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

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Biology

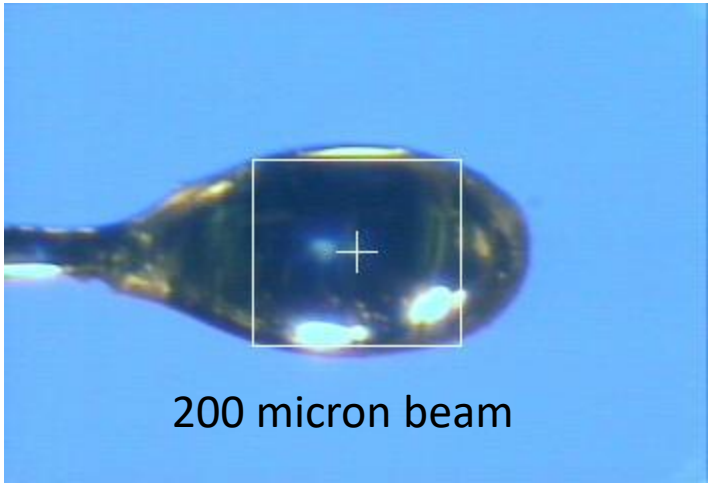
- Yeast *Saccharomyces cerevisiae is* a well-established model system for understanding fundamental cellular processes of higher eukaryotic organisms.
- Our target today is Glutaminyl tRNA synthetase (Gln4) from yeast *Saccharomyces cerevisiae*

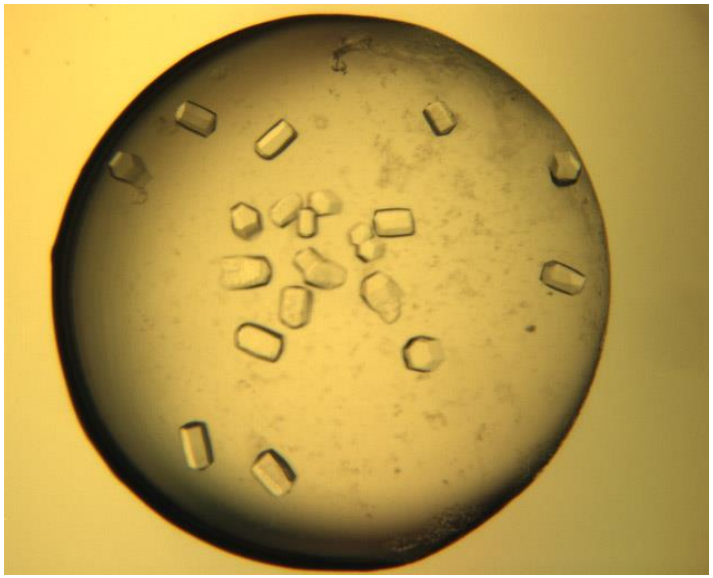
- Many eukaryotic tRNA synthetases like Gln4 differ from their prokaryotic homologs by the attachment of an additional domain appended to their N or C-terminus. It is not known, why these additional domains are not found in prokaryotes.
- The additional domain in Gln4 is known to have weak non-specific RNA binding activity.
- 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 synthetases

- The 228 amino acid N-terminal domain of Gln4 is among the best studied of these domains, but is structurally uncharacterized.
- The yeast Gln4 N-terminal domain is functionally important, since its addition to *E. coli* Gln synthetase allows the *E. coli* enzyme to function in yeast.

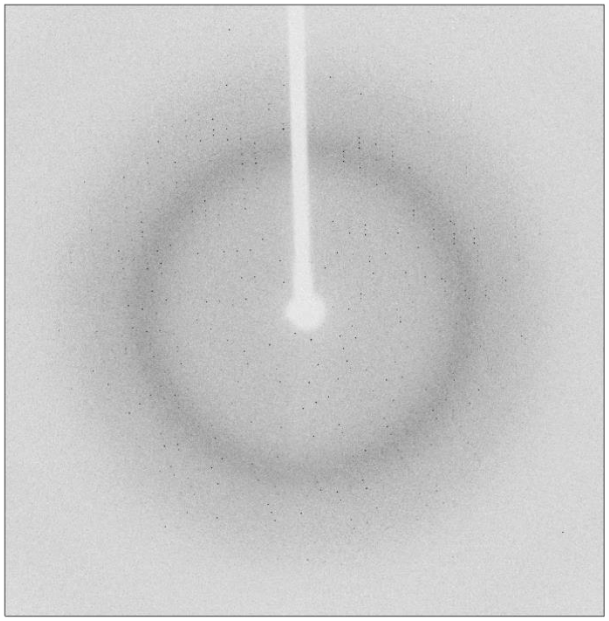
Crystallization/Data collection

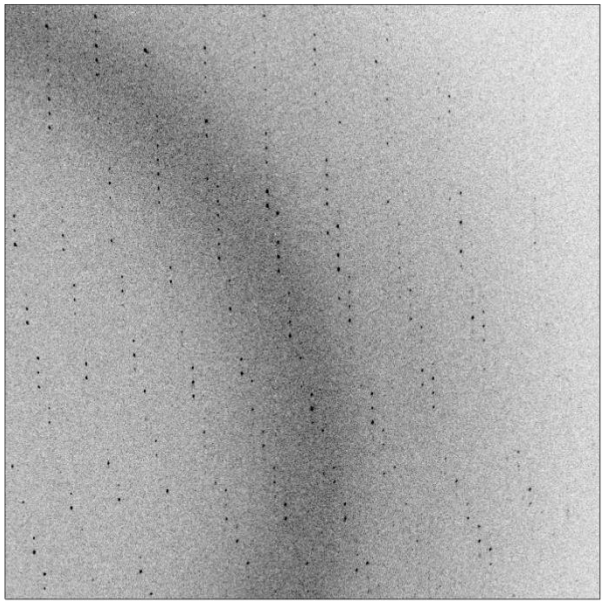
- Gln4 Screened against 1536 different biochemical conditions, ~1000 forming an incomplete factorial of chemical space and ~500 representing commercially available screens.
- Crystal leads seen, several were chosen based on ease of cryoprotection of the native hit.
- Crystals were optimized with a Drop 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

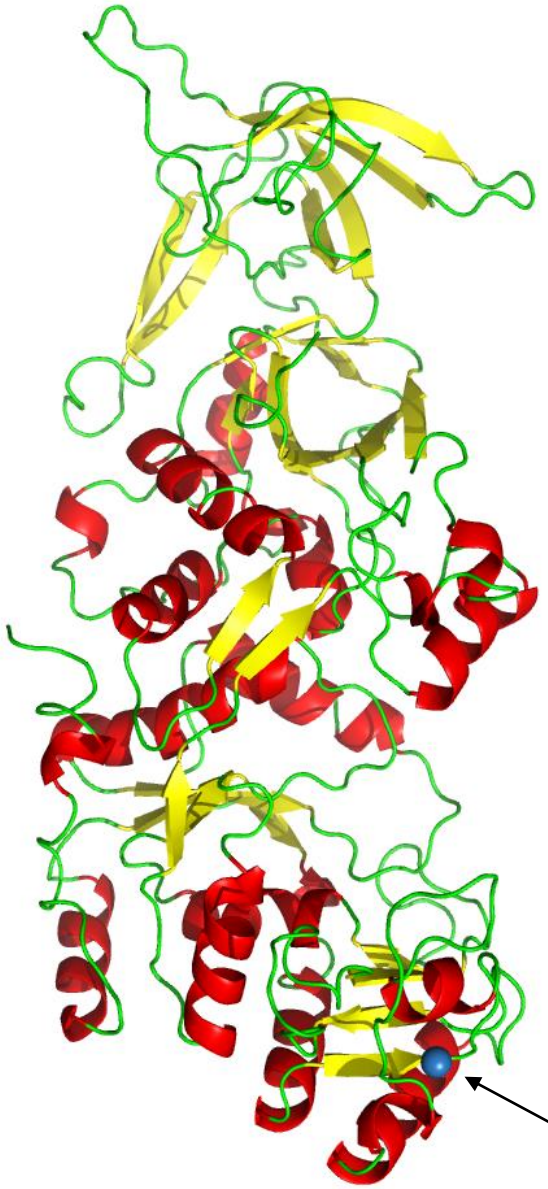




Data collection/Processing

- 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˚

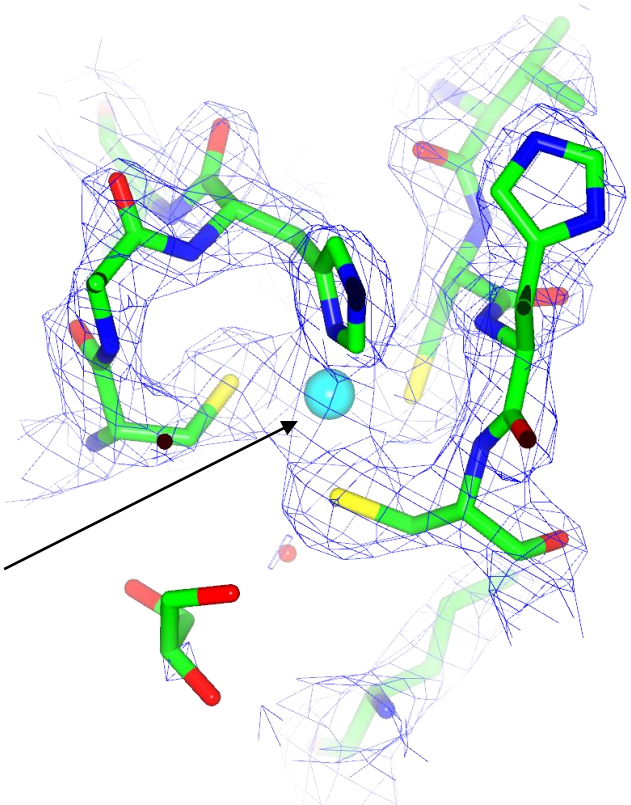
	Overall	Inner Shell	Outer Shell
Low resolution limit (Å)	40.00	40.00	2.64
High resolution limit (Å)	2.5	7.91	2.5
R_{merge}	0.104	0.036	0.743
R_{pim}	0.032	0.011	0.273
	3.2%	1.1%	27.3%
Total number of observations	508484	17694	51511
Total number unique	44752	1523	6332
Mean((I)/sd(I))	24.6	86.6	2.2
Completeness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

