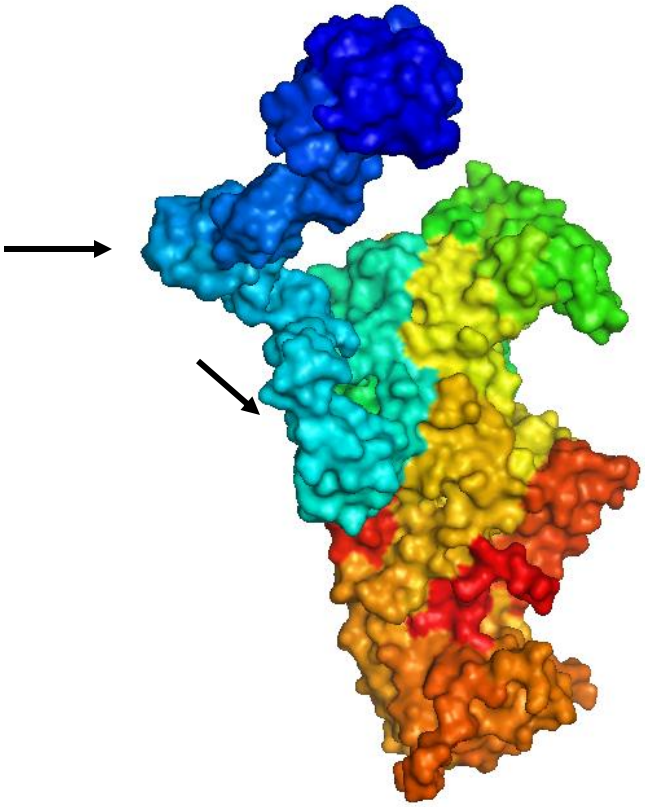
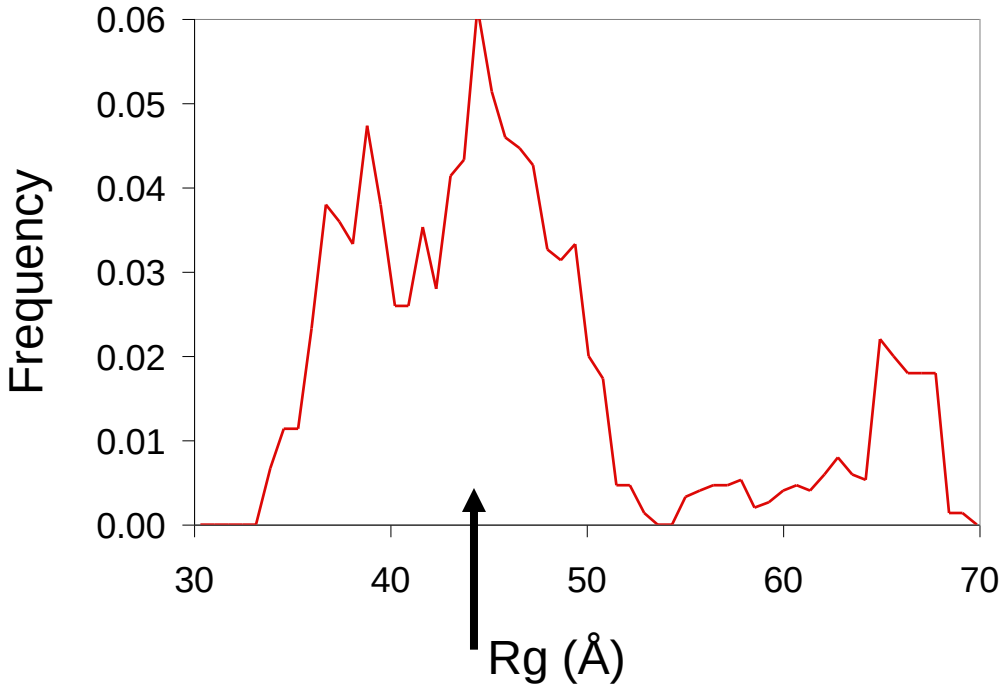
0.02
0.01
0.00
Frequency
30
40
50
60
70
Rg (Å)




0.06
0.05
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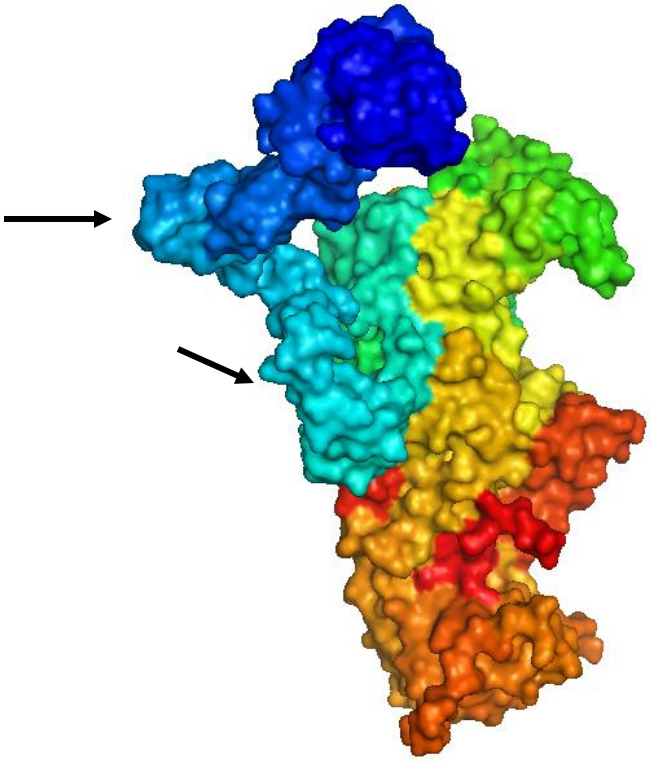
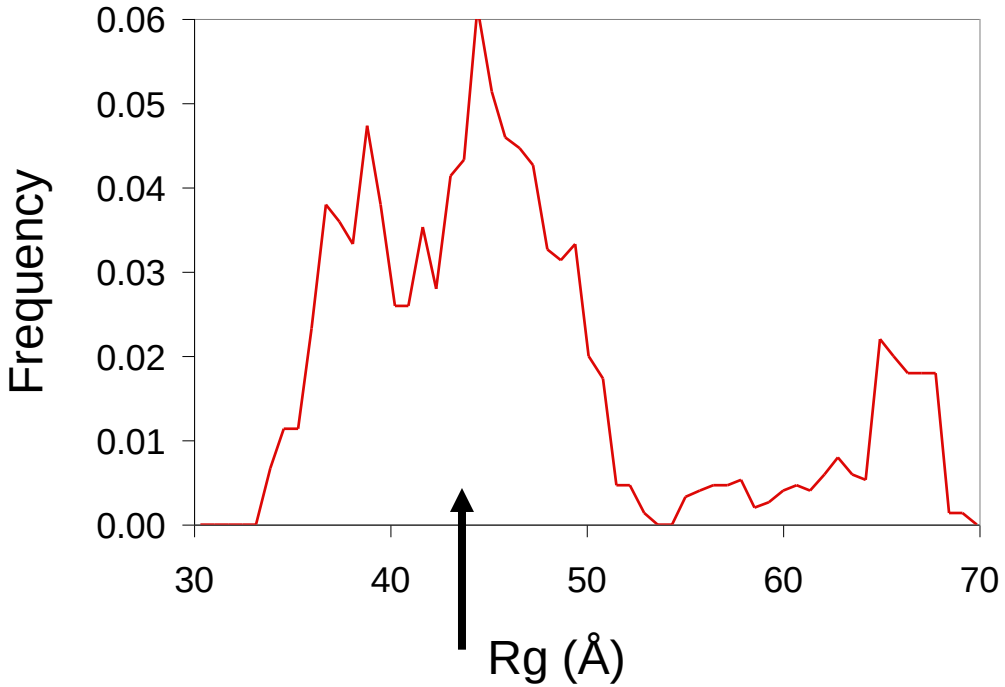
0.03
0.02
0.01
0.00
Frequency
30
40
50
60
70
Rg (Å)




