Developing tools to transition high-throughput crystallization to high-output crystallography

A case study with eukaryotic Glutaminyl-tRNA synthetase

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An introduction to the screening laboratory at the Hauptman-Woodward Medical Research Institute

Since February of 2000 the High Throughput Search (HTS) laboratory has been screening potential crystallization conditions for the general biomedical community and two Protein Structure Initiative large-scale structure production centers (NESG, Montelione, PI; SGPP/MSGPP, Hol, PI) and one PSI specialized PSI-2 center (CHTSB, DeTitta, PI).

The HTS lab screens samples against an incomplete factorial screen of two categories of crystallizing agents:

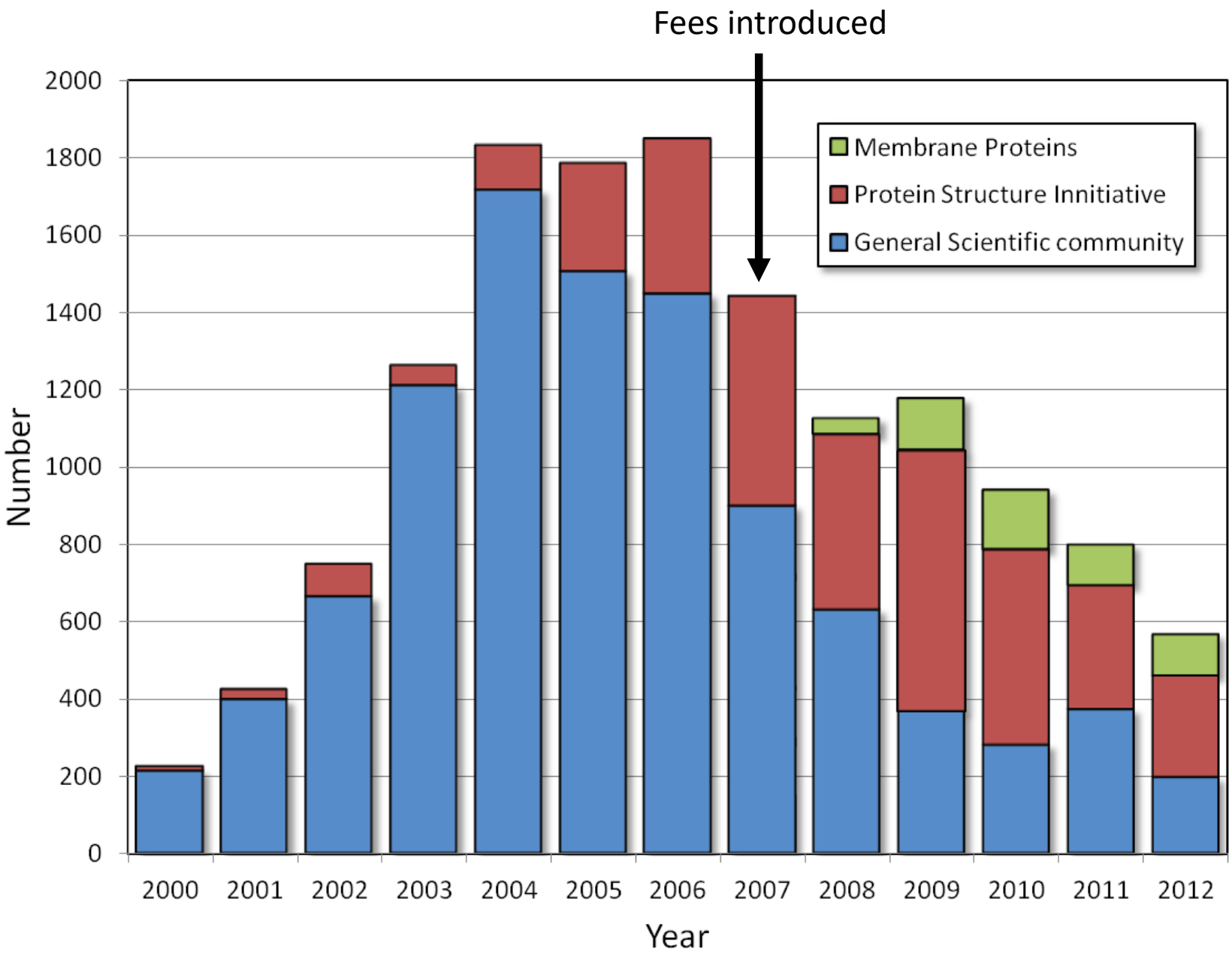
1. buffered (4<pH< 10), highly concentrated salts (35 salts total, sampling 18 different cations and 20 anions) – 229 conditions.
2. PEG/salt/buffer solutions (eight buffers (4<pH< 10), six molecular weight PEGs at three concentrations, and 35 salts at fixed 200 mM concentration) – 721 conditions.

Added to this is a screen of some 586 conditions encompassing screens commercially available from Hampton Research.

The crystallization method used is micro-batch under oil with 200 nl of protein solution being added to 200 nl of precipitant cocktail in each well of a 1536 well plate.

Wells are imaged before filling, immediately after filling then weekly for six weeks duration with images available immediately on a secure ftp server.