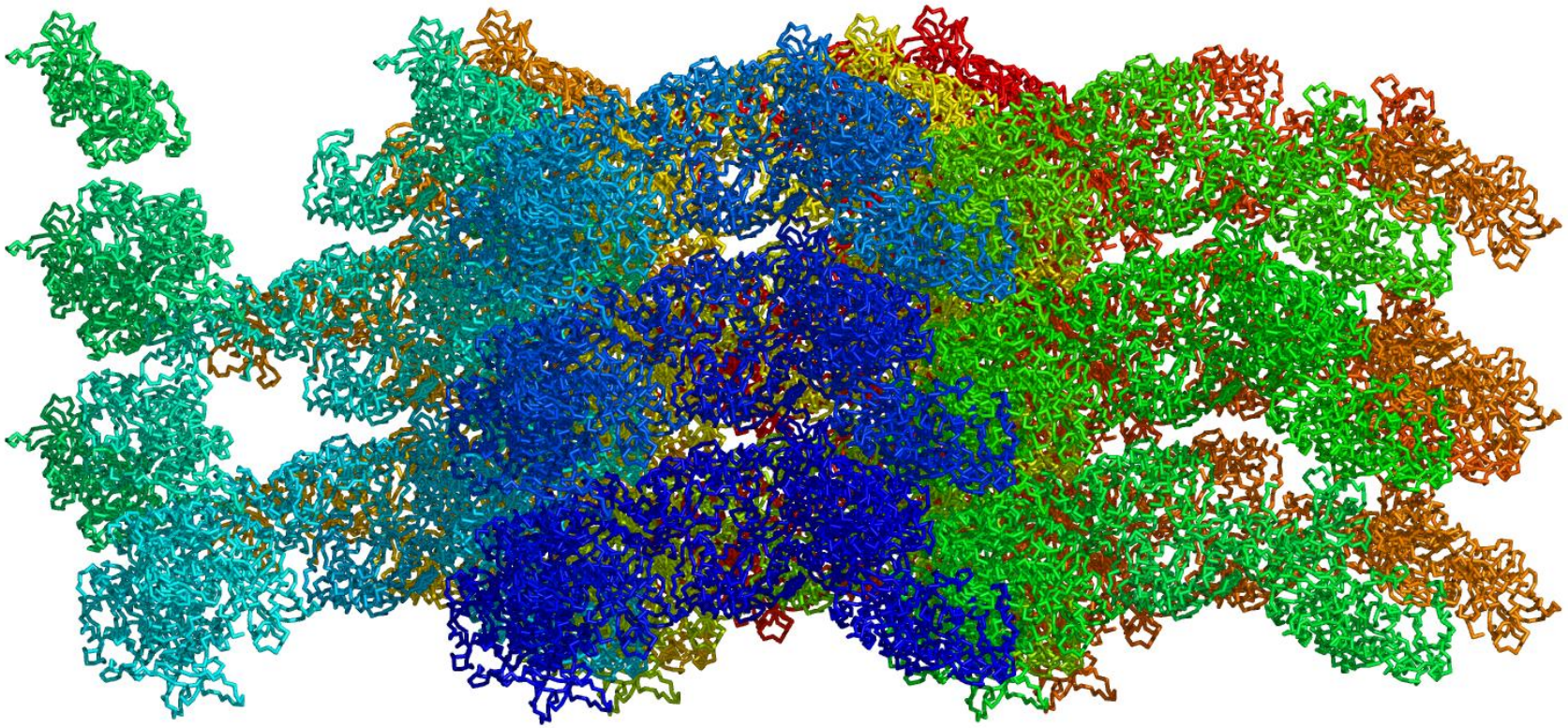


Zinc

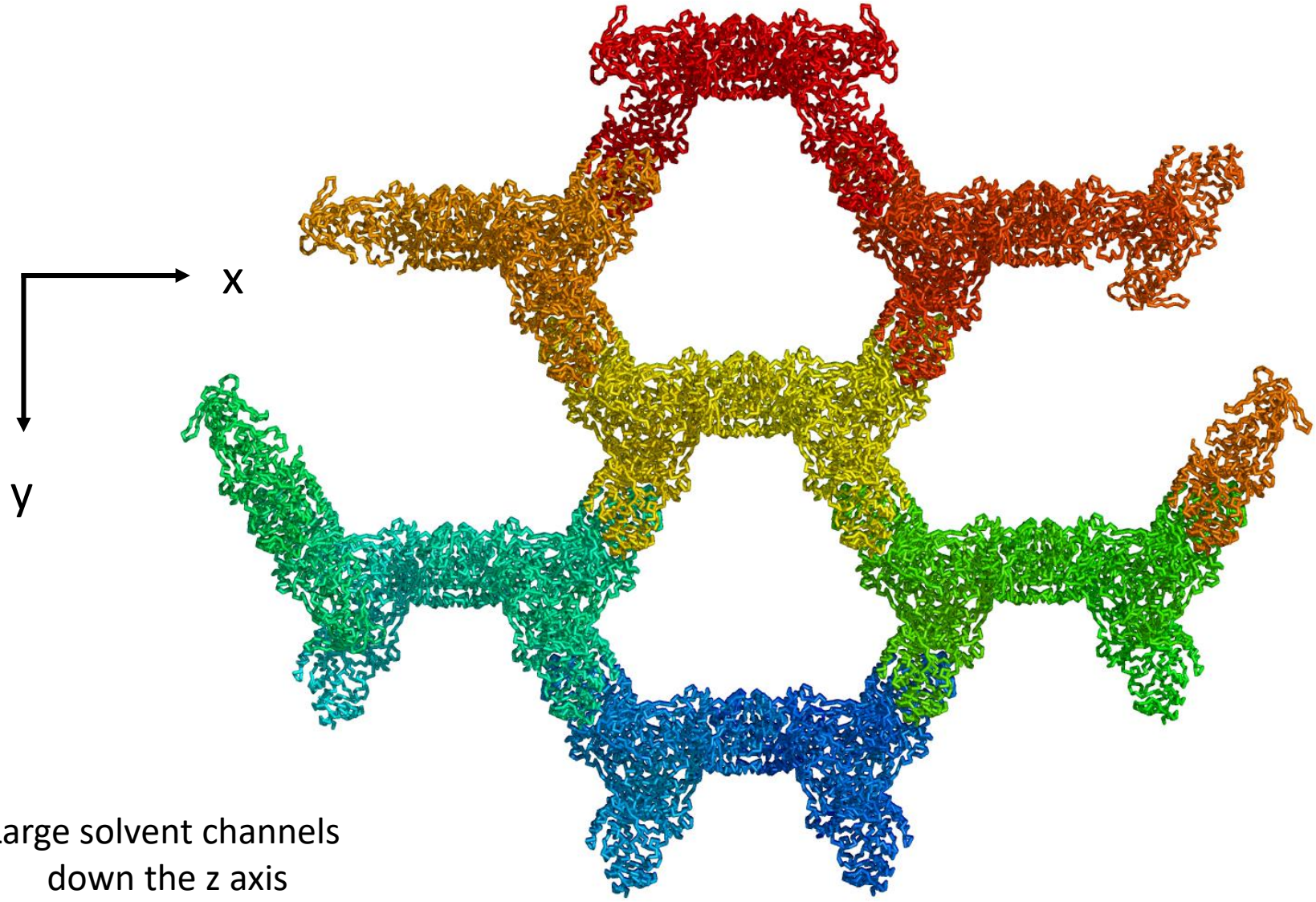
Structure solved (with help of the zinc signal) and refined with Phenix.

Overall R and R_{free} are 14.2 and 19.8% respectively.

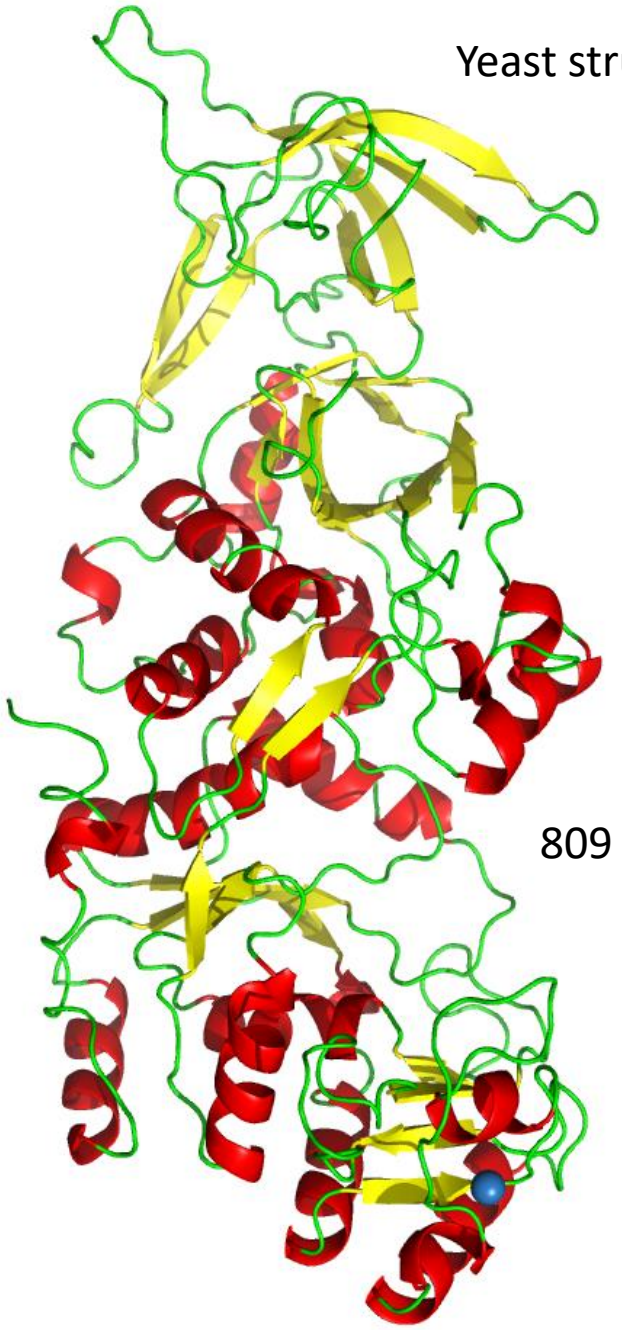




Tight packing in z and y



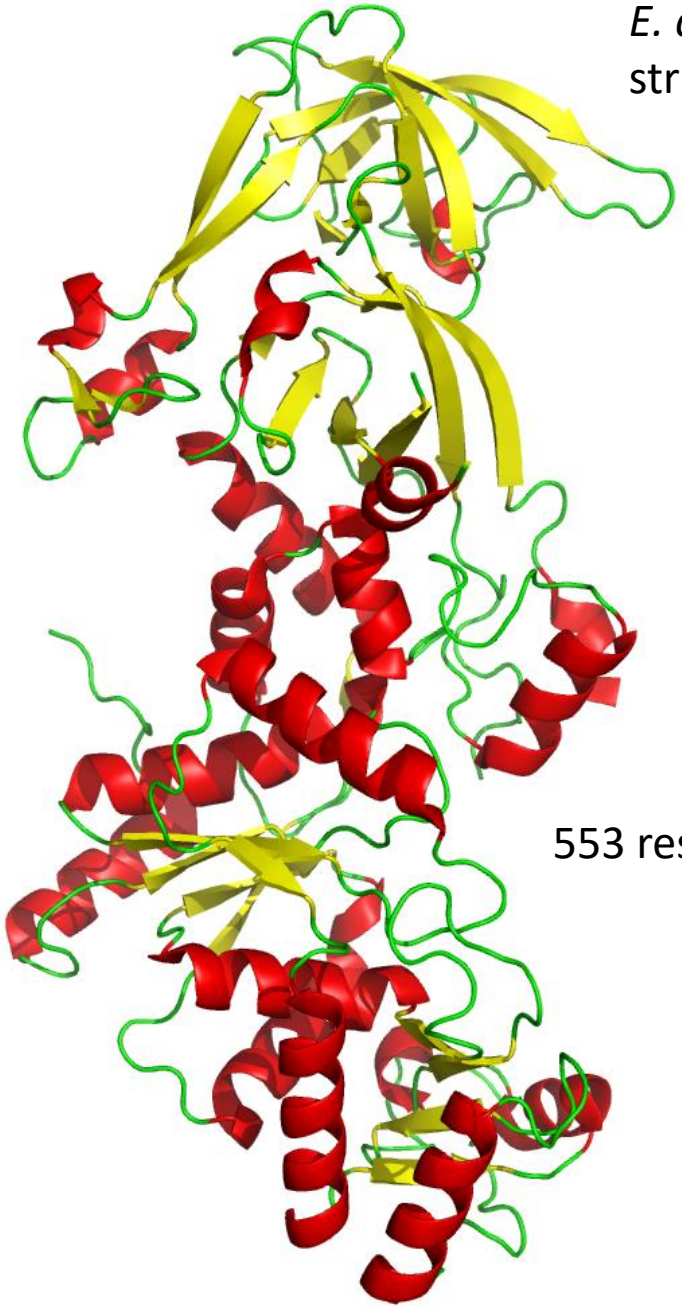
Large solvent channels
down the z axis



Yeast structure

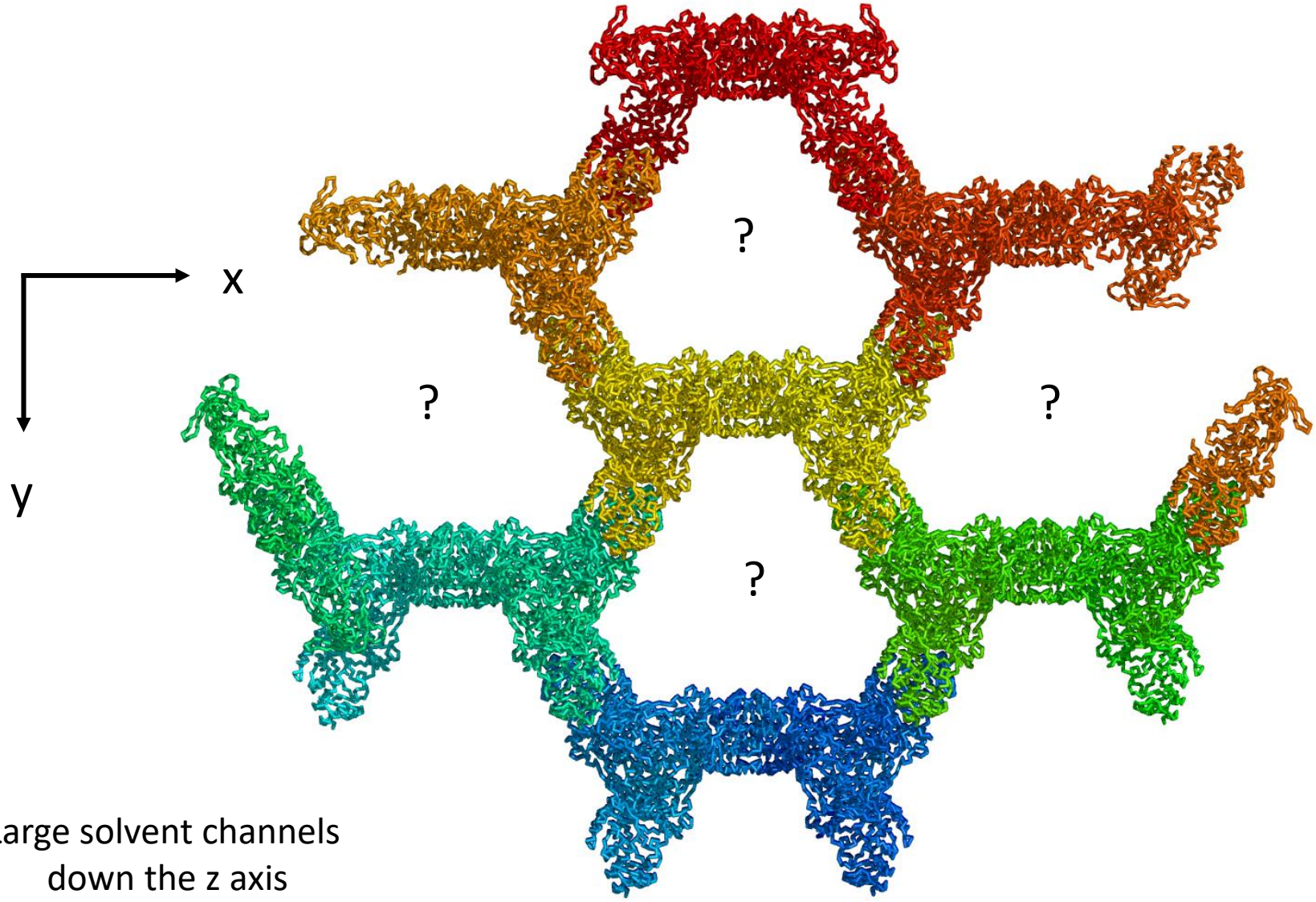
?