0.06
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Frequency
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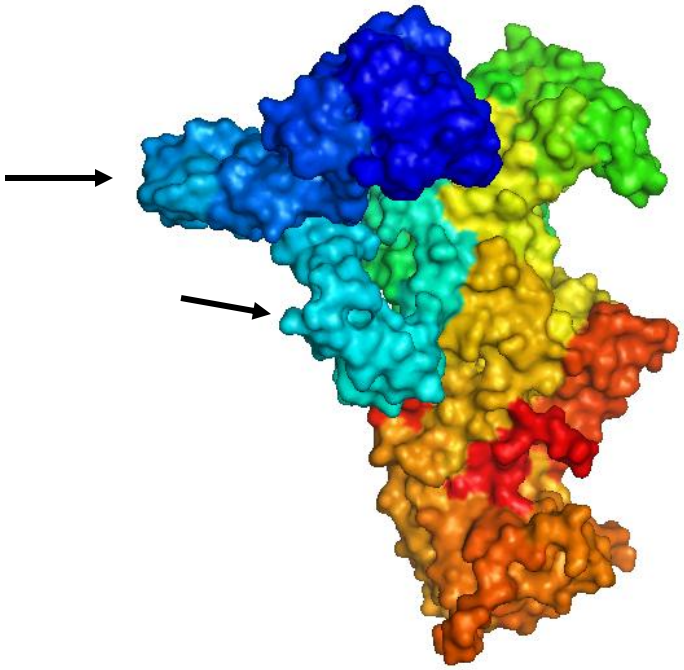
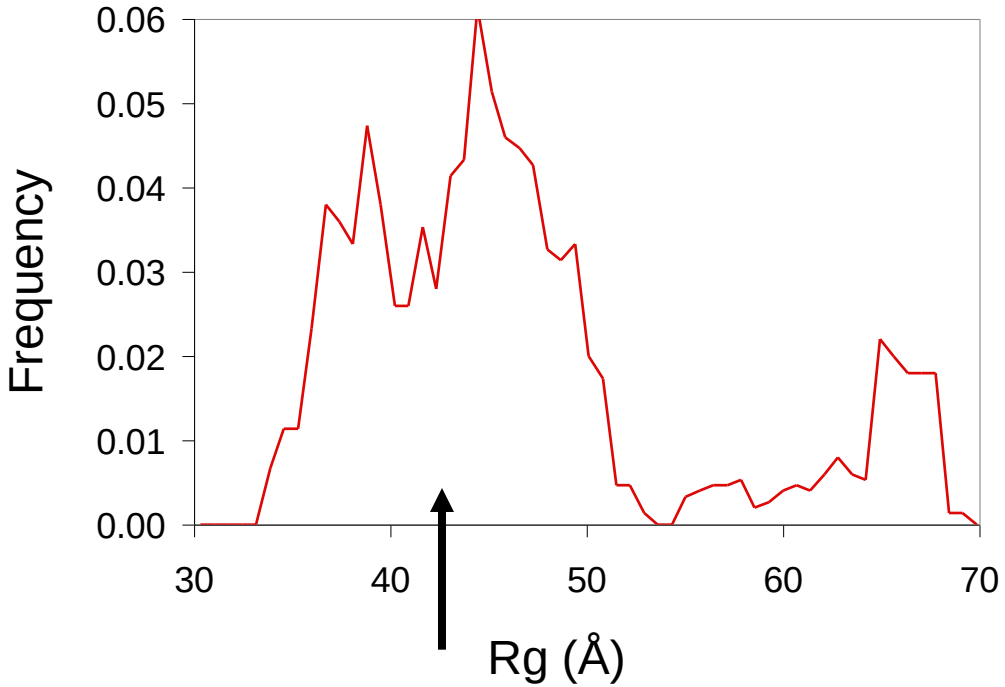
Rg (Å)

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Frequency
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60
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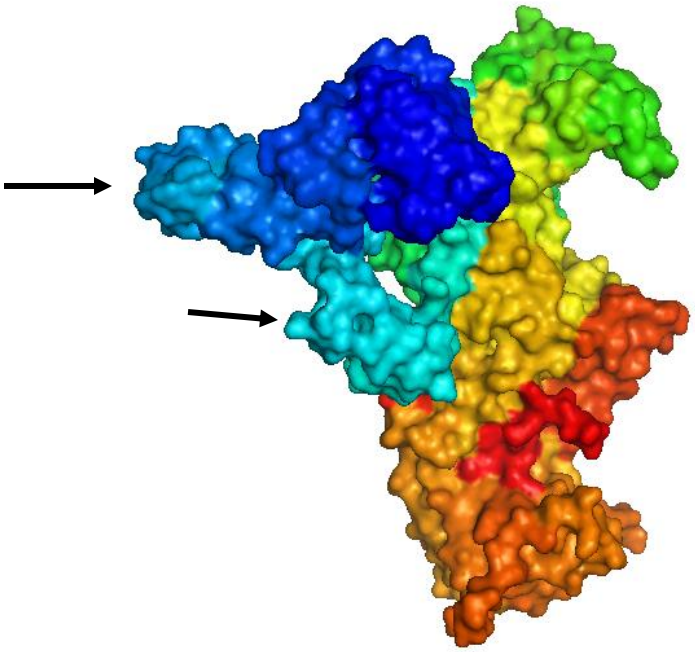
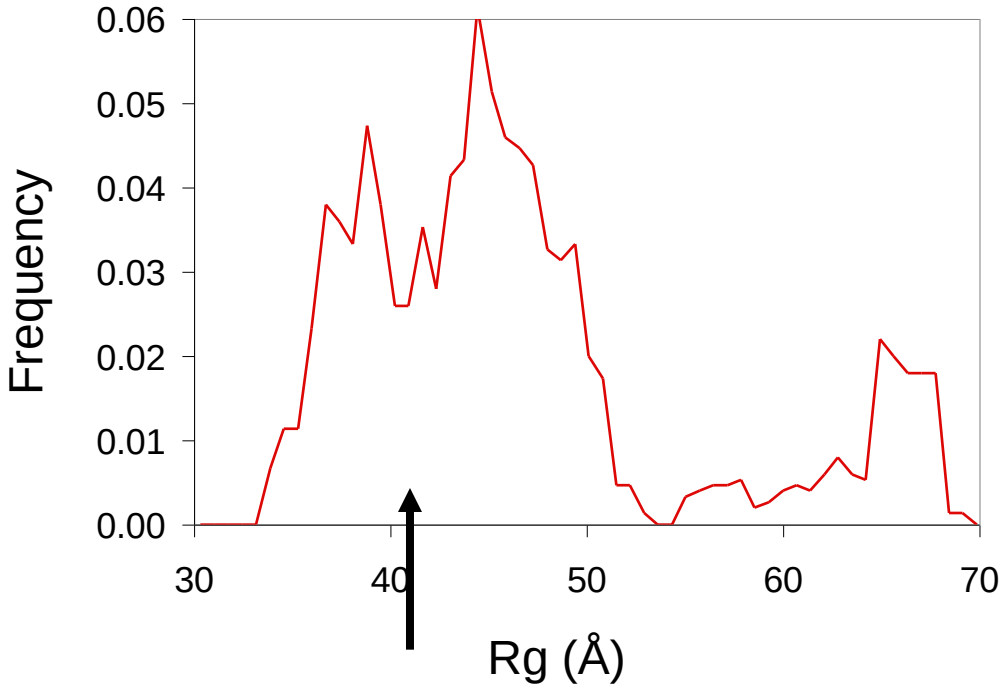
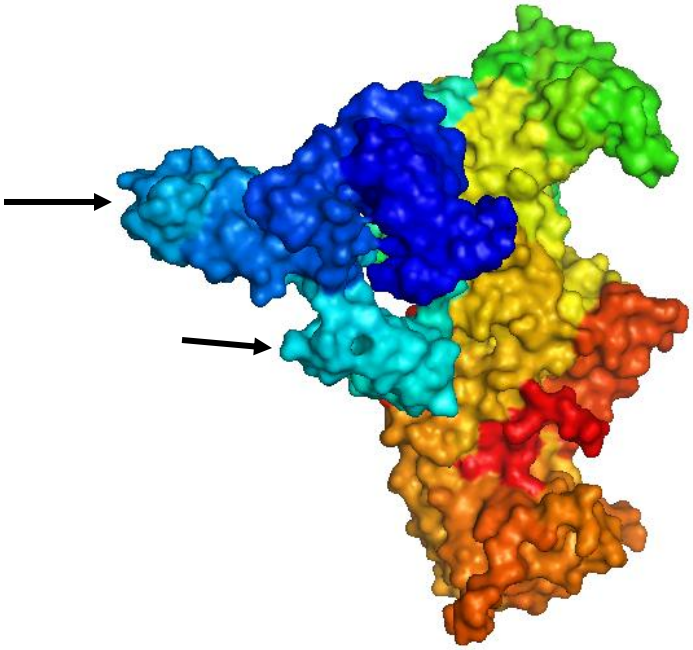
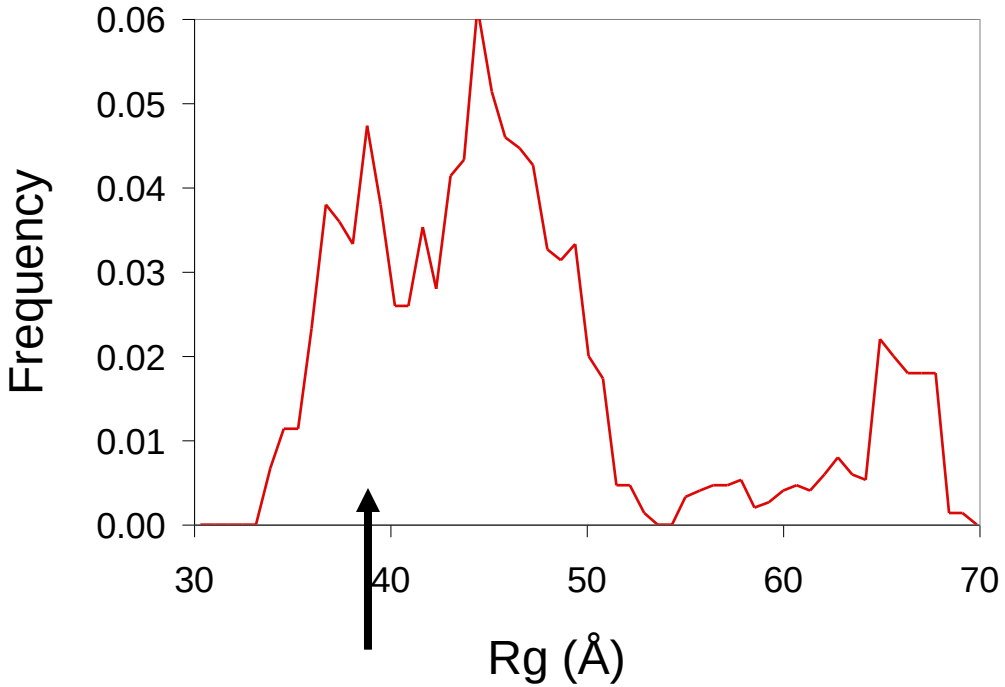
Rg (Å)