The HTSlab has investigated the crystallization properties of over 13,900 individual proteins archiving over 115,000,000 images of crystallization experiments.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Protein Structure Innitiative
General Scientific community
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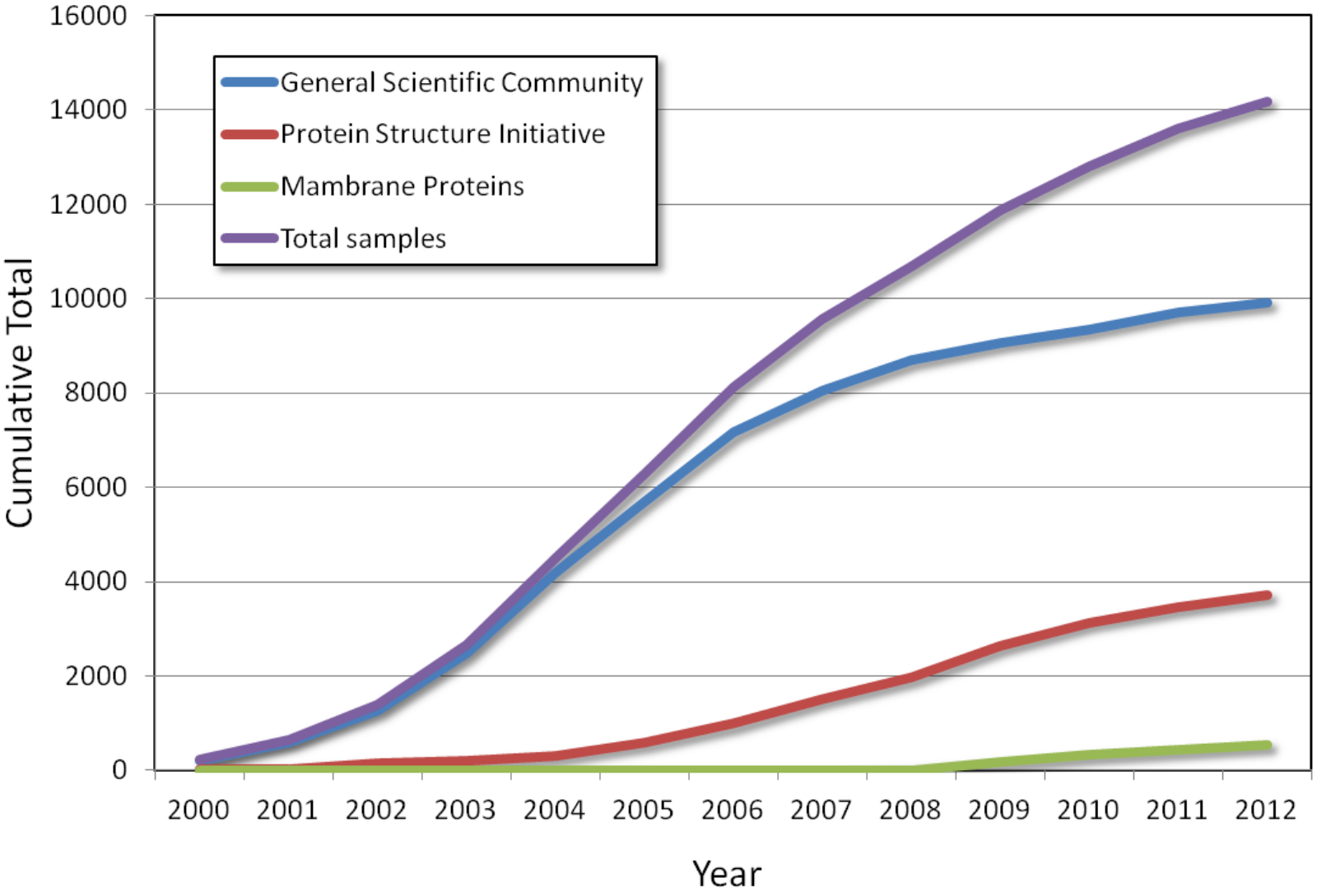
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Cumulative Total
General Scientific Community
Protein Structure Initiative
Mambrane Proteins
Total samples
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2003
2004
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2012
Year

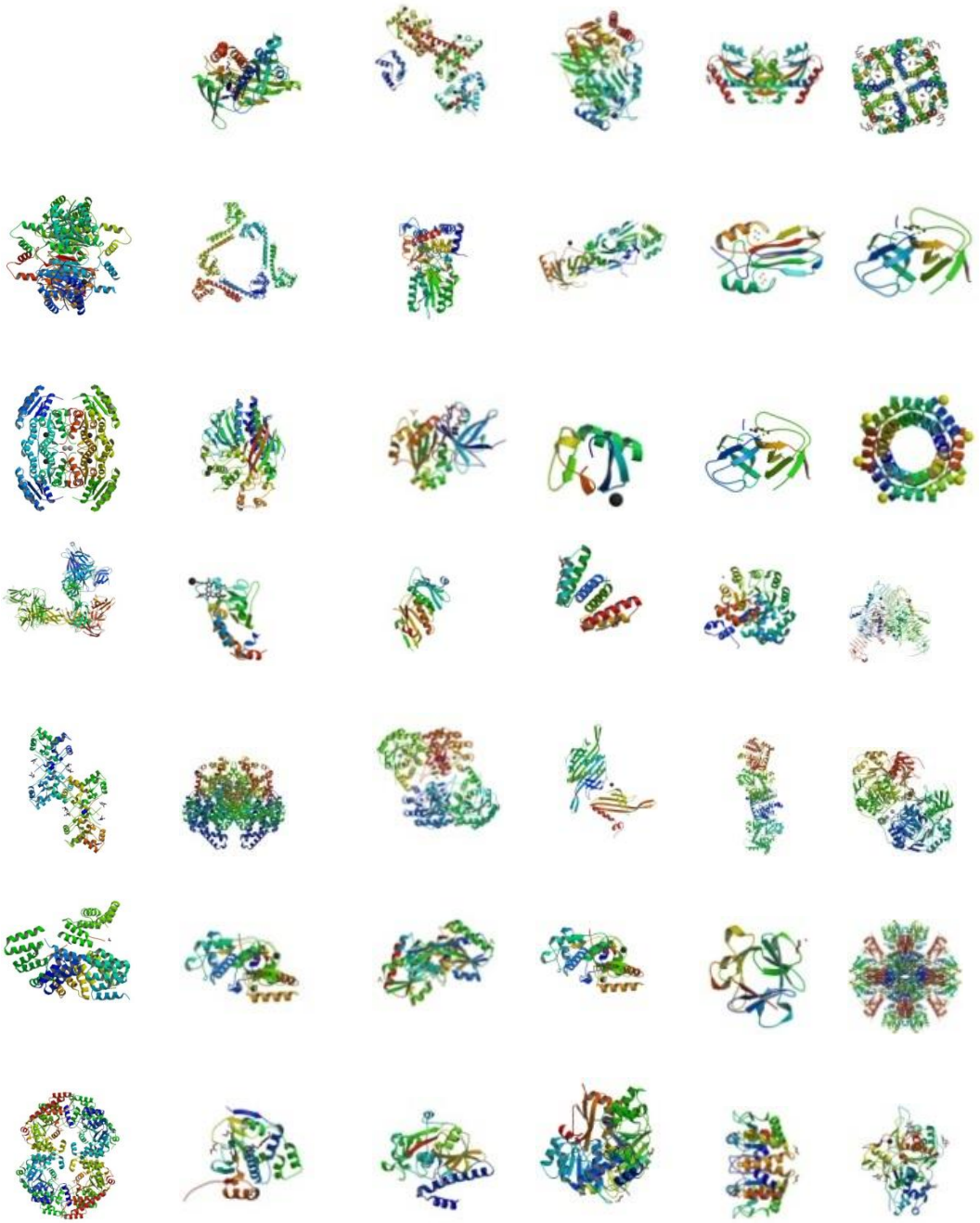
Born in Buffalo

Over 1,000 general biomedical laboratories world wide use the crystallization screening service with approximately 2,000 unique investigators.