809 residues



E. coli. structure

553 residues

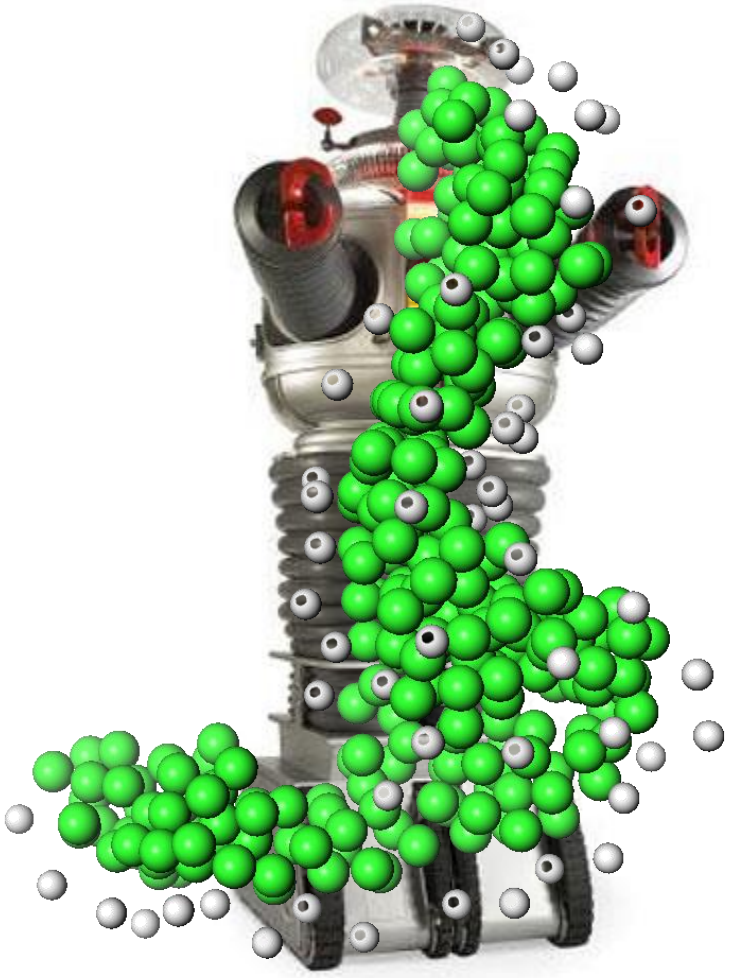


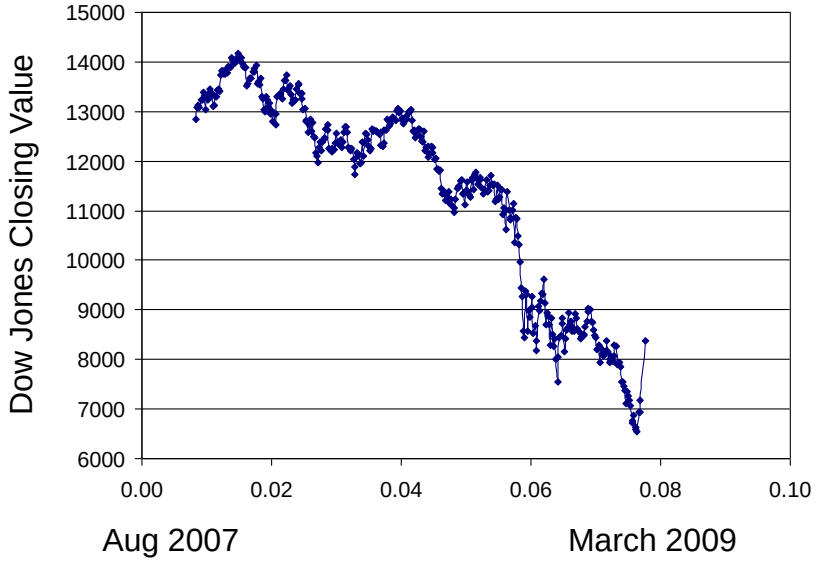
Large solvent channels
down the z axis

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.

Can we use X-ray solution scattering?



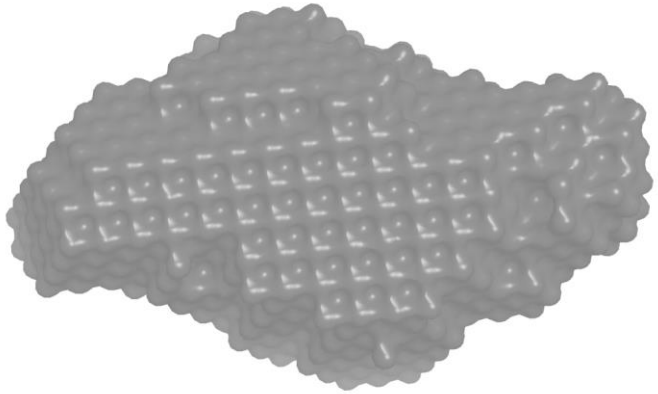


The scattering data from SAXS provides a 1D Fourier transform of the envelope of the particle.

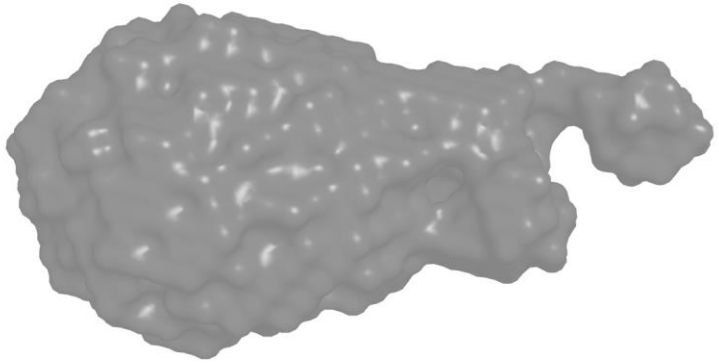
It's possible to fit multiple envelopes to the data.

You will always get an envelope despite the data!

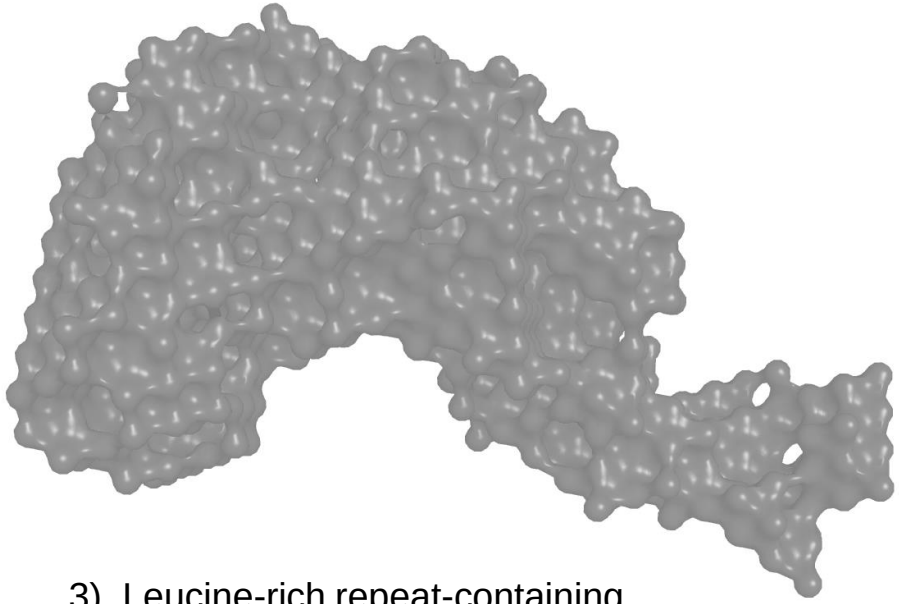
Ab intio envelopes from samples generously supplied by NESG



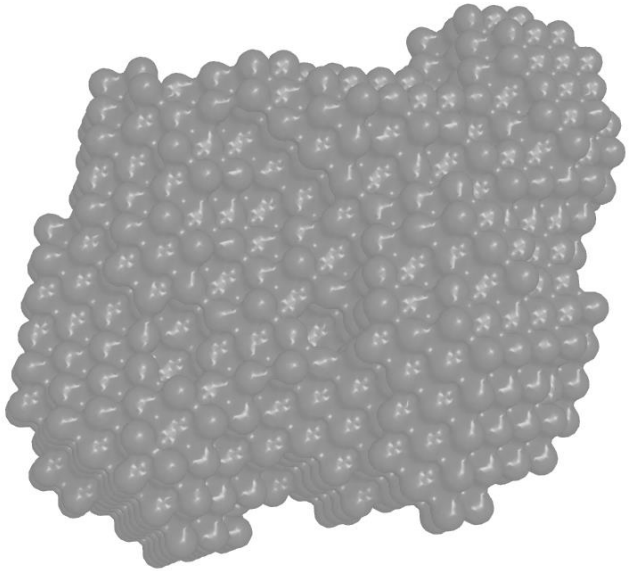
1). alr0221 protein from Nostoc (18.6 kDa)



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

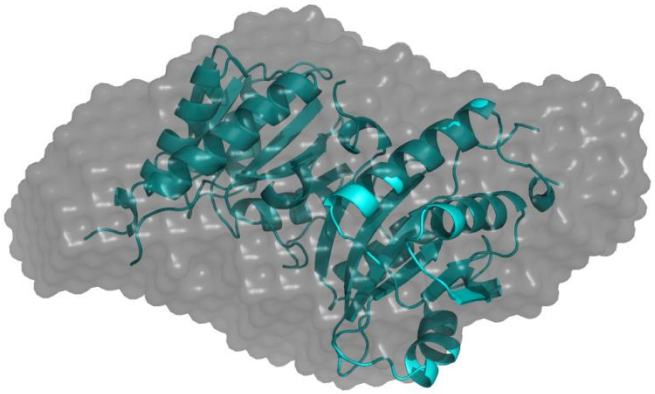


3). Leucine-rich repeat-containing protein LegL7 (39 kDa)