0.06
0.05
0.04
0.03
0.02
0.01
0.00
Frequency
30
40
50
60
70
Rg (Å)

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Frequency
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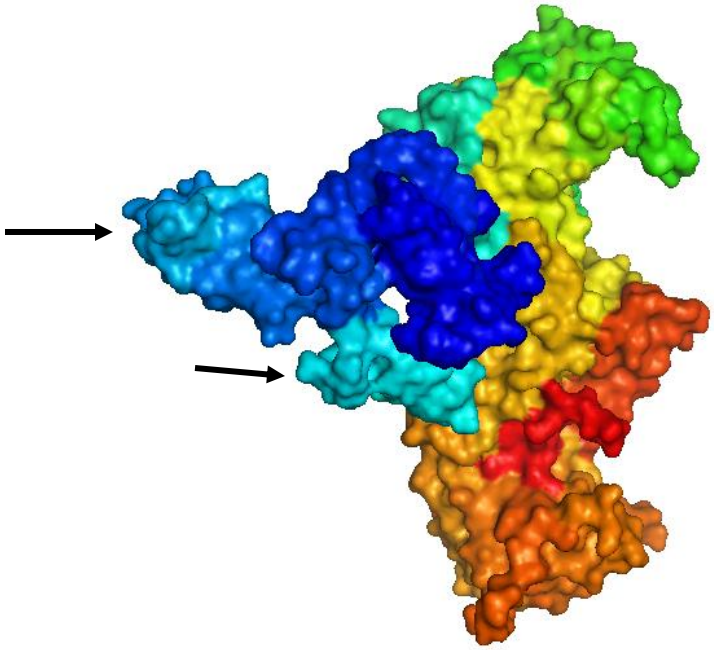
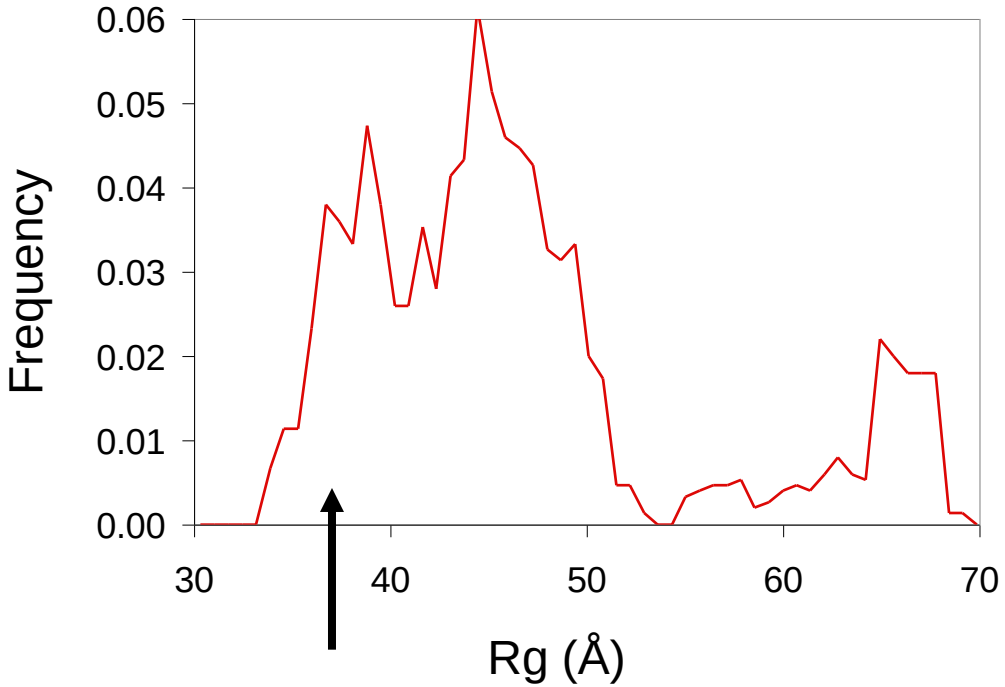
Rg (Å)

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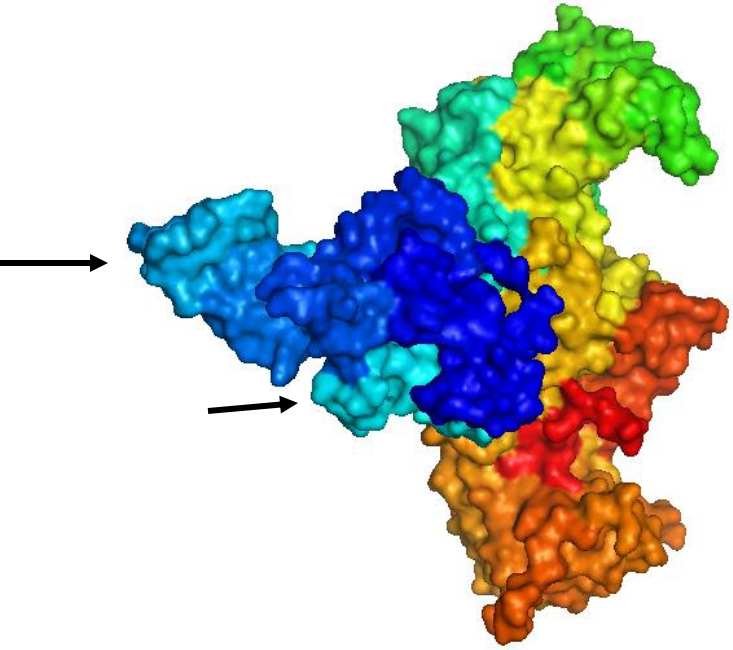