Investigators are sent photographs of the results, analyze these images and perform their own optimization of any hits observed.

No information is released on targets. Progress is tracked by acknowledgements and citation searches. Currently no other metrics are used to measure success rates for the general biomedical community.

These images represent examples of structures from initial hits in the HTS laboratory.



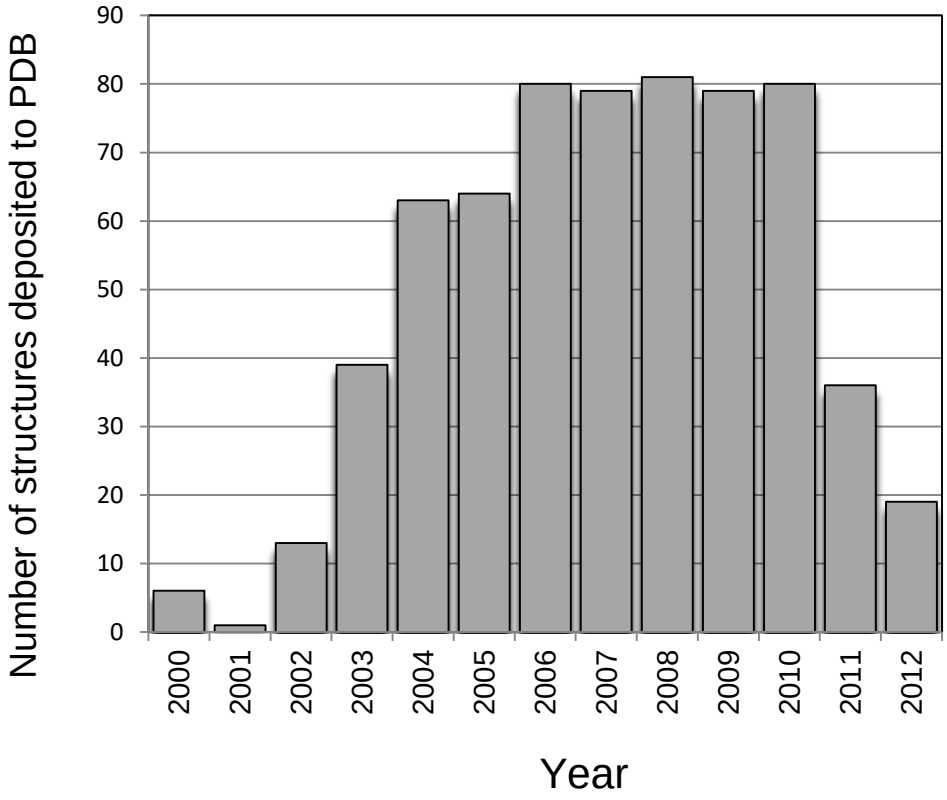
Where success is tracked.

For our Protein Structure Initiative partners both success and failure is tracked. In the case of NESG our initial screening hits enable on average 80 structures per year to be deposited to the PDB.

The graph demonstrates the ramp up of operations with maximum success reached from 2006 onward.

Our success rate from protein in the door to a crystallization hit leading to a PDB deposition is **22%**.

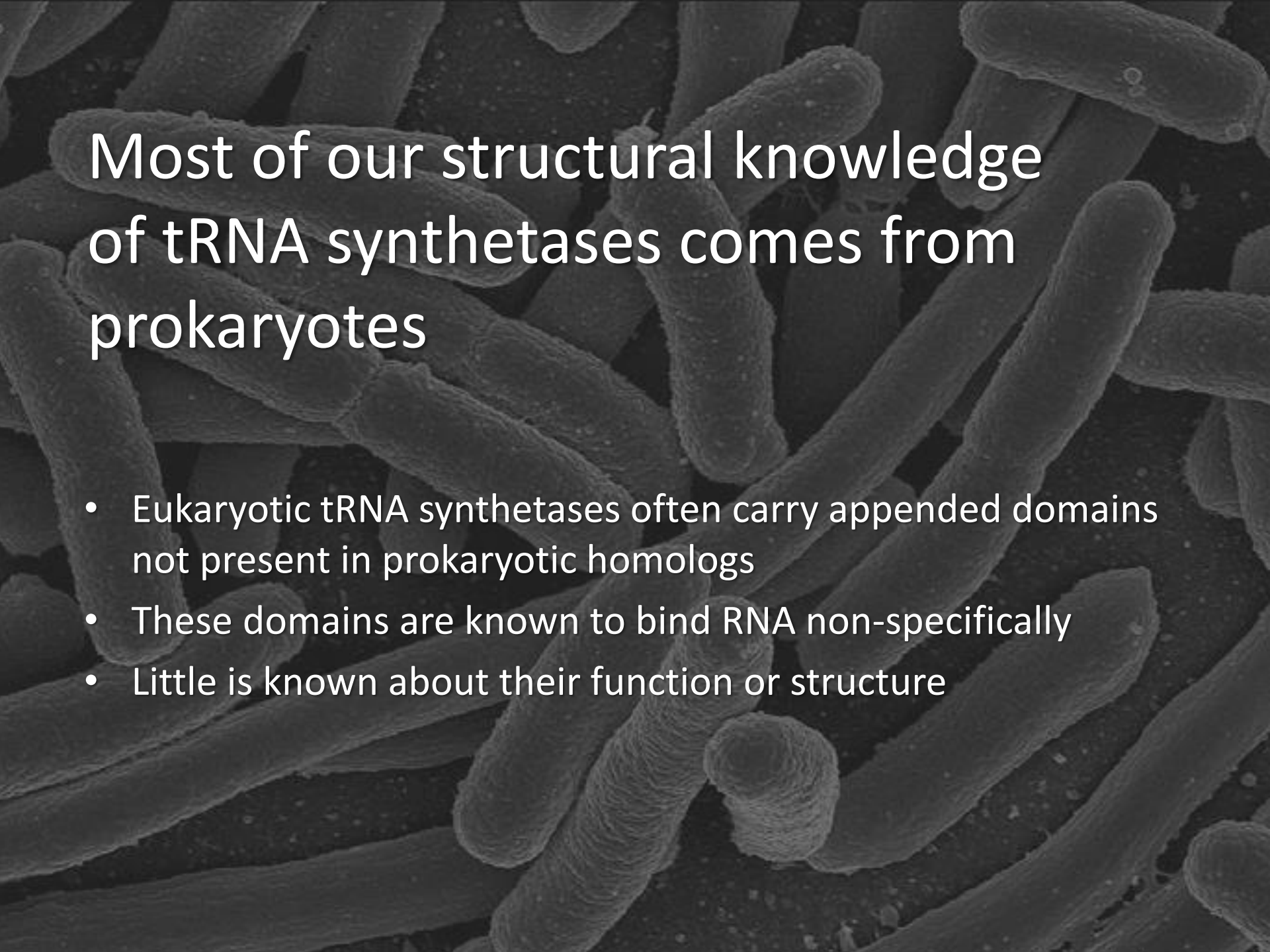
The NESG samples represent a special case in that they are well characterized beforehand – size exclusion chromatography, mass spec analysis and dynamic light scattering studies.