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

Overlaid with subsequent X-ray structures



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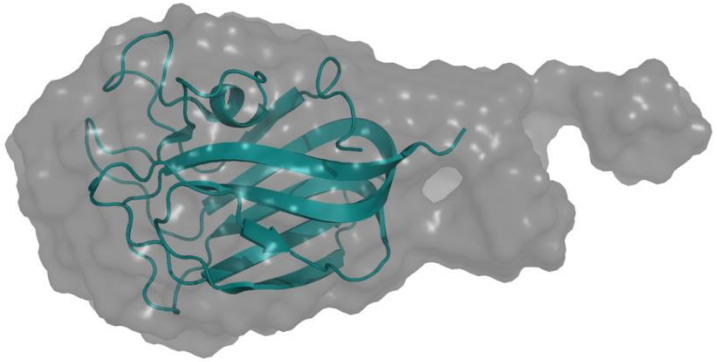
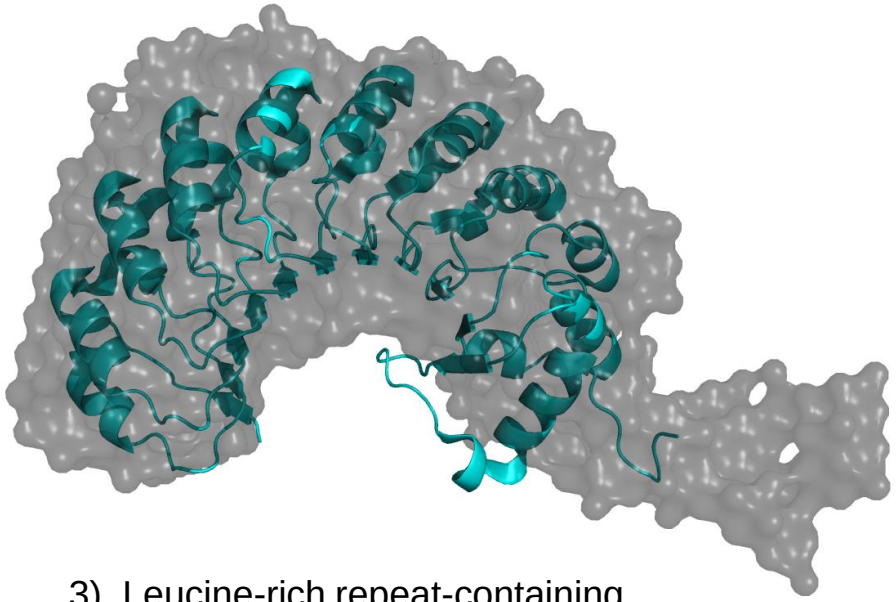


2). C-terminal domain of a chitobiase (17.9 kDa)



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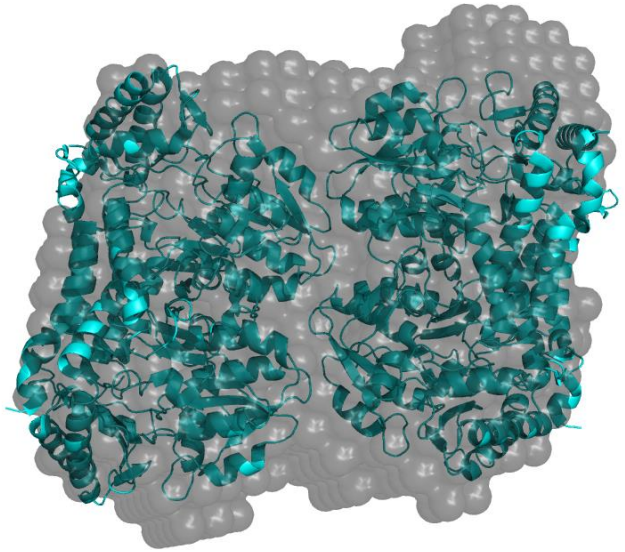

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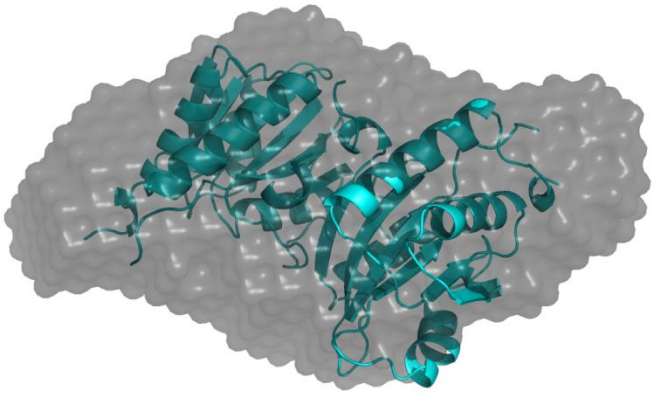
And data on what was missing … (in our case SAXS is reliable)



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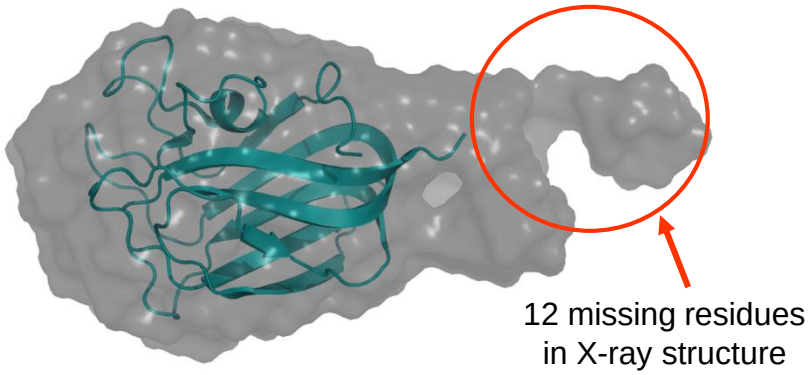
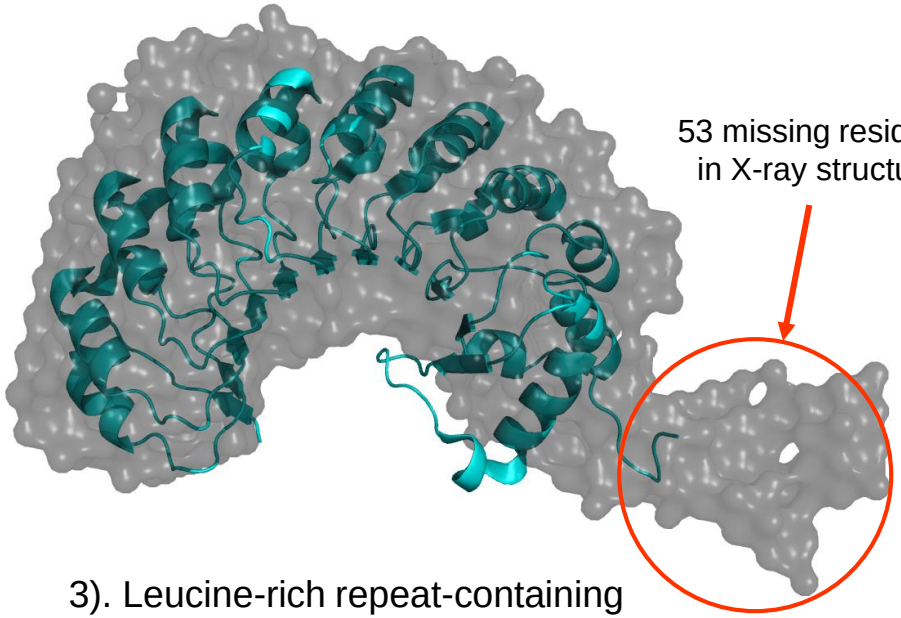


2). C-terminal domain of a chitobiase (17.9 kDa)



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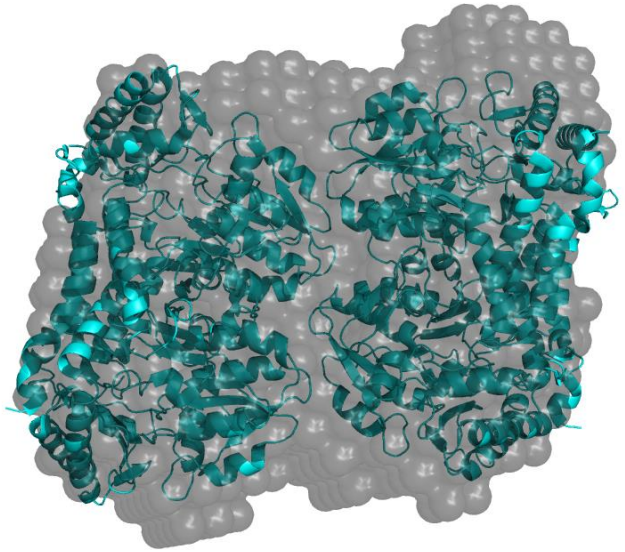
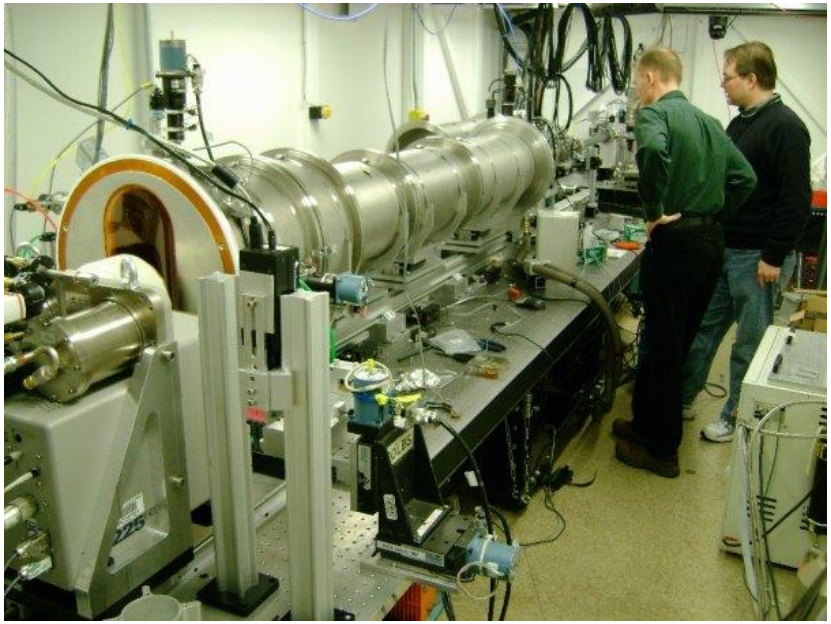


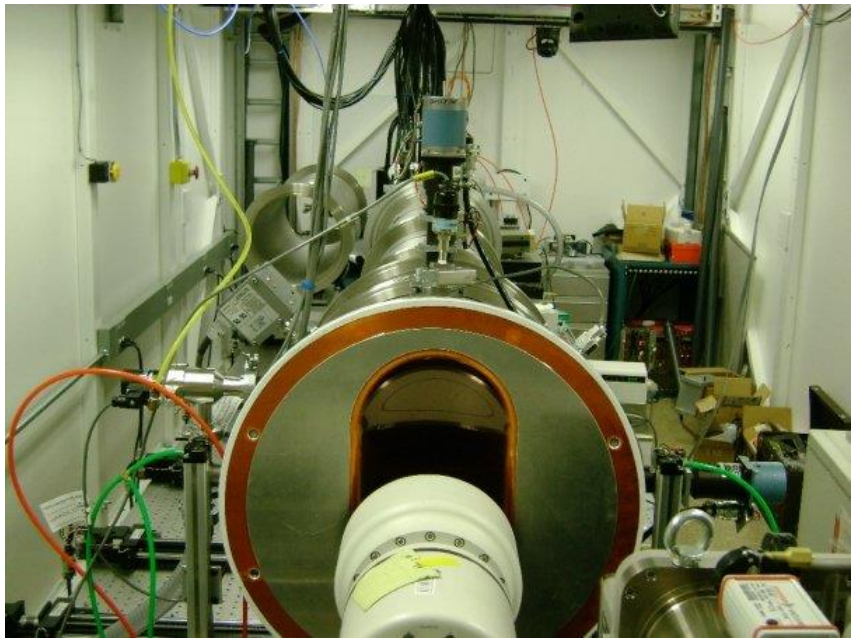
4). *E. Coli.* Cystine desulfurase activator complex (170 kDa)

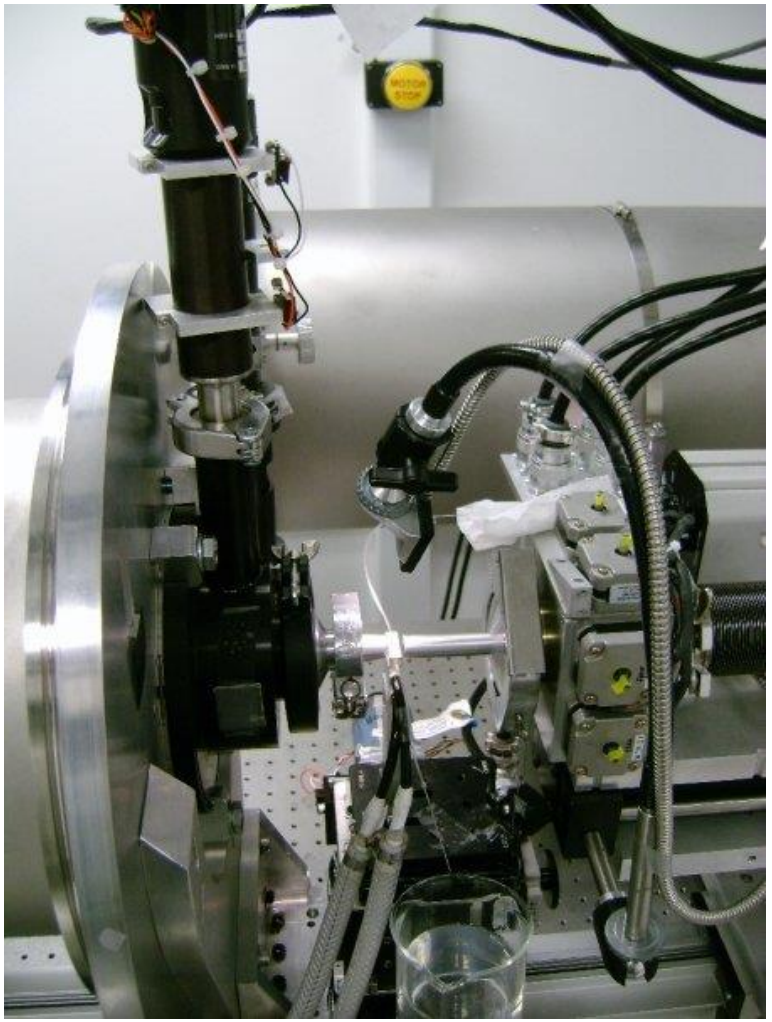
SAXS is complementary to crystallography and ideal for the Gln4 study

- The SAXS solution can be validated when other information is present (**we have crystallographic information**).
- In a non-symmetrical case (most examples) the X-ray derived structure can be fitted to the envelope (**our system is non-symmetrical, at least the known part**).
- 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, i.e. **we can visualize the part missing in the crystal structure**).