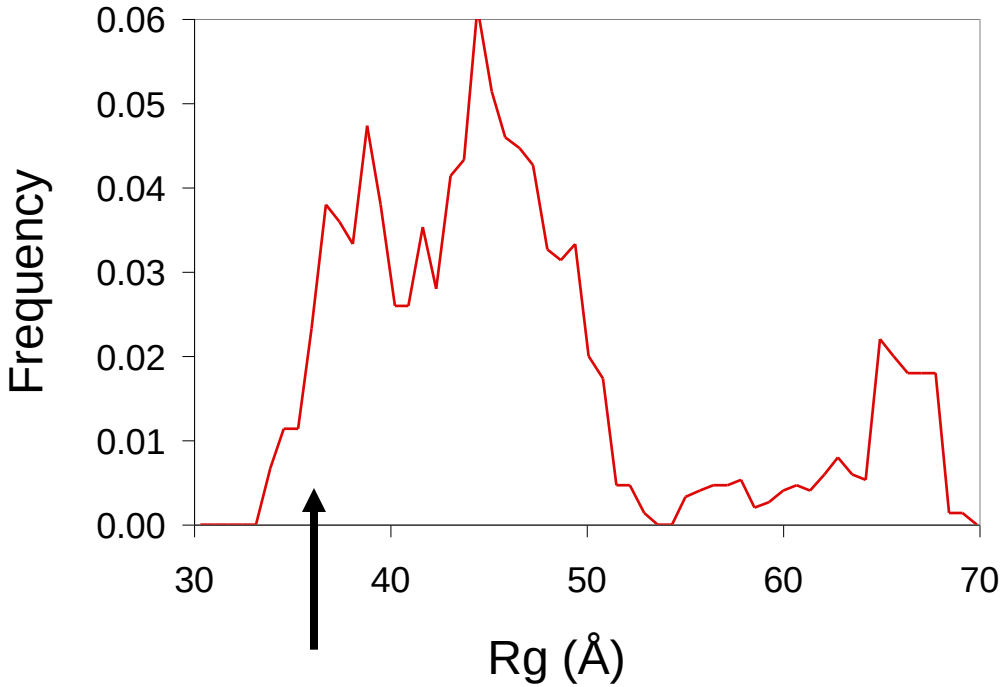
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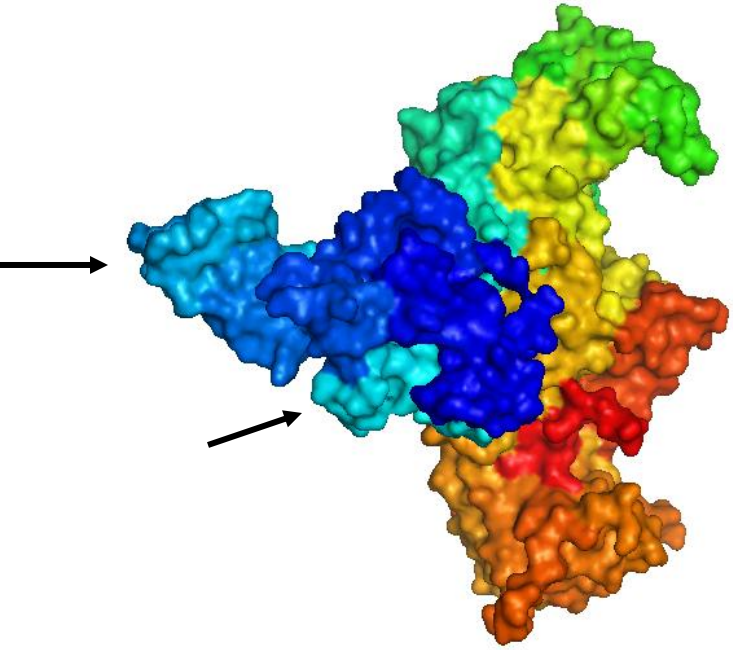

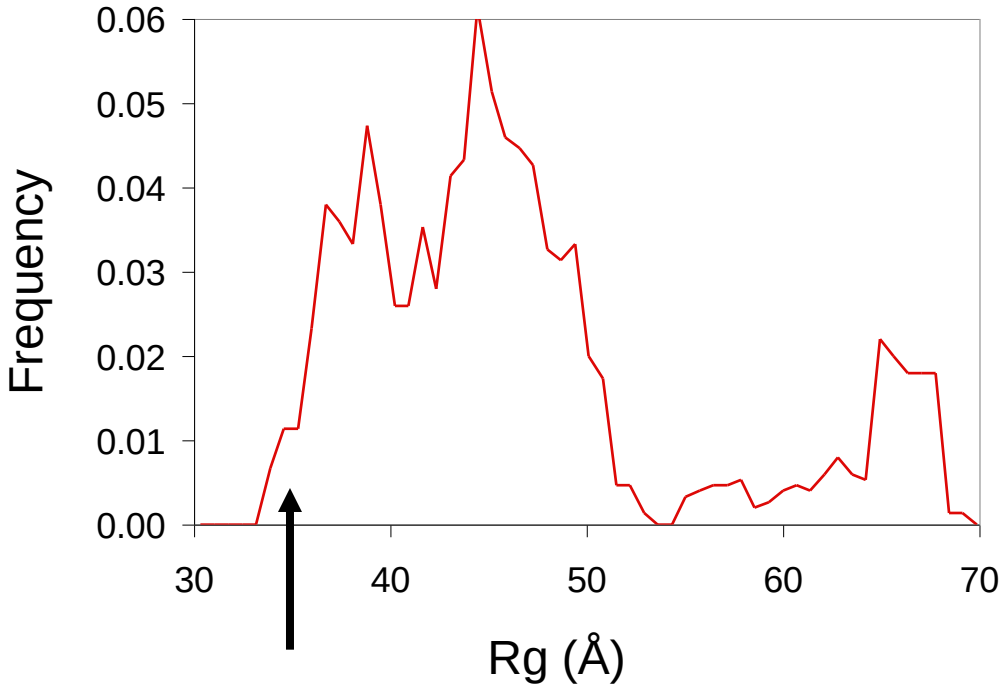
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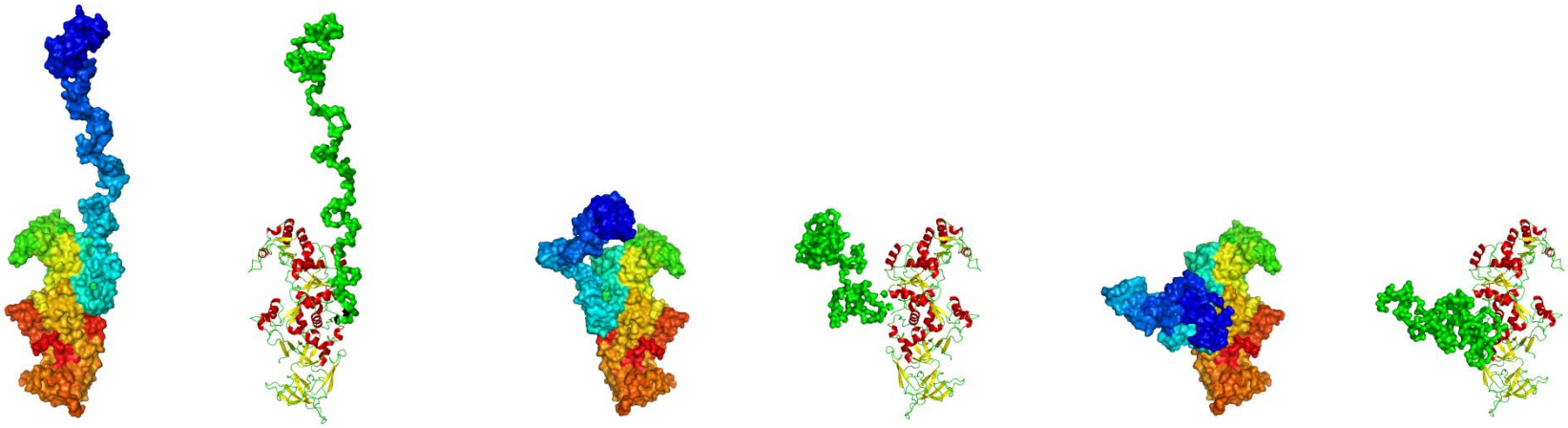
Outline

- Biological dynamics
- Crystallography
- Small Angle X-ray Scattering
- A biological case
- A structure, a puzzle, a question?
- A solution
- Computational analysis
- **An answer, more questions, more solutions**
- Where are we going next?