In 2011 we switched to PSI Biology – More difficult targets

We are now working with more difficult proteins:

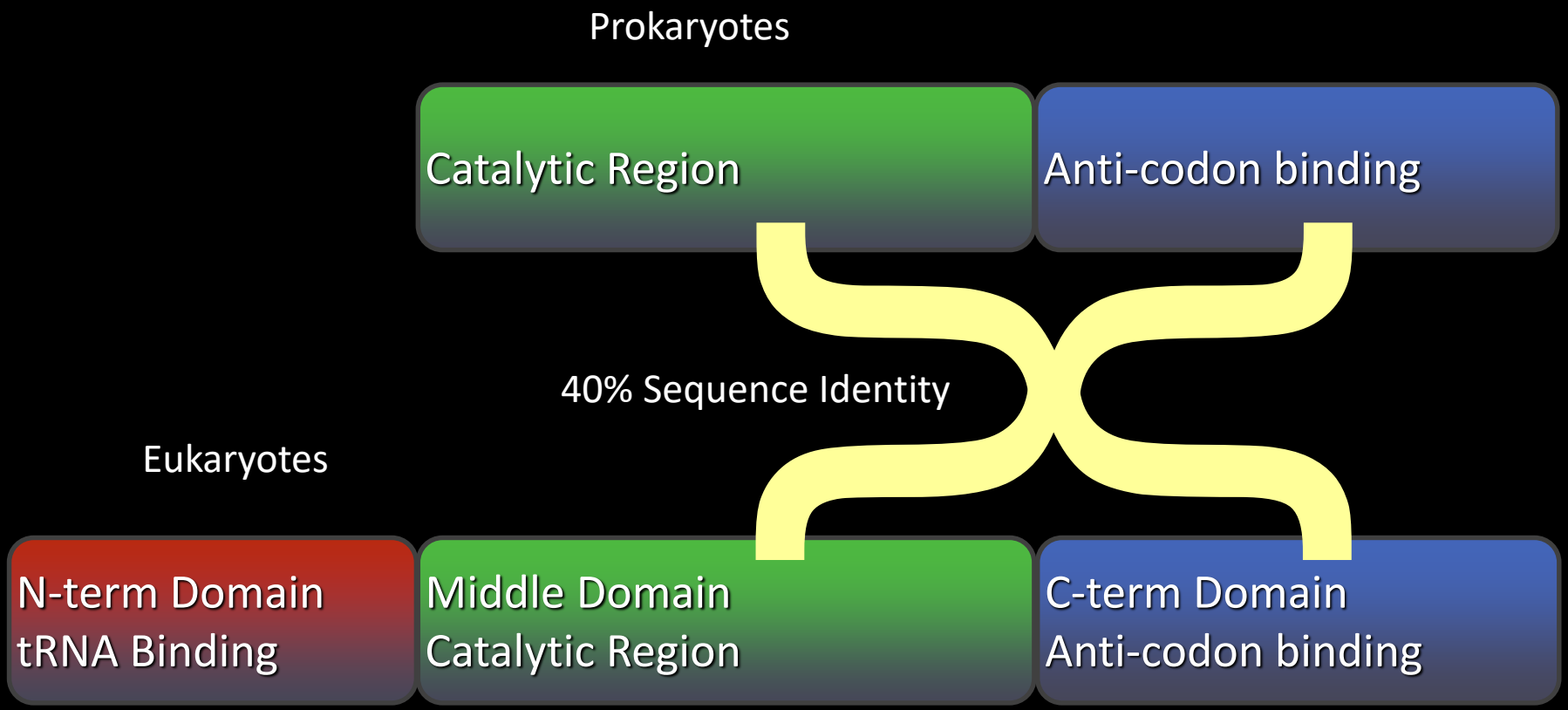
Complexes and disordered systems and membrane proteins