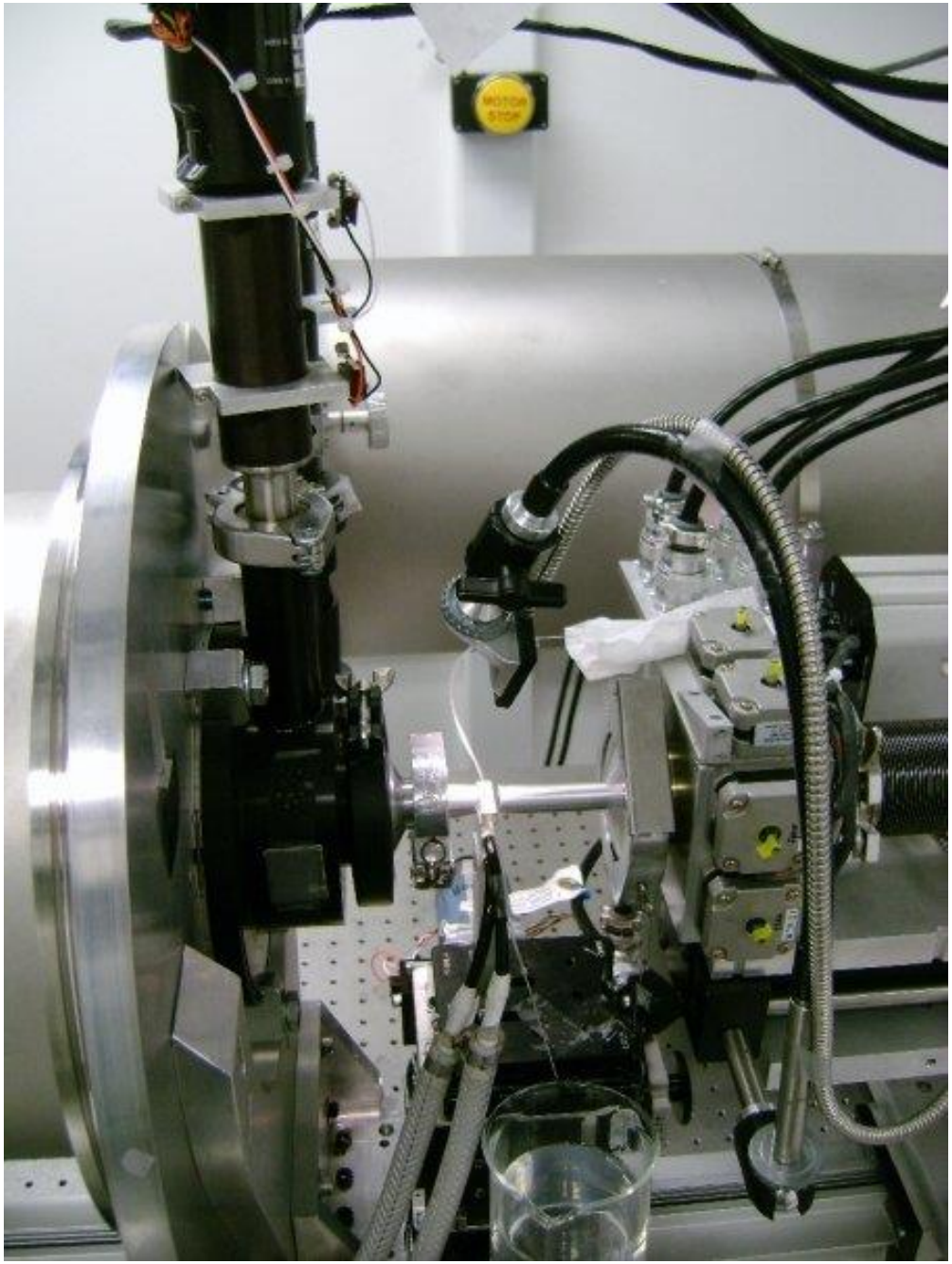
- SAXS is sensitive to information that crystallography does not see.
- SAXS is sensitive to dynamics.
- SAXS is a low-resolution technique - Crystallography is sensitive to information SAXS cannot see.
- **Crystallography and SAXS are complementary.**







SSRL Beamline 4-2 (Being developed with a high-throughput capability)



Initial protocol

5 concentrations

Start with buffer then lowest concentration first

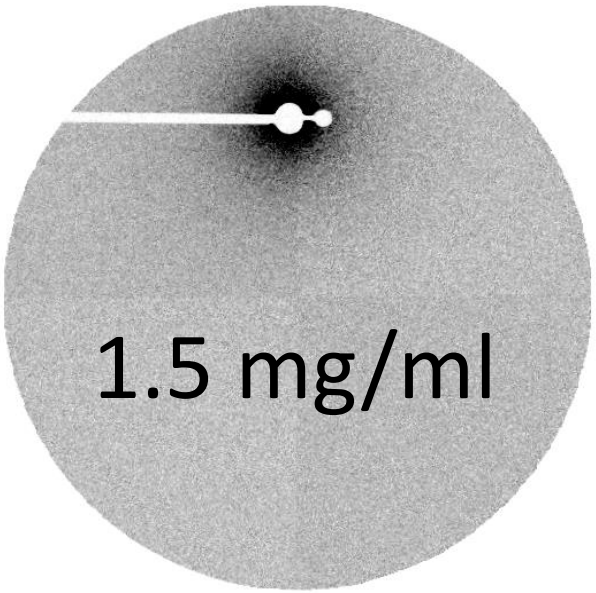
Using the lowest first means that residual protein in the capillary would not alter the assumed concentration greatly.

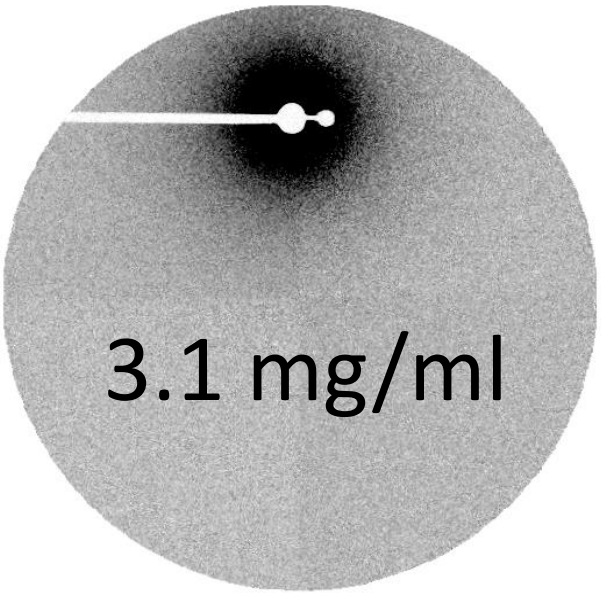
Up to 12 exposures, 1.5s each.

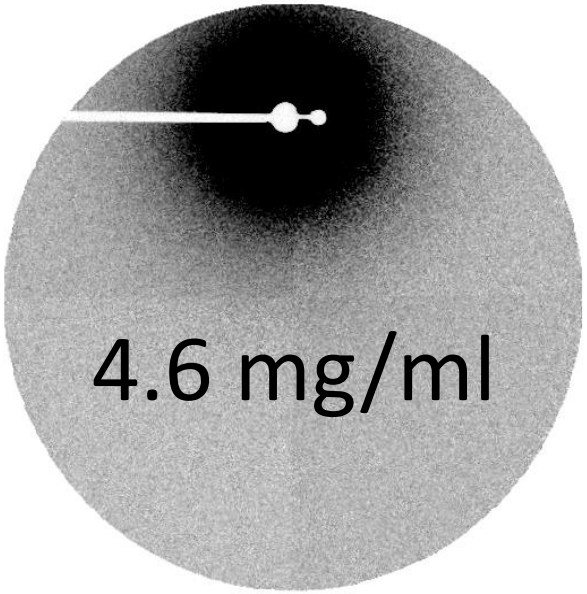
Load next concentrations and repeat.

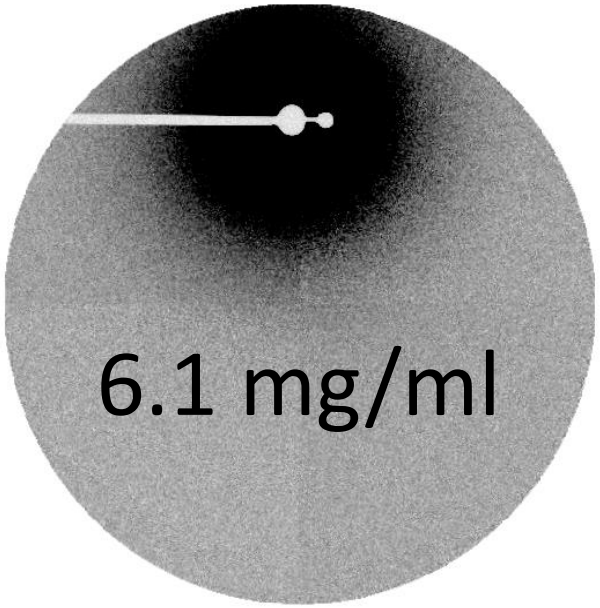
Repeat the buffer.

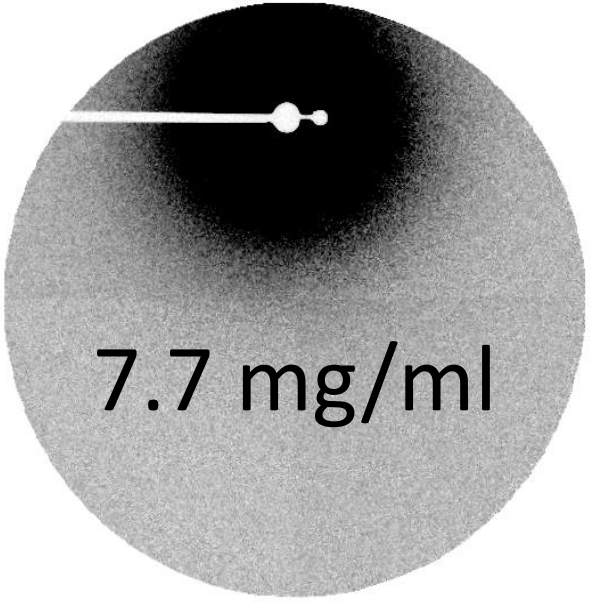
Clean the capillary with bleach followed by water.