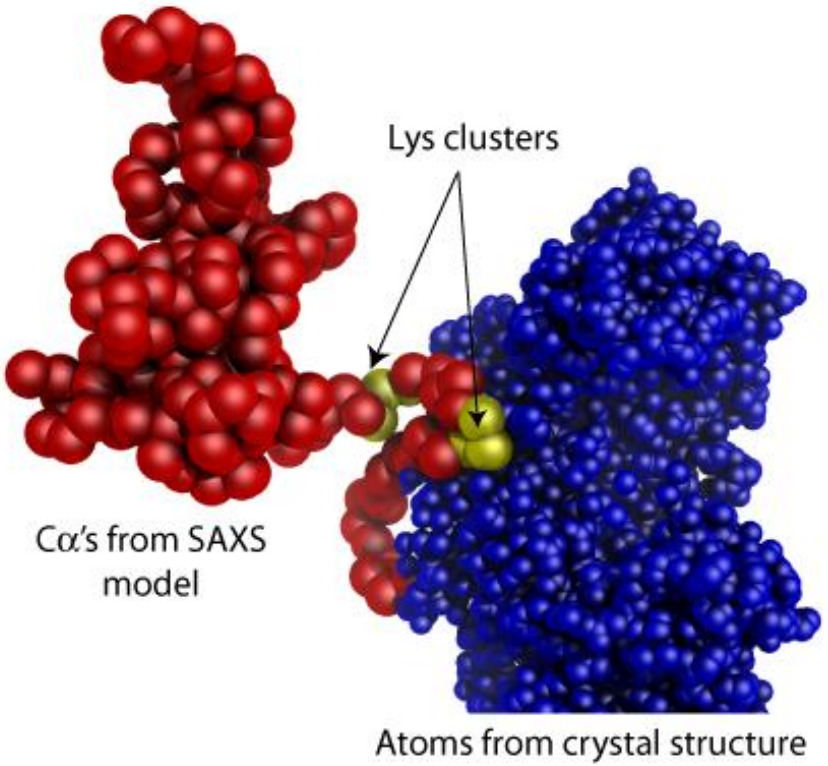
Compare the model with the experimental SAXS data

- The Ensemble Optimization Method derived model structures all fit within the envelope of the computational dynamic model derived from simple consideration of the most compact and most extended conformer.
- The experimentally derived data between the two extremes used to model the dynamic pathway showed good agreement with the resulting model.



- The computational model predicts regions to mutate and confirm both the dynamic model and the SAXS model experimentally.

Compare with the experimental SAXS model with biochemistry

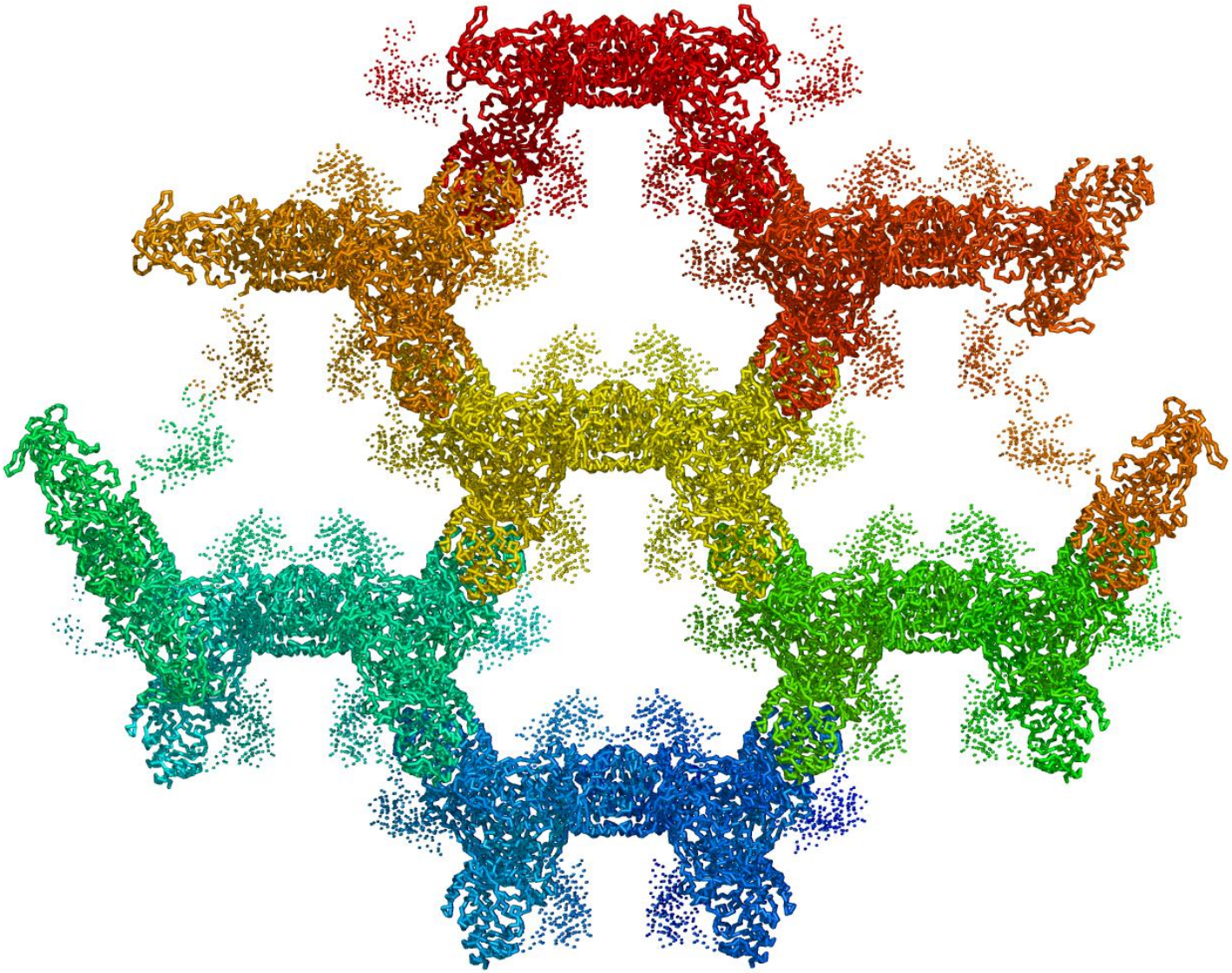


Mutations in 2 pairs of lysine residues simultaneously inactivate the rescue function of the hybrid yeast-*E. coli* gene and also cause a 10-fold reduction in the affinity of the N-terminal domain for $tRNA^{Gln}$