Most of our structural knowledge of tRNA synthetases comes from prokaryotes

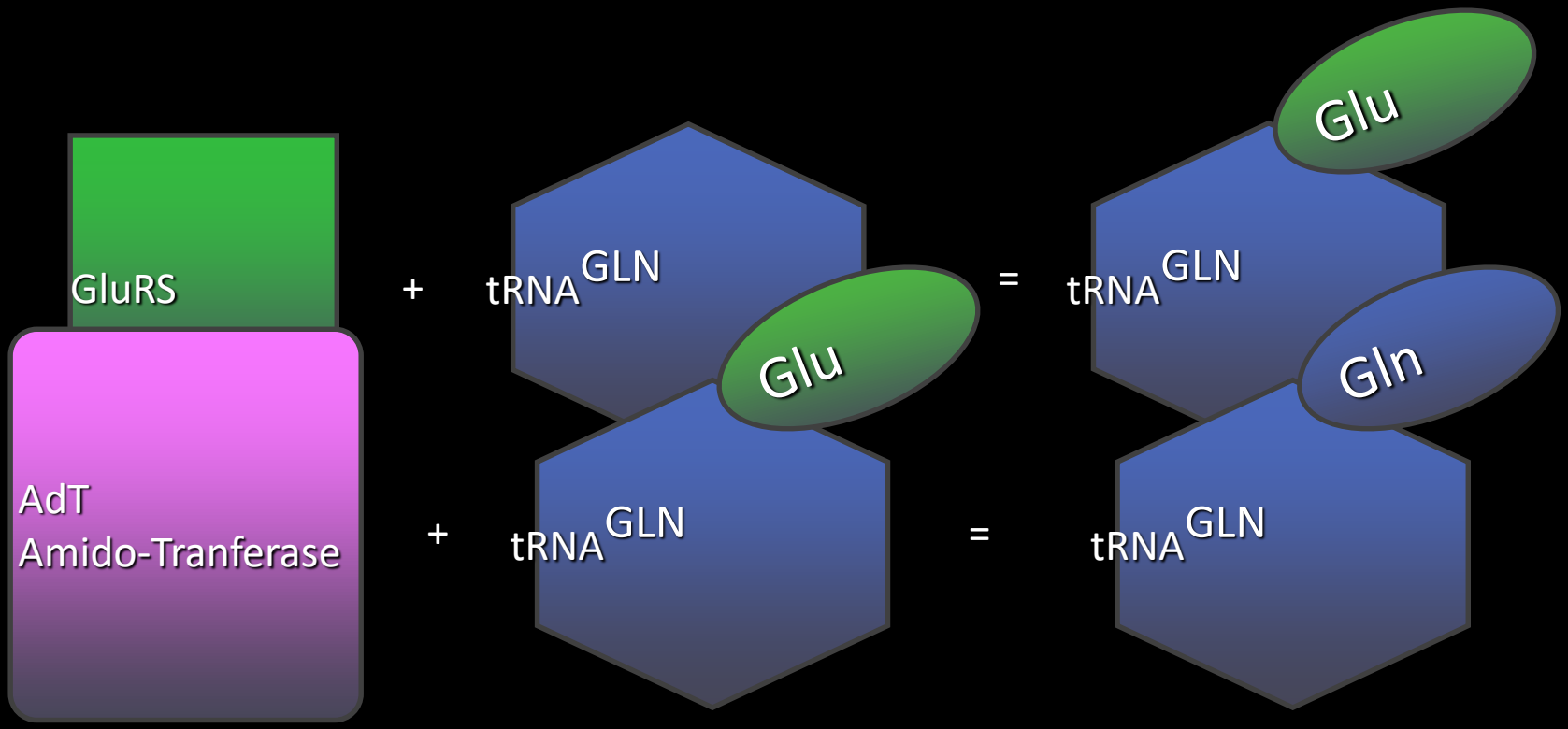
- Eukaryotic tRNA synthetases often carry appended domains not present in prokaryotic homologs
- These domains are known to bind RNA non-specifically
- Little is known about their function or structure

Glutamine tRNA Synthetase



Two routes of gln-tRNA^{GLN} Formation

Indirect Route: Archaea and Most Bacteria



Two routes of gln-tRNA^{GLN} Formation

Direct Route: Eukaryotes and few bacteria

Target

- Our target is Glutaminyl tRNA synthetase (Gln4) from yeast *Saccharomyces cerevisiae*

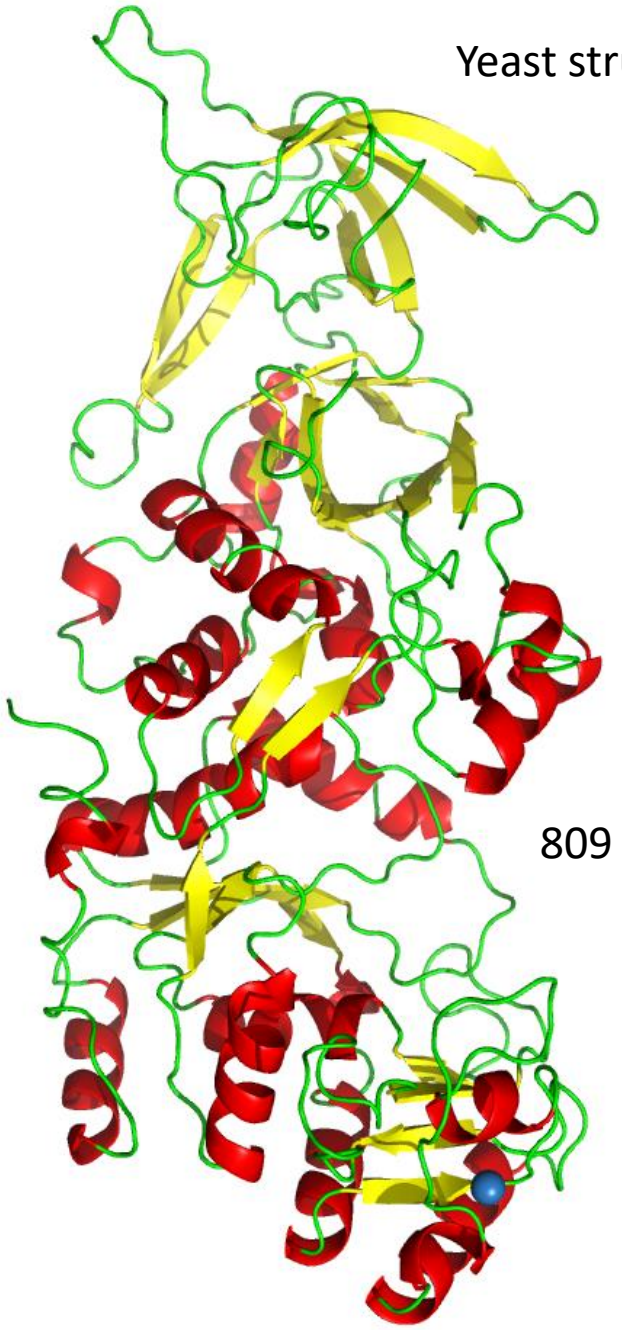
- Yeast *Saccharomyces cerevisiae is* a well-established model system for understanding fundamental cellular processes of higher eukaryotic organisms.