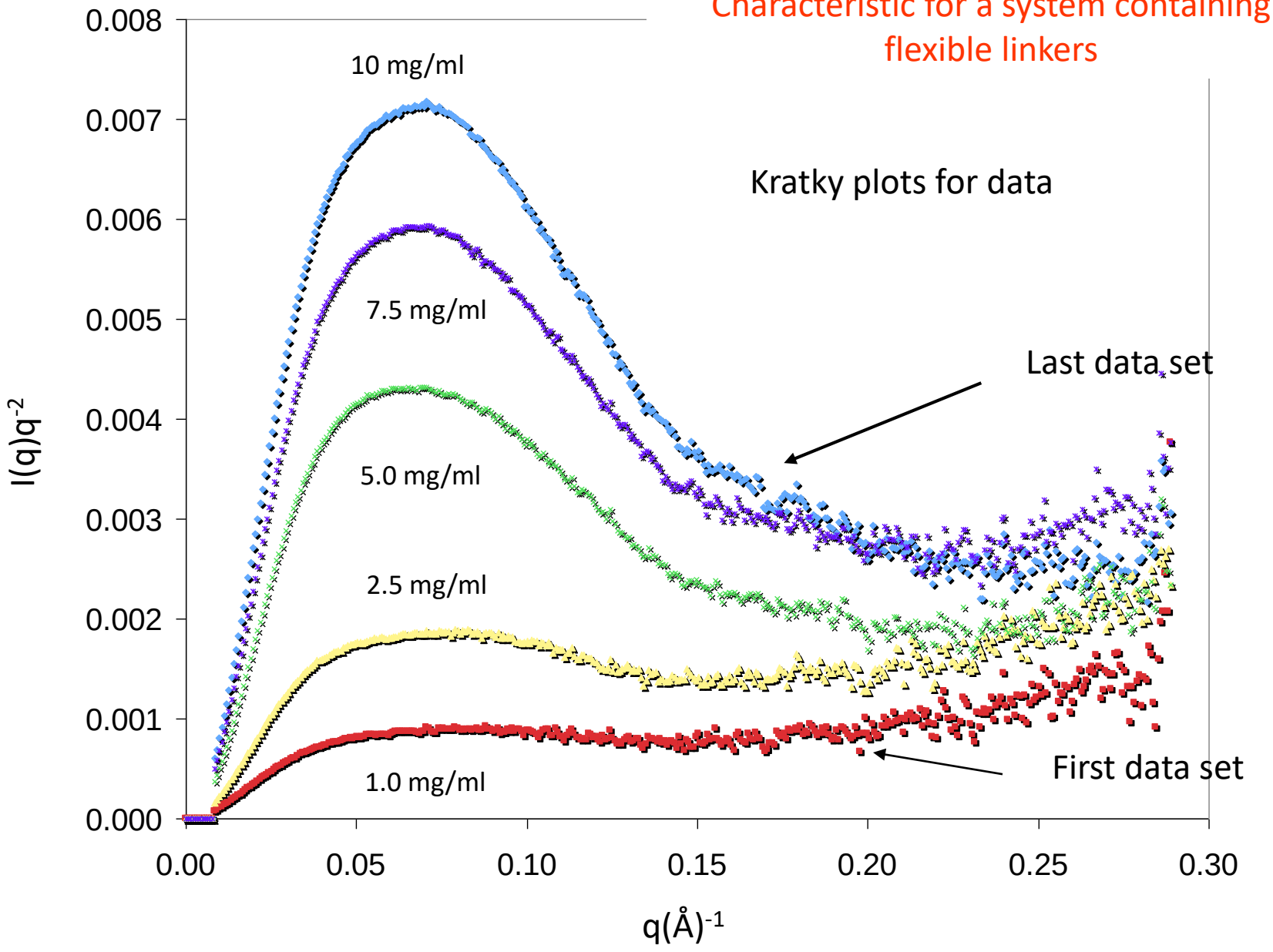

1.5 mg/ml

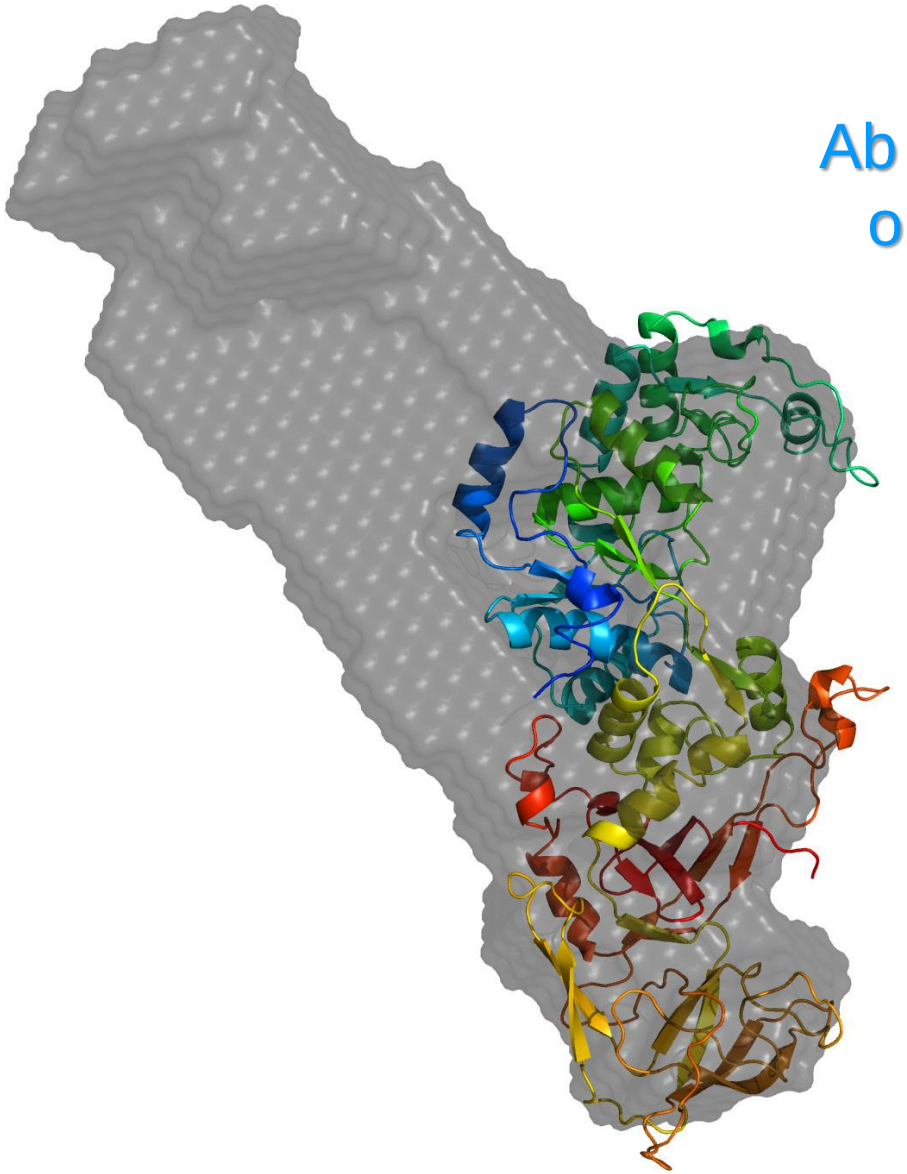

3.1 mg/ml


4.6 mg/ml


6.1 mg/ml

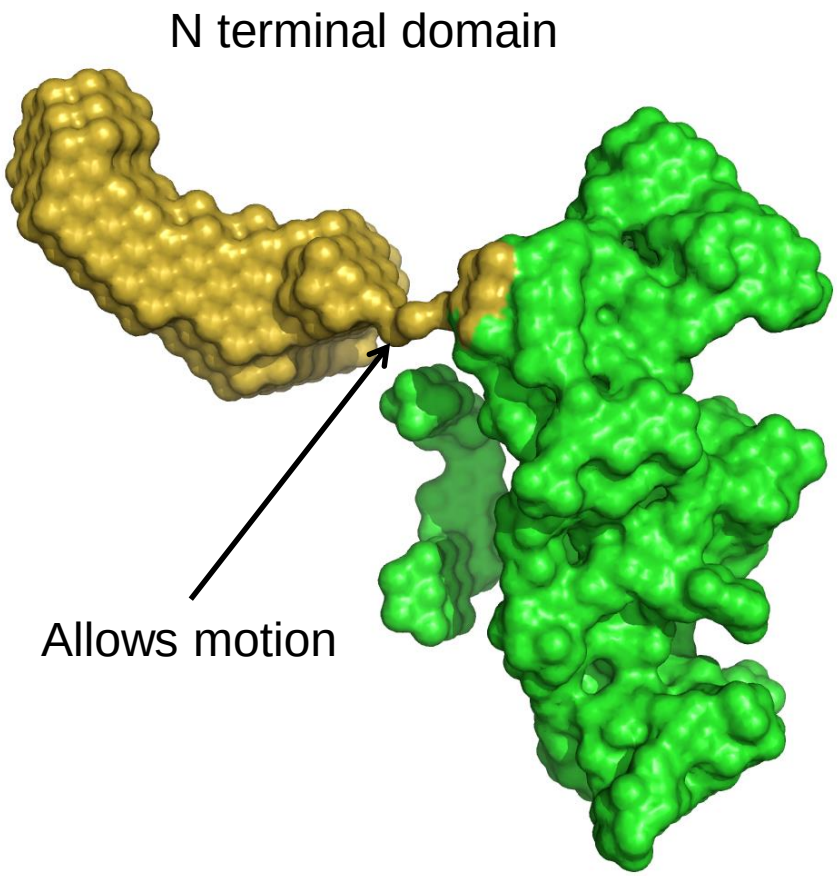

7.7 mg/ml

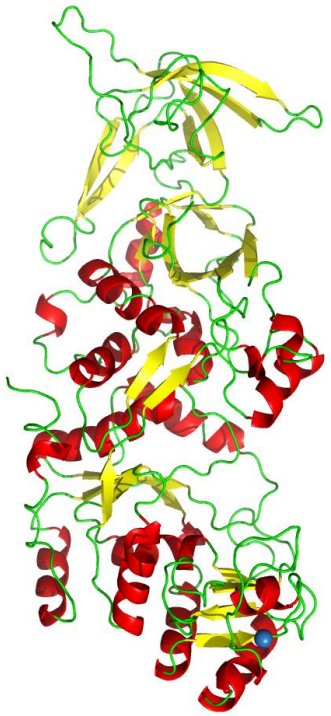

Characteristic for a system containing flexible linkers
Kratky plots for data
10 mg/ml
7.5 mg/ml
5.0 mg/ml
2.5 mg/ml
1.0 mg/ml
Last data set
First data set
I(q)q-2
q(Å)-1
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.00
0.05
0.10
0.15
0.20
0.25
0.30



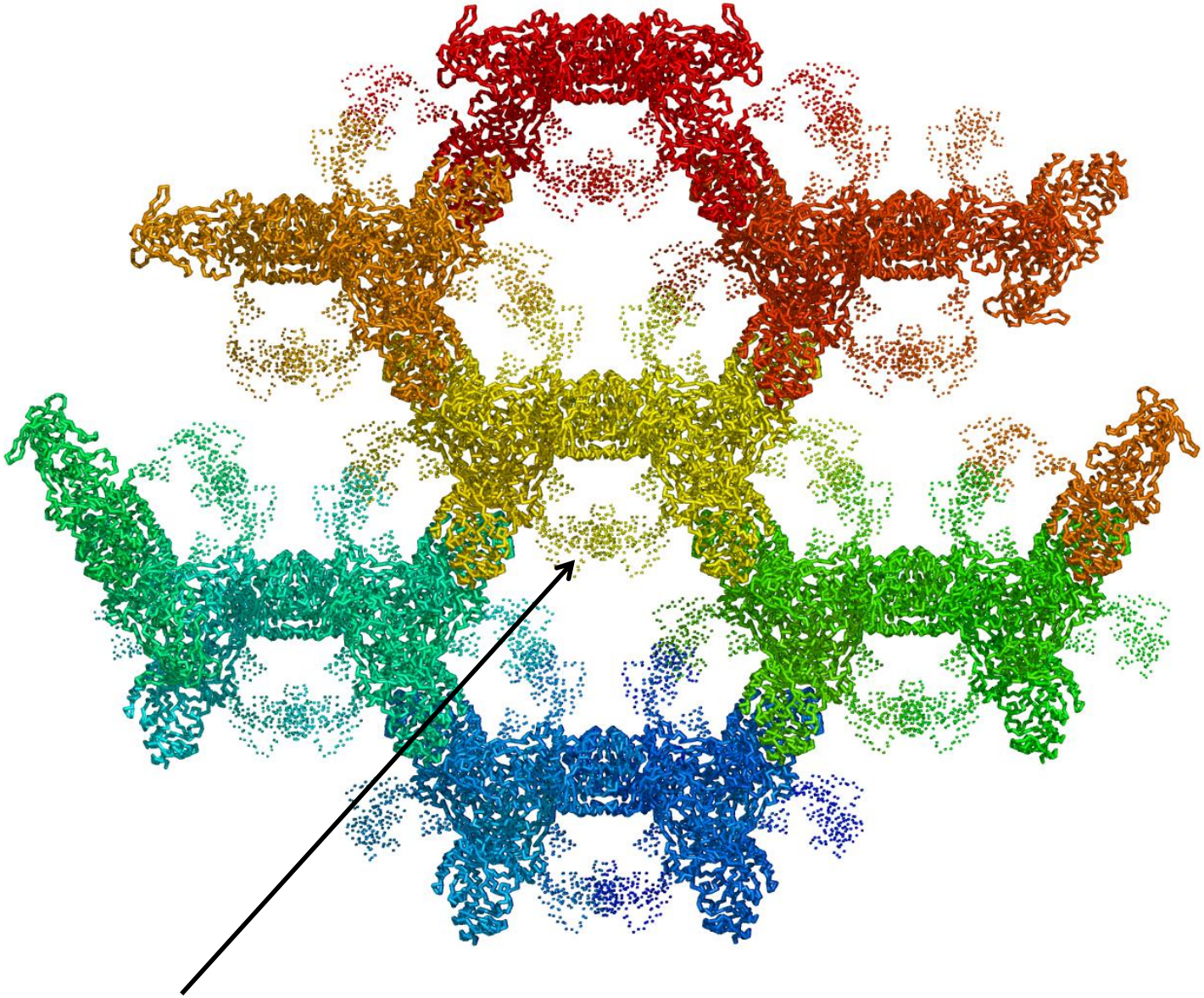
Ab initio structure overlaid on the crystallographic structure