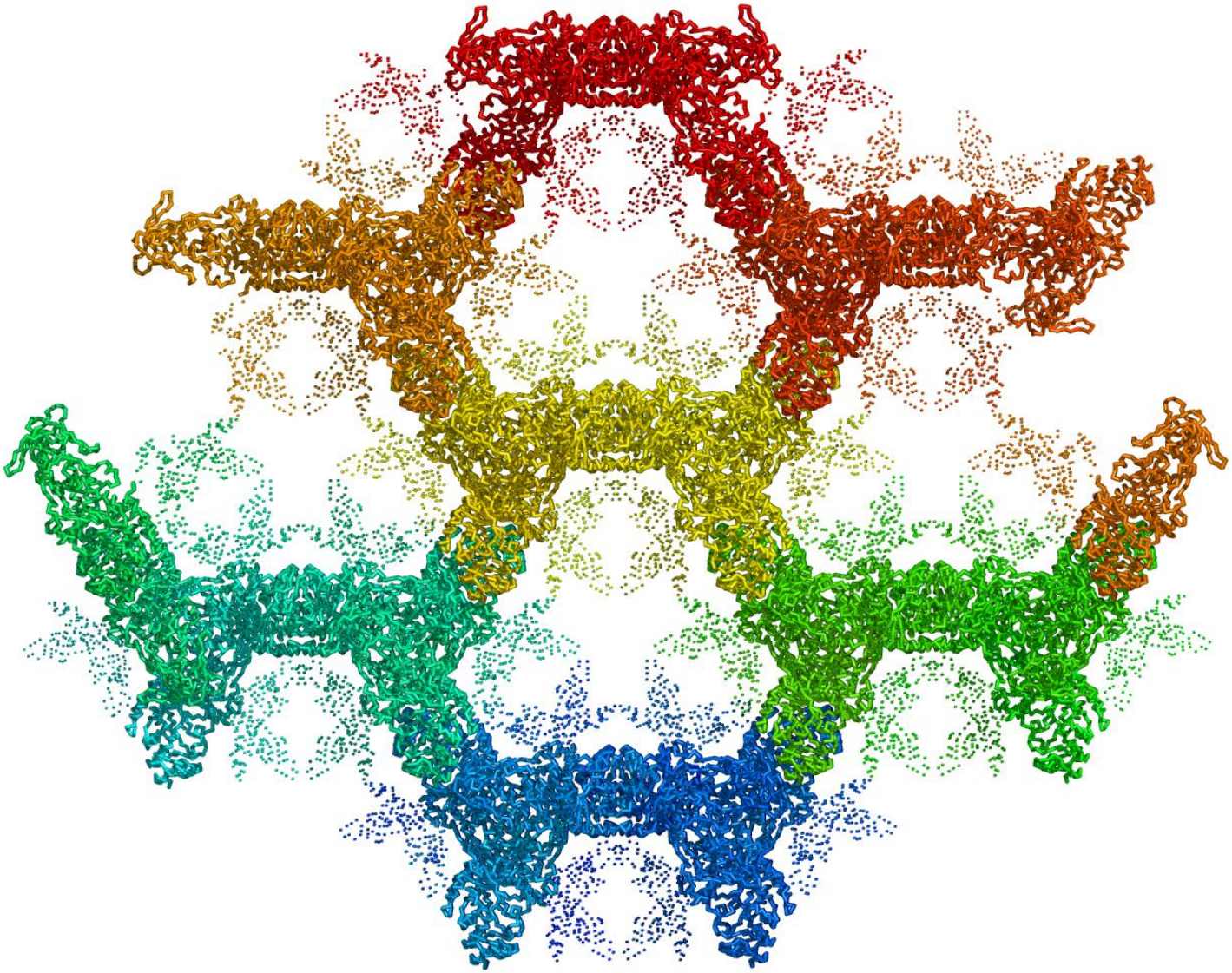
Wang et al.,JBC 275, 17180 (2000)

SAXS derived models can be used to explain biochemical data and develop new hypothesis that are testable. The model is low-resolution and care has to be taken to keep that in mind.

Is the model compatible with the crystallographic data?



The N-terminal ‘arm’ is completely compatible with the crystal structure



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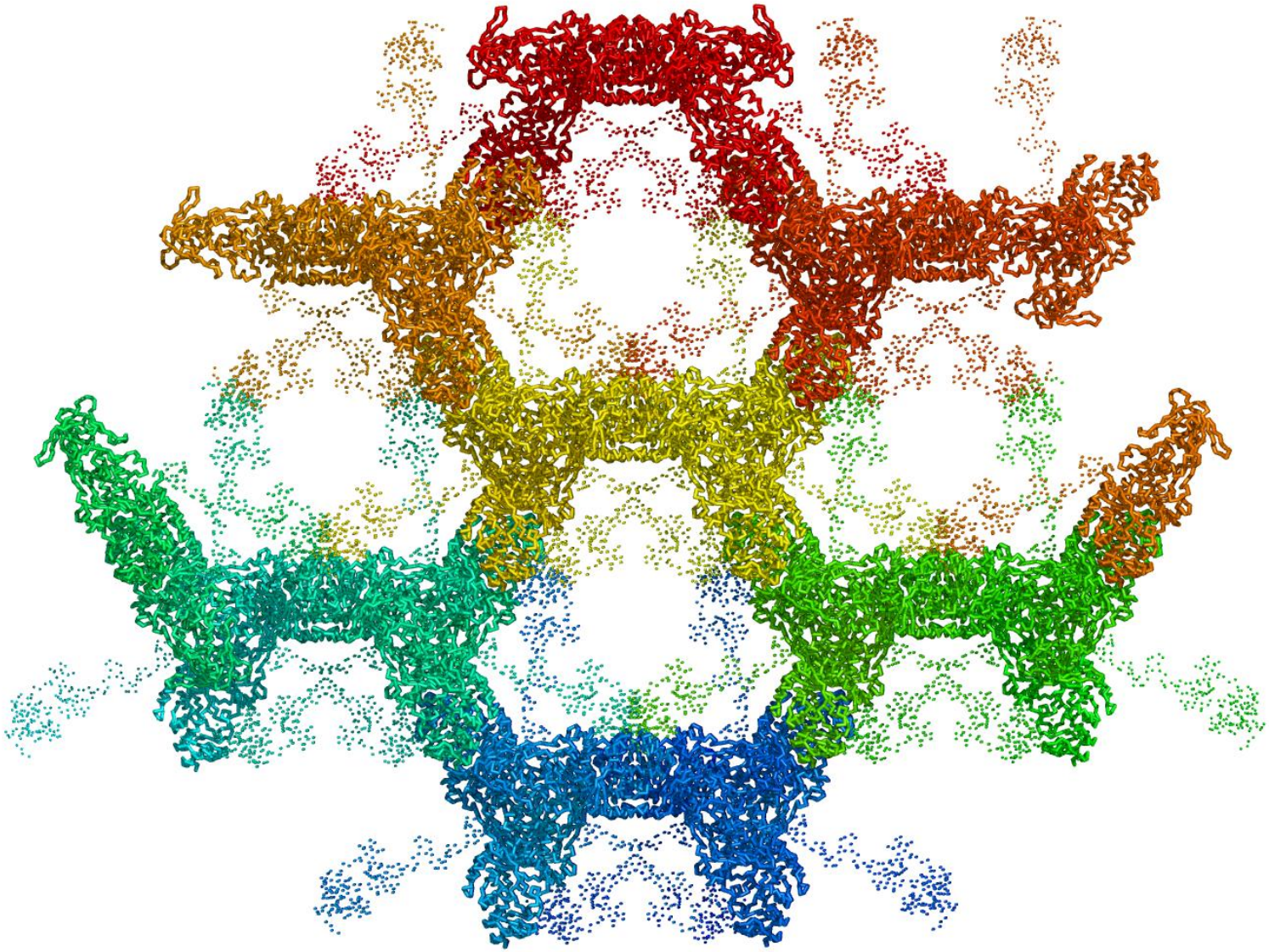

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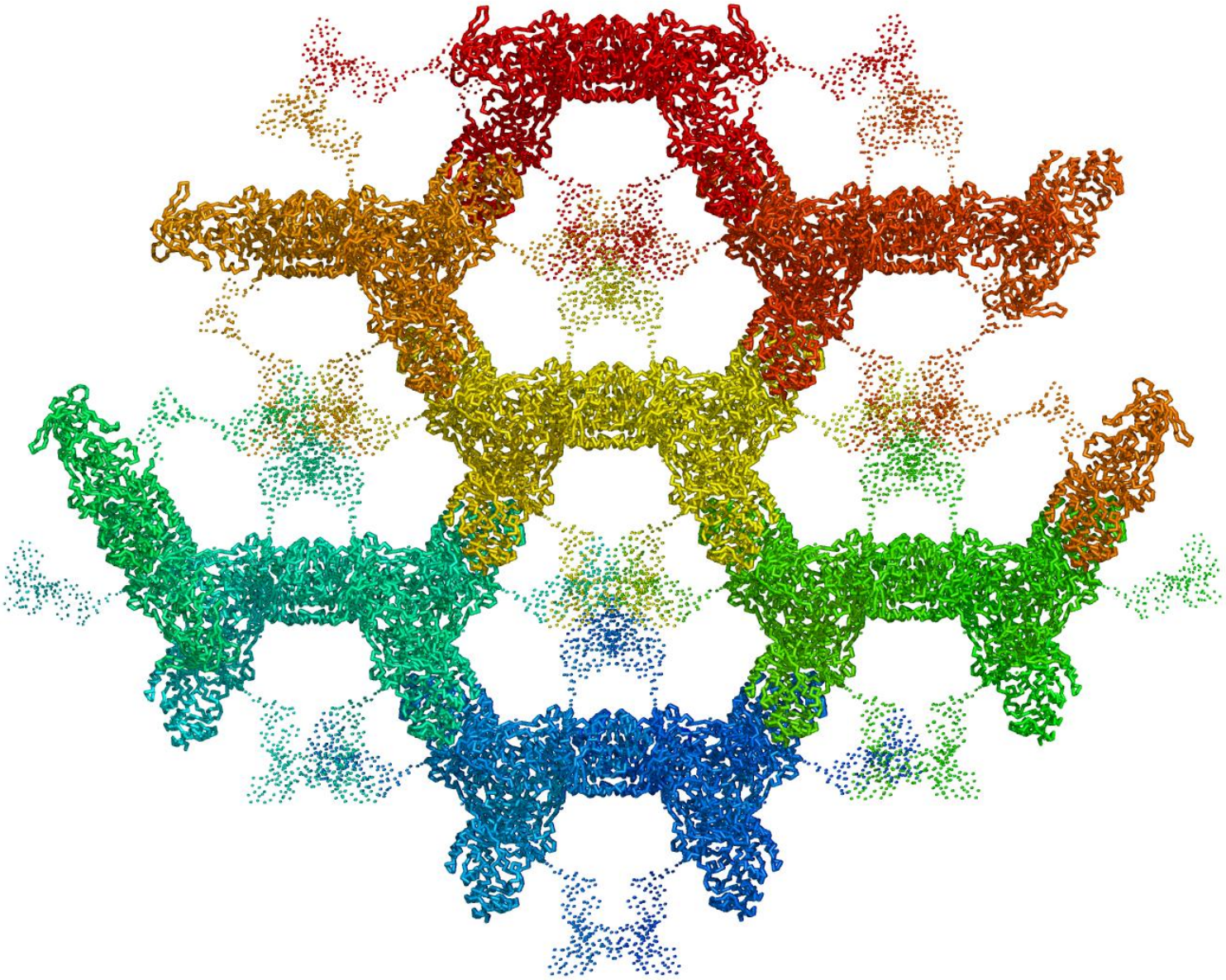