- 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.

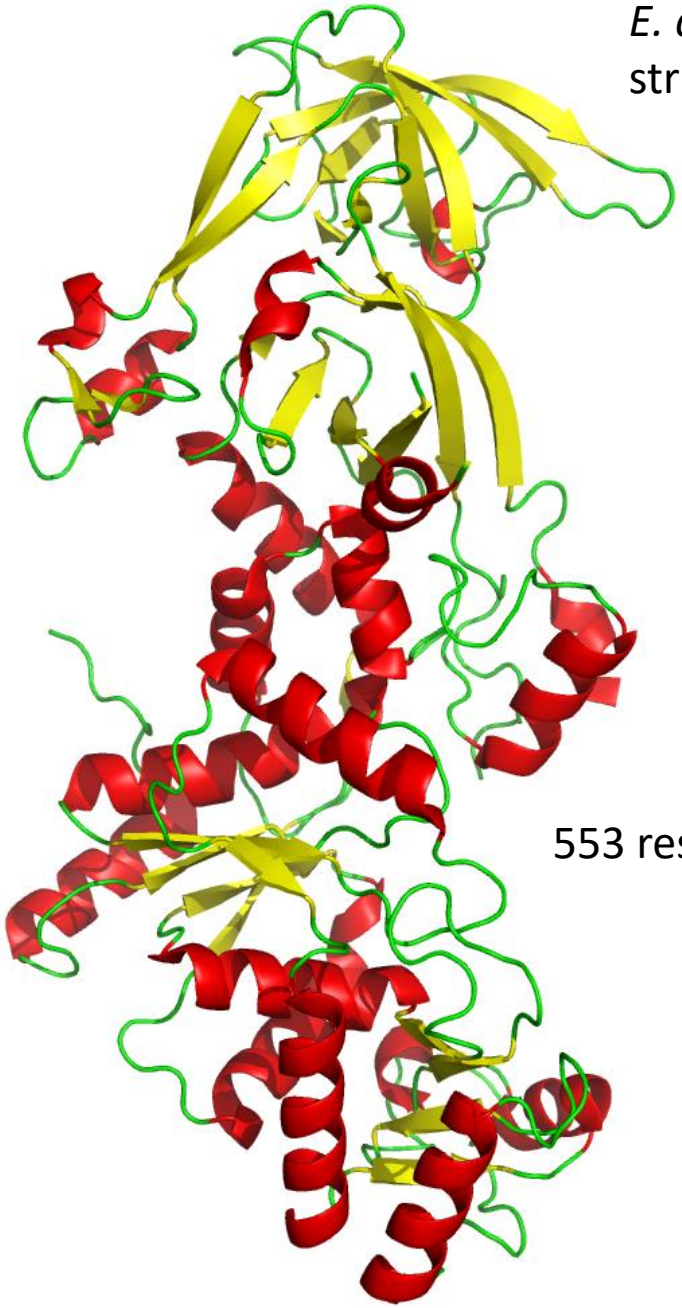
- The role of a nonspecific RNA binding domain in the function of a highly specific RNA binding enzyme is baffling, but clearly crucial given its prevalence among tRNA



Yeast structure

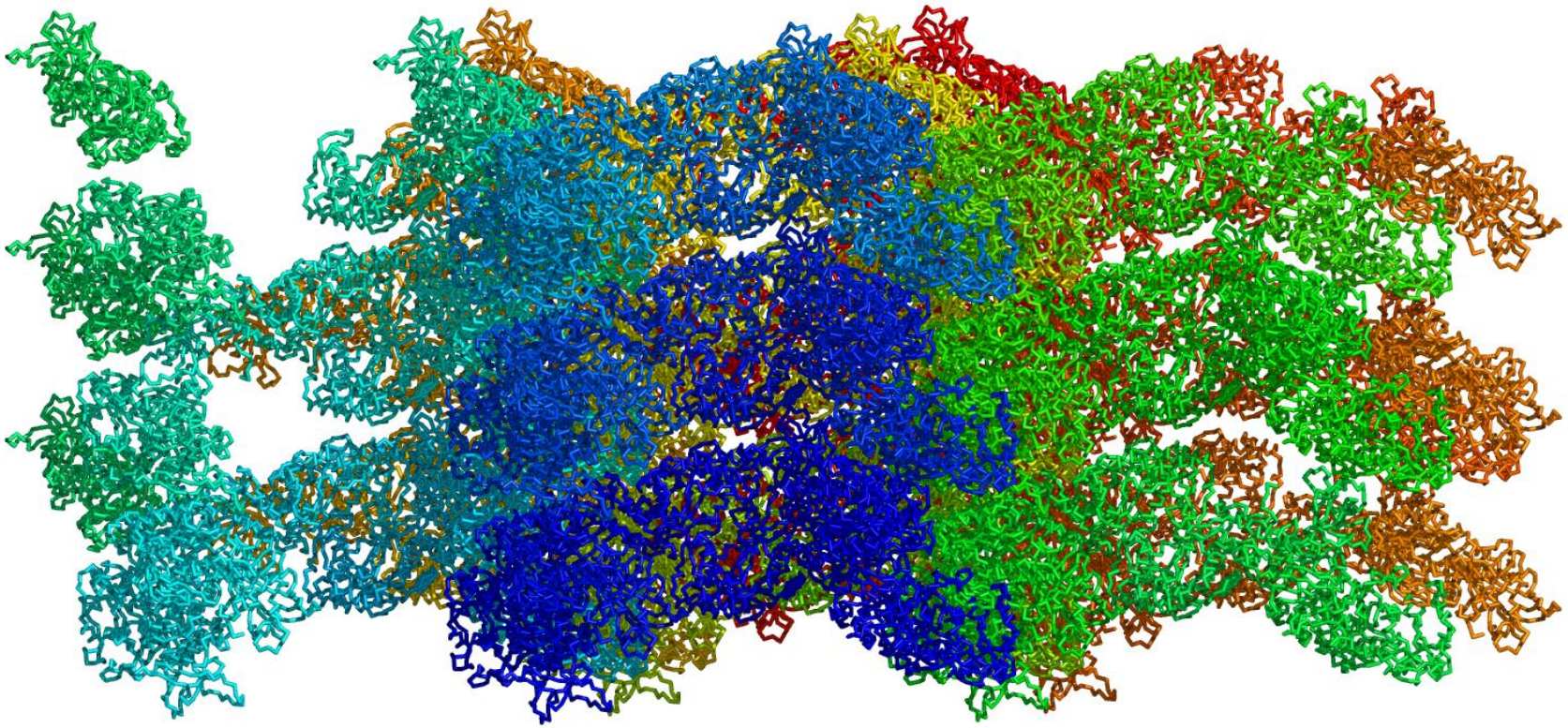
?