Envelope reconstruction using the crystallographic structure



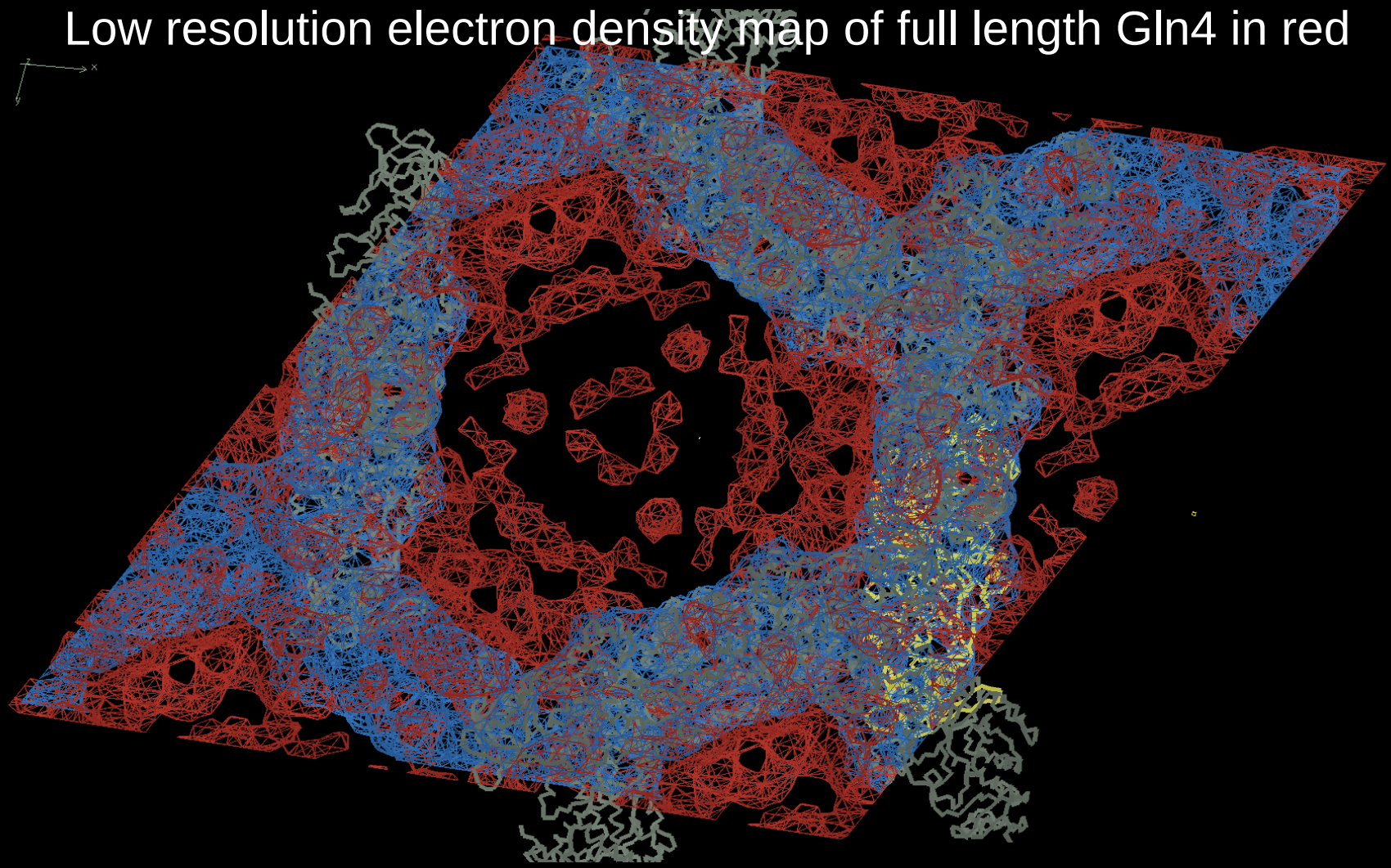


The crystal structure (which shows only the C-domain)



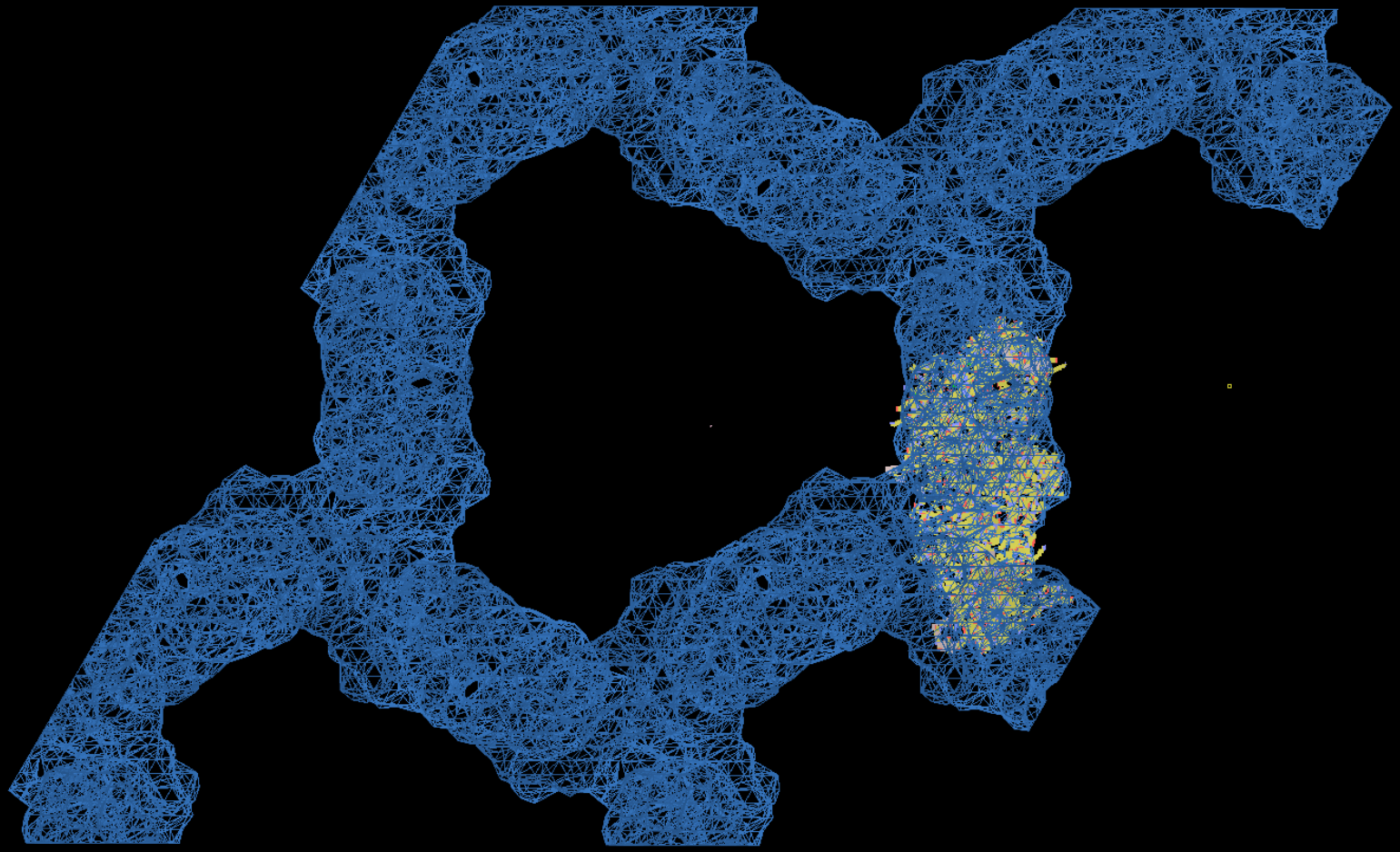
The N-terminal ‘arm’ is completely compatible with the crystal structure

Low resolution electron density map of full length Gln4 in red

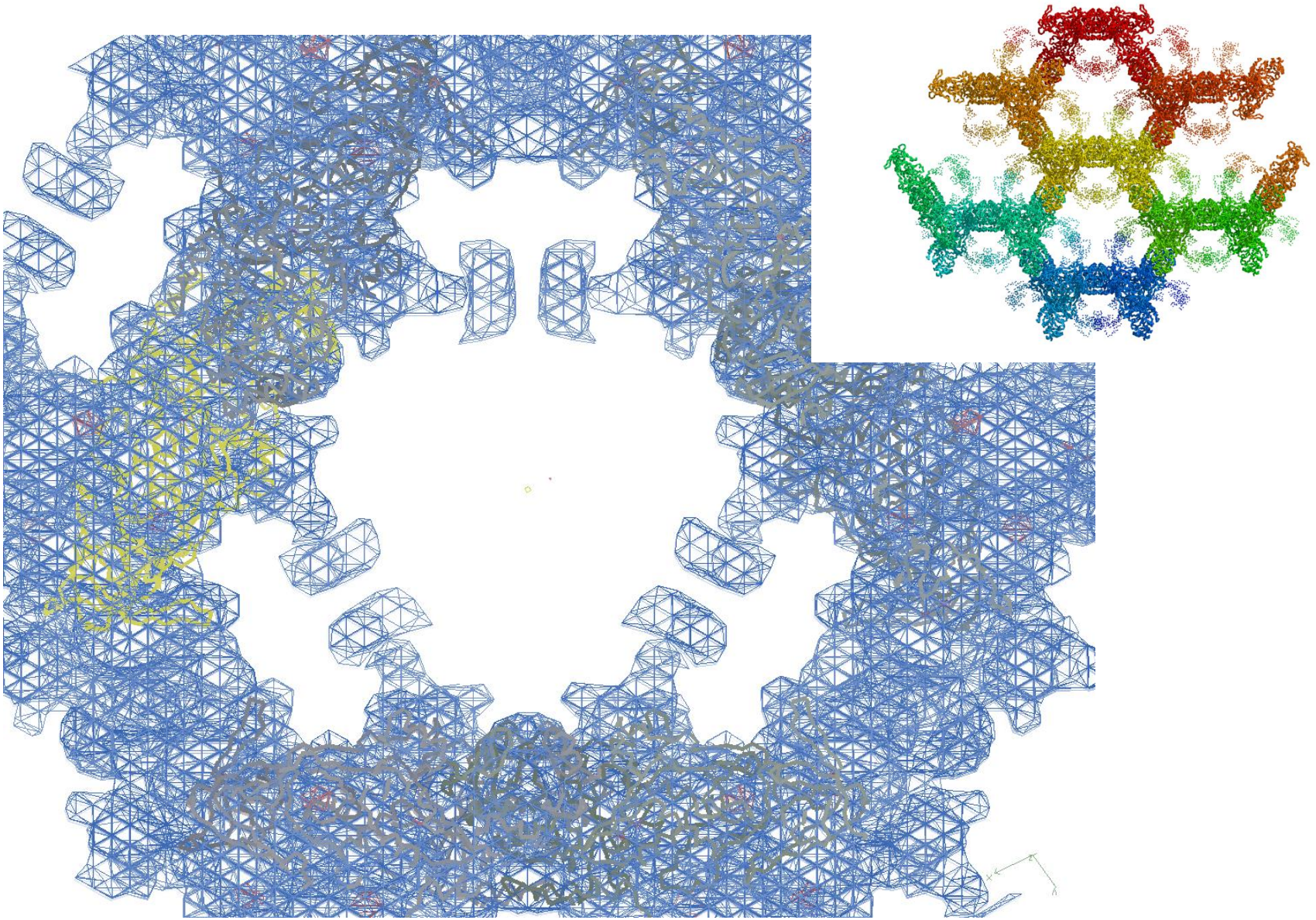


Data truncated to 20A (data to 78A still plenty of reflections due to geometry and wavelength used purposely used for data collection)