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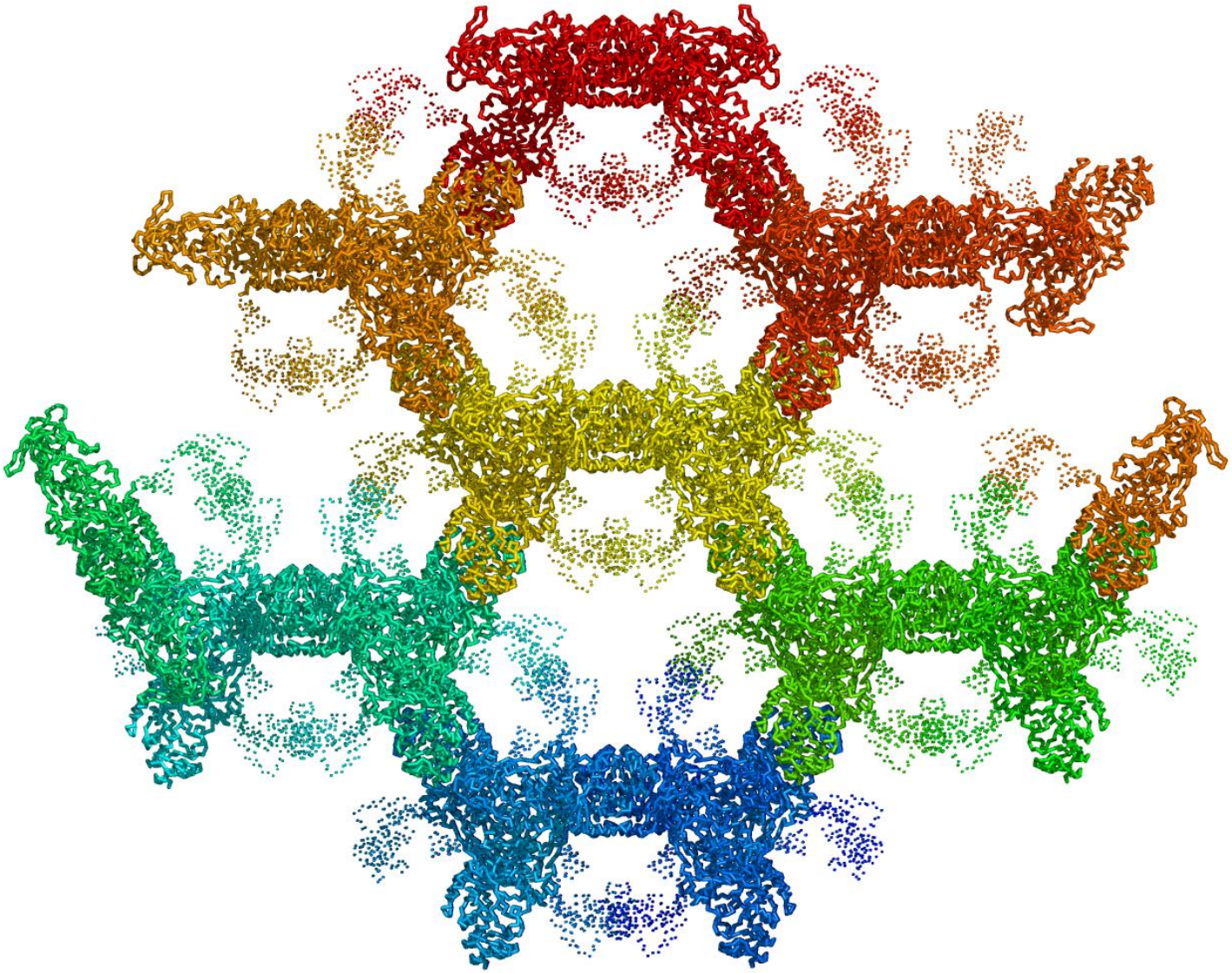

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- How general is this, is it typical?
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  - Studies on the N-terminal domain separately
  - Rational mutations
  - Orthologs

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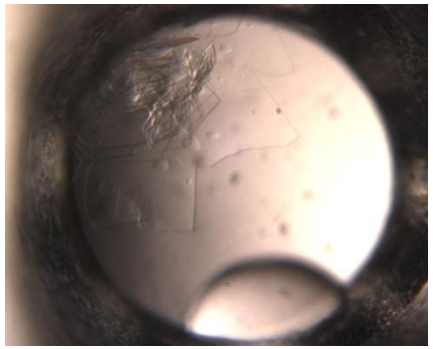
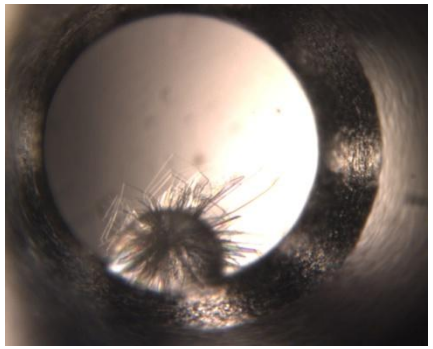
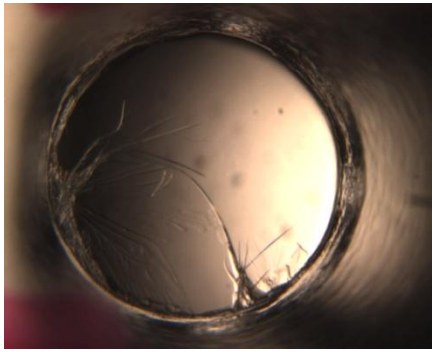
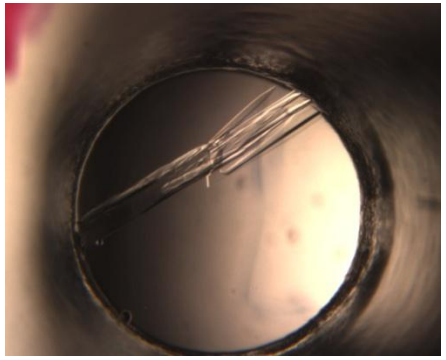
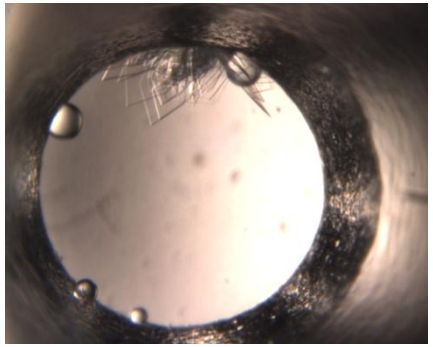
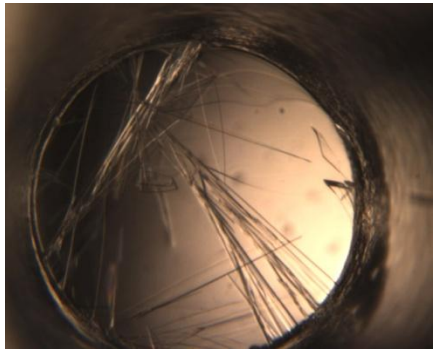
Studies on the N-terminal domain separately

32 hits at 4 weeks.

pH 4-10 sampled equally

Distributed as:

- 8 hits in pH 7
- 10 hits in pH 8
- 11 hits in pH 9
- 3 hits in pH 10.