809 residues

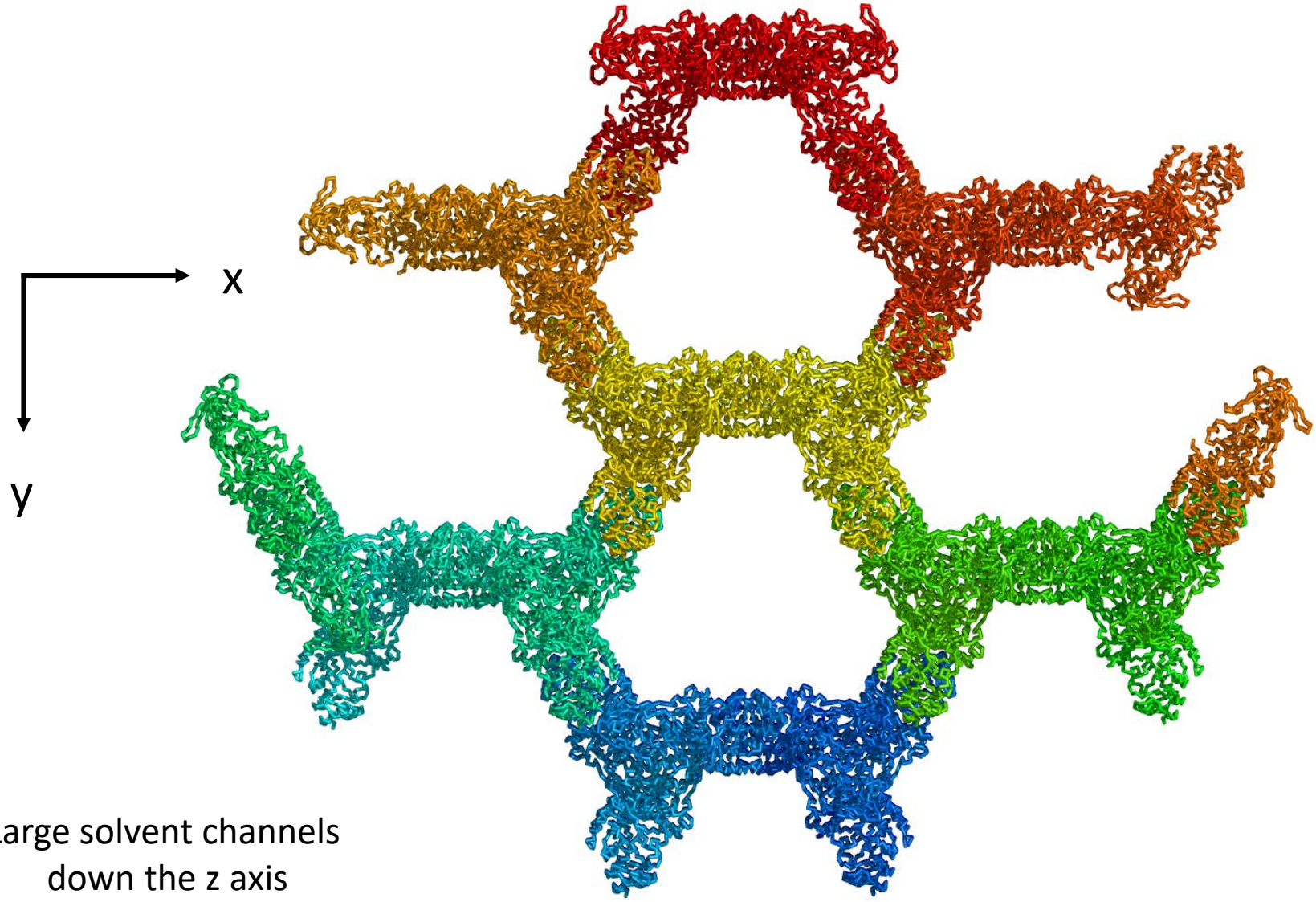


E. coli. structure

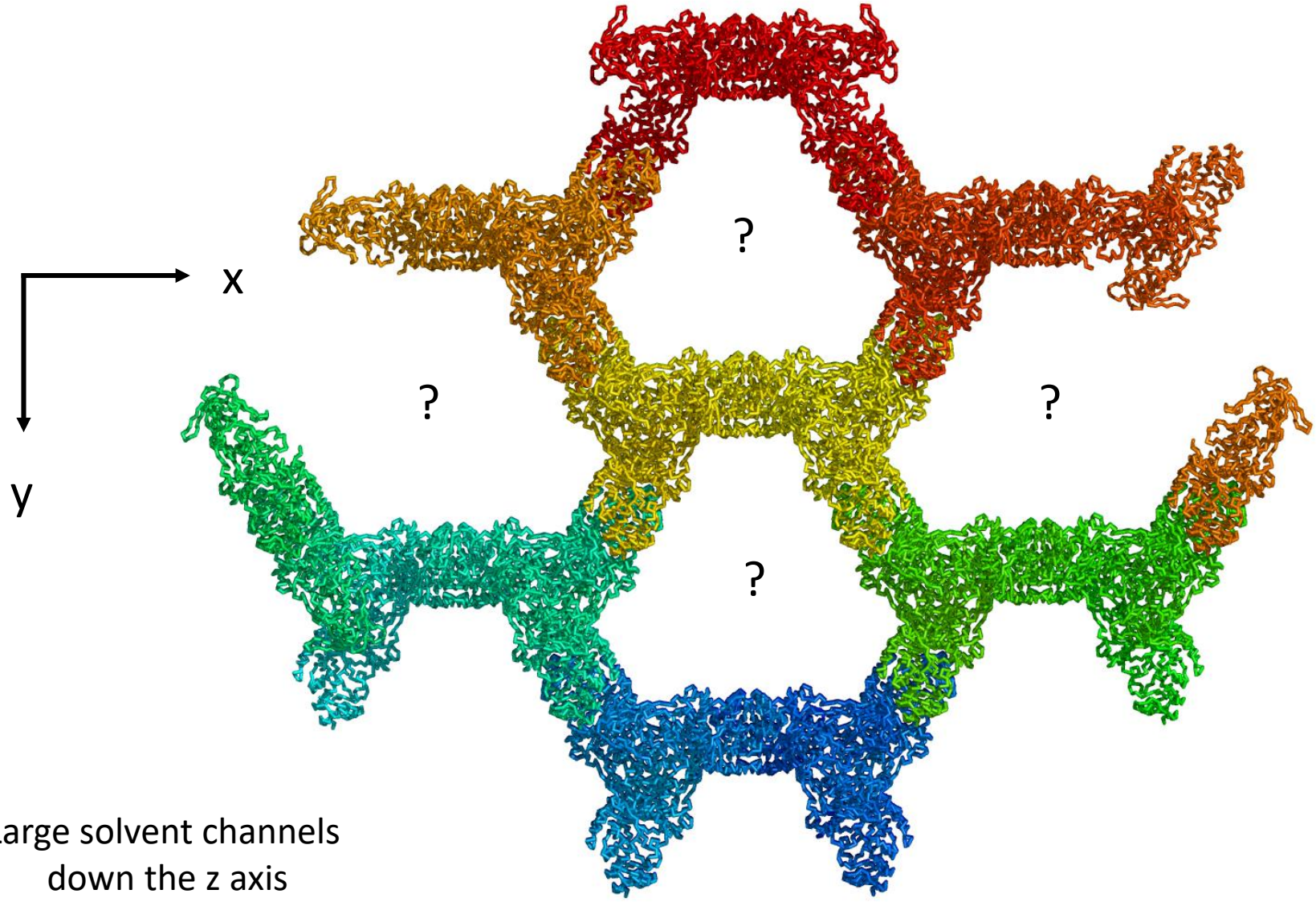
553 residues



Tight packing in z and y



Large solvent channels