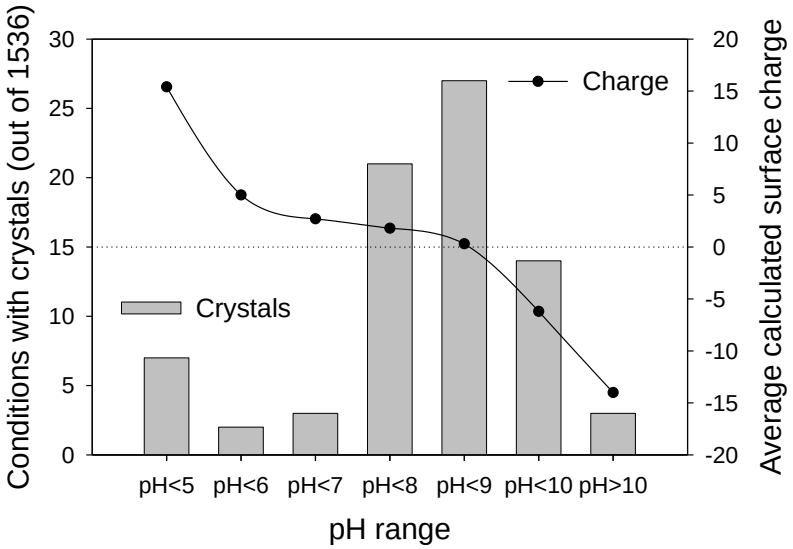
Gln-4 with N-terminal arm cleaved



Crystallized, data truncated to 20A (data to 78A still plenty of reflections due to geometry and wavelength used purposely used for data collection)



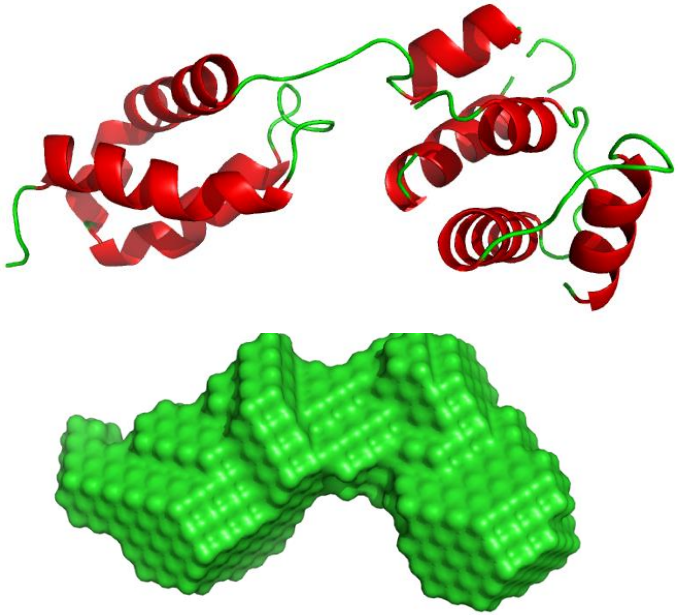
Low resolution truncation (15 Å) of the single crystal data, 1σ, real but not traceable?

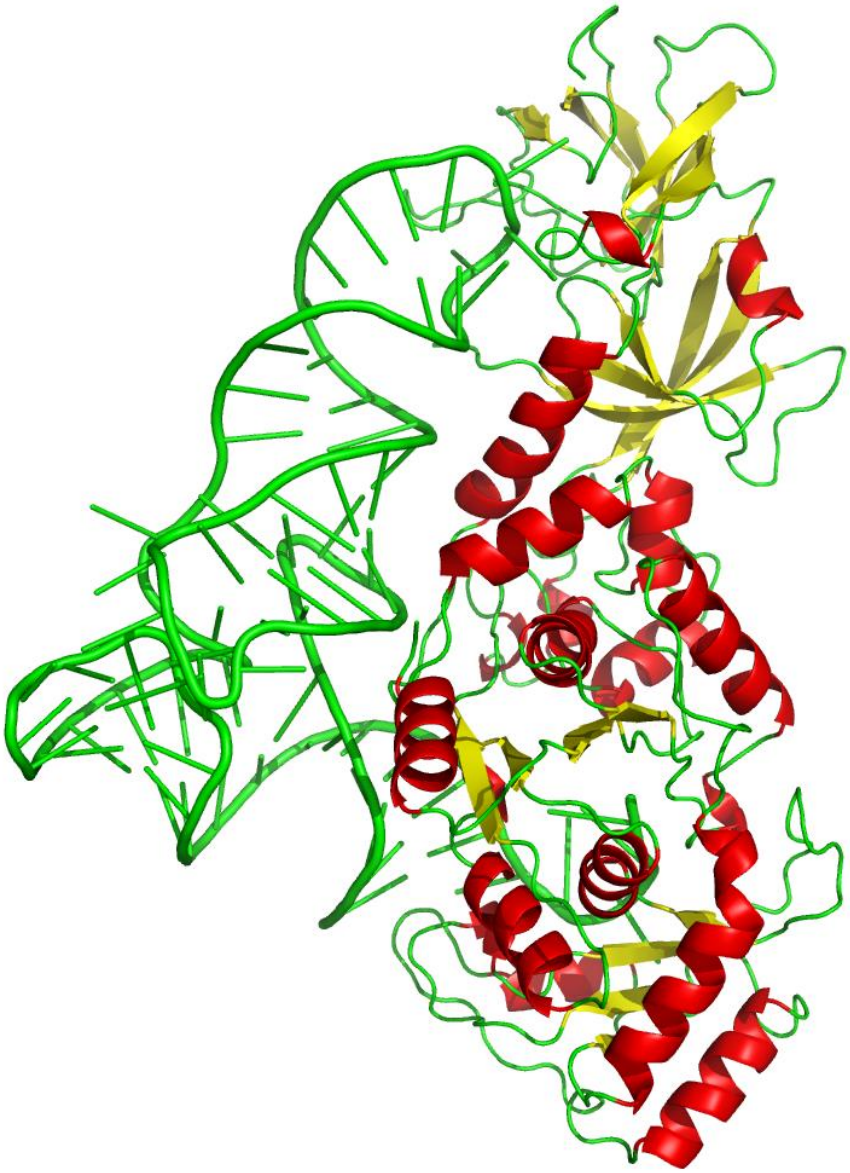


Initial screening of the n-terminal 187 amino acid component gave multiple hits. Using a recently developed mounting method we were able to salvage SeMet crystals of the N-term from several 400 nl drops out of the 1536 screening plate. X-ray data was collected.

A MAD solution was obtained (7/13/10) and is currently being refined.

SAXS data of the arm was also recorded along with that for the truncated C-terminal.





The E-coli structure with bound tRNA is available.

Is the N-terminal arm in *Saccharomyces cerevisiae Gln4* a common feature, i.e. is it important?

Yes – we also see it in SAXS data for *C. glabrata.*

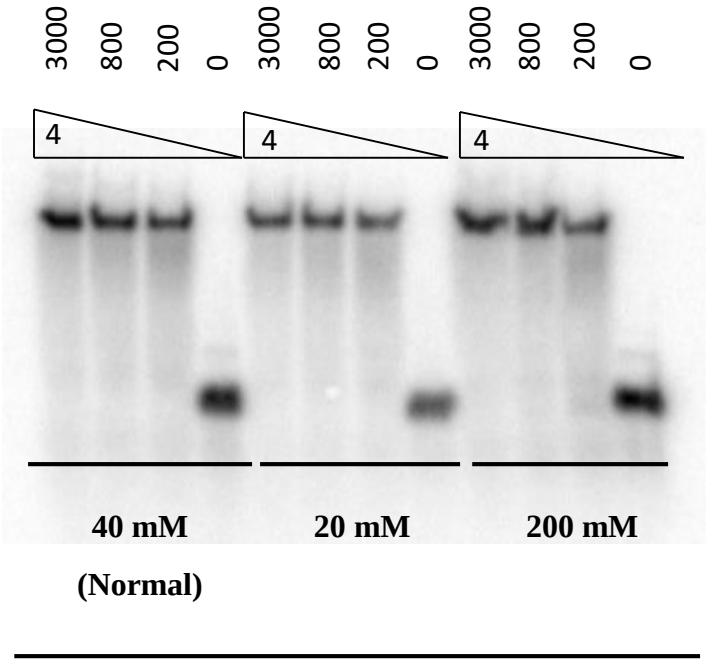
What is the function of the extra N-terminal arm in Gln-4?

Possibilities are recruitment or regulation?

We don’t know until we see how it interacts with the tRNA.

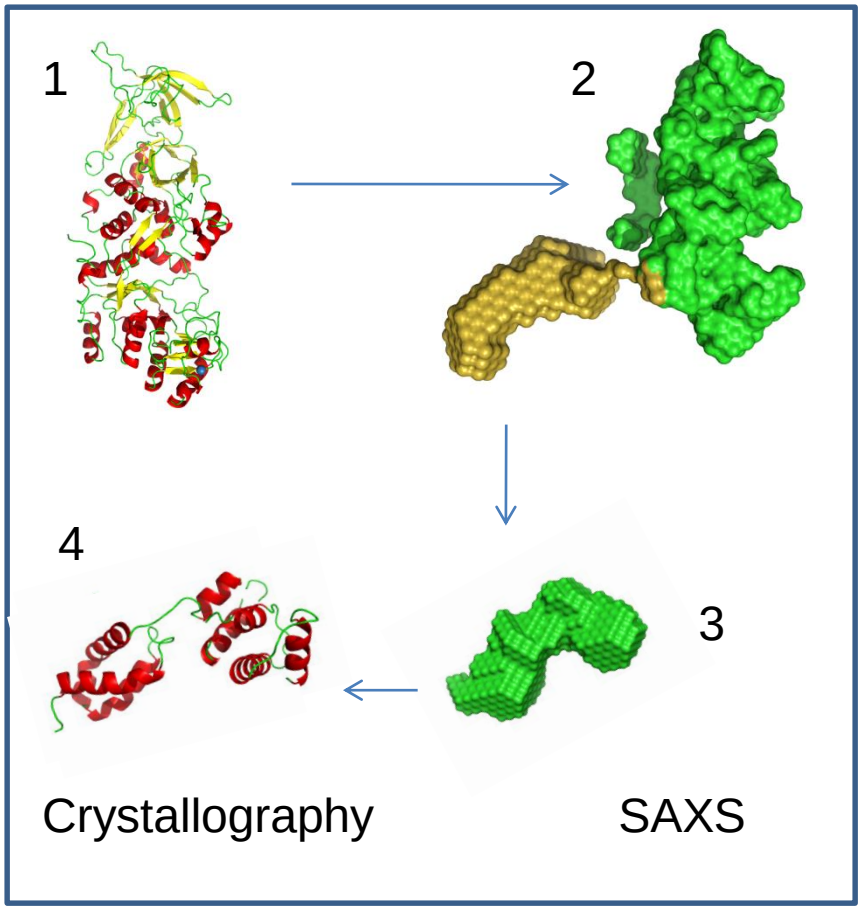
Probing the interaction of full length Gln-4 and tRNA

Gln4 Protein (nM)



We have been able to obtain tight binding with native tRNA and can now produce the complex in large quantities.

Summary



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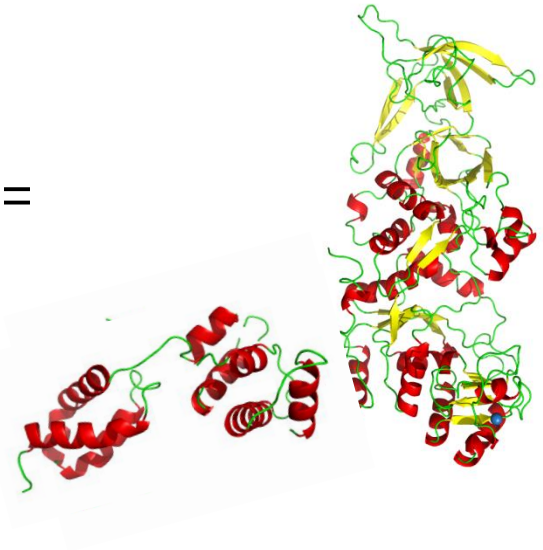

+ tRNA (in progress)

and similar data from an ortholog (not shown)

//

An answer to the role of the additional domain appended to C-terminus of Gln4 and to why these appended C and N terminal domains are important in eukaryotic tRNA synthetases compared to their prokaryotic homologs.

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Questions?

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