Laboratory SAXS data indicates globular components with flexible linkers.

Synchrotron single crystal and SAXS data next month

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In progress

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Orthologs

Currently, laboratory SAXS data on two.

Similar results to the Gln4

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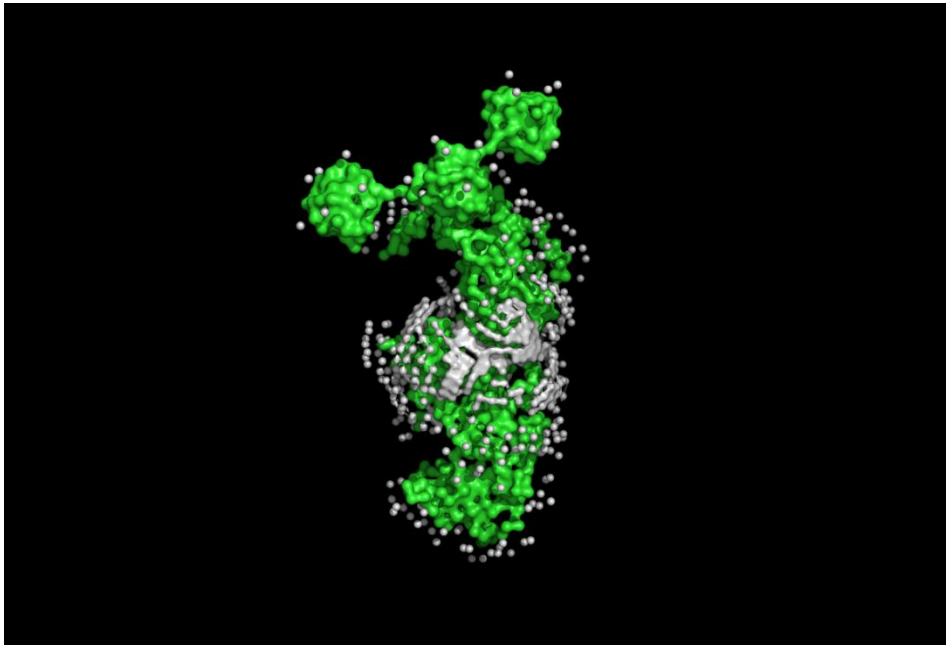

First reconstruction from *C. Glabrata*

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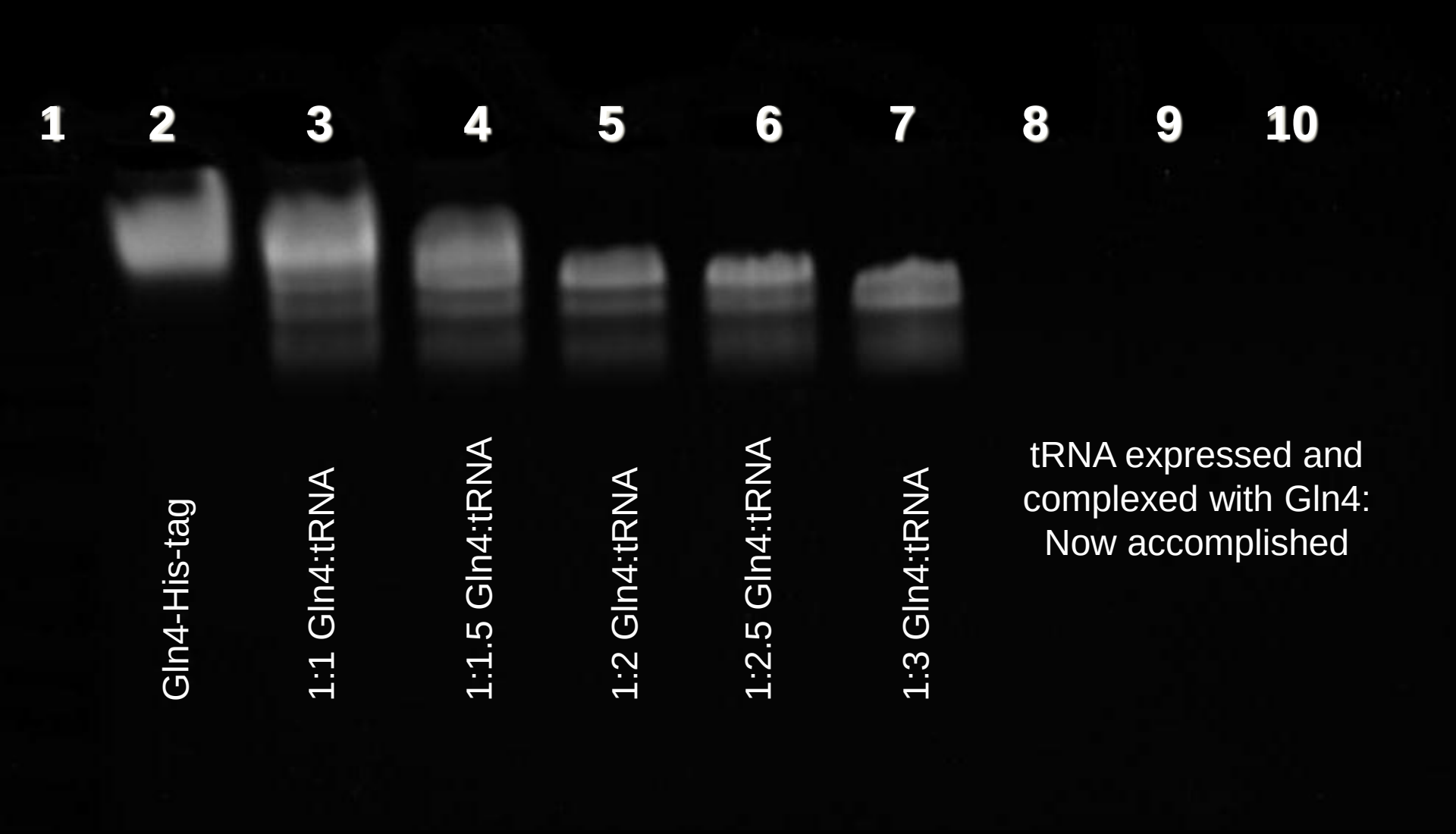

Next step, bind tRNA to the protein and do the SAXS experiment

Summary

- Through a population distribution analysis we have defined a unique signature to identify distinct and possibly functional conformations within dynamic regions of the macromolecule
- For Gln4, we propose that the N-terminal region of Gln4 has a dynamic motion that aids in its function.
- Our experimental evidence and modeling supports this but does not completely rule out multiple solution states
- Similar structurally uncharacterized domains are found on higher eukaryotic glutamyl-, isoleucyl, lysyl-, mehtionyl- and valyl-synthetases
- We propose that these may have a structurally similar role.
- By combining the strengths of crystallography and SAXS we have produced information that could not be obtained by either in isolation.
- Crystallography and SAXS are not the only techniques that can be used to produce the whole picture. A full functional and dynamic understanding requires multiple approaches.

Roles and Acknowledgements

Molecular biology, protein production
Beth Grayhack, Eric Phizicky, Erin Quartley, Stephanie Corretore

Crystal growth, X-ray crystallography
Edward Snell, Joseph Luft, Jen Wolfley, Elizabeth Snell

Small Angle X-ray Scattering
Tom Grant, Edward Snell, Joseph Luft, Hiro Tsuruta

Computational modeling
Tom Grant, Edward Snell

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NIH PSI support to George DeTitta

With special thanks to the core team now analyzing these results:
Tom Grant, Joseph Luft, Eric Phizicky, and Beth Grayhack

Questions?

esnell@hwi.buffalo.edu