down the z axis



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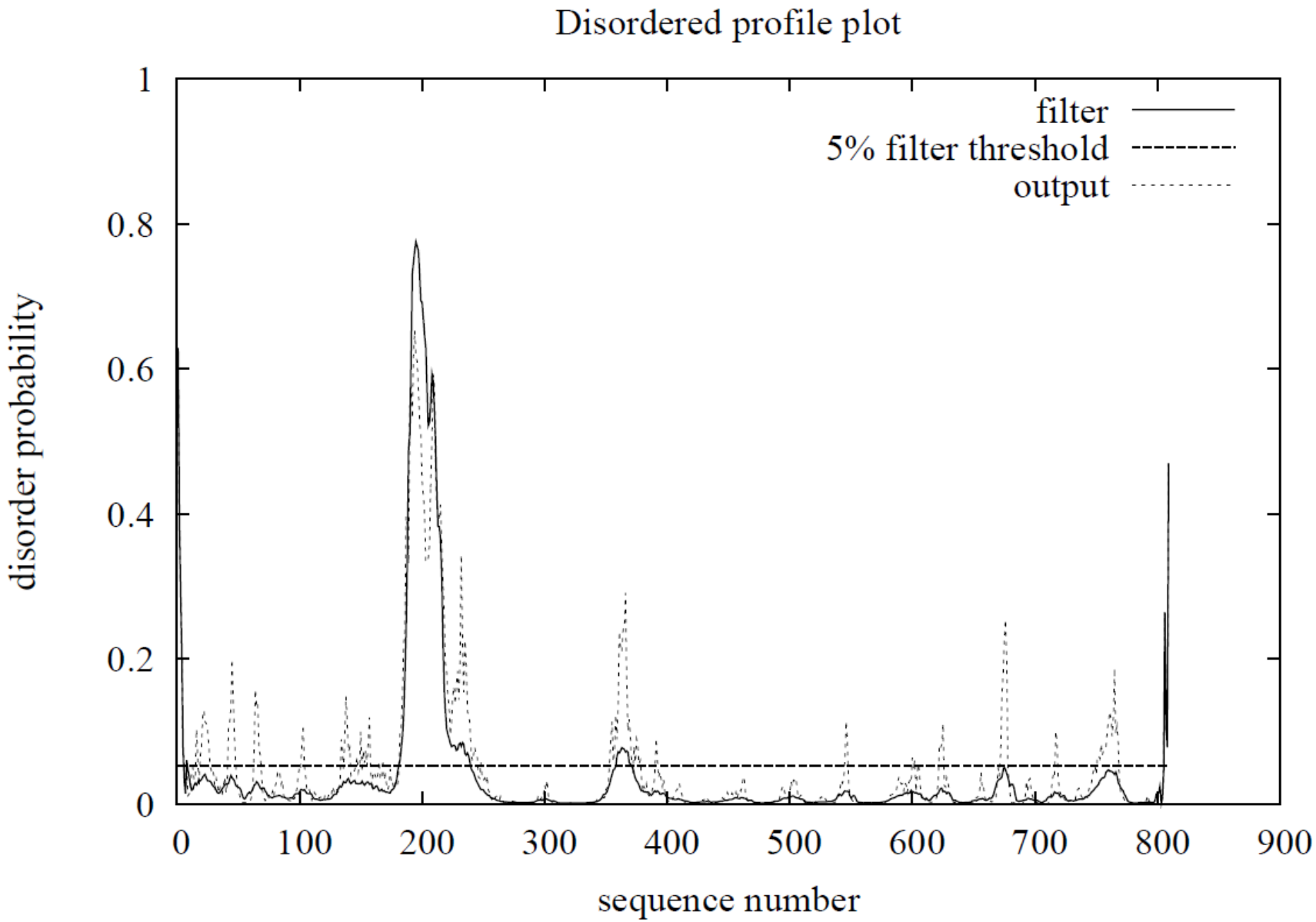
Disordered profile plot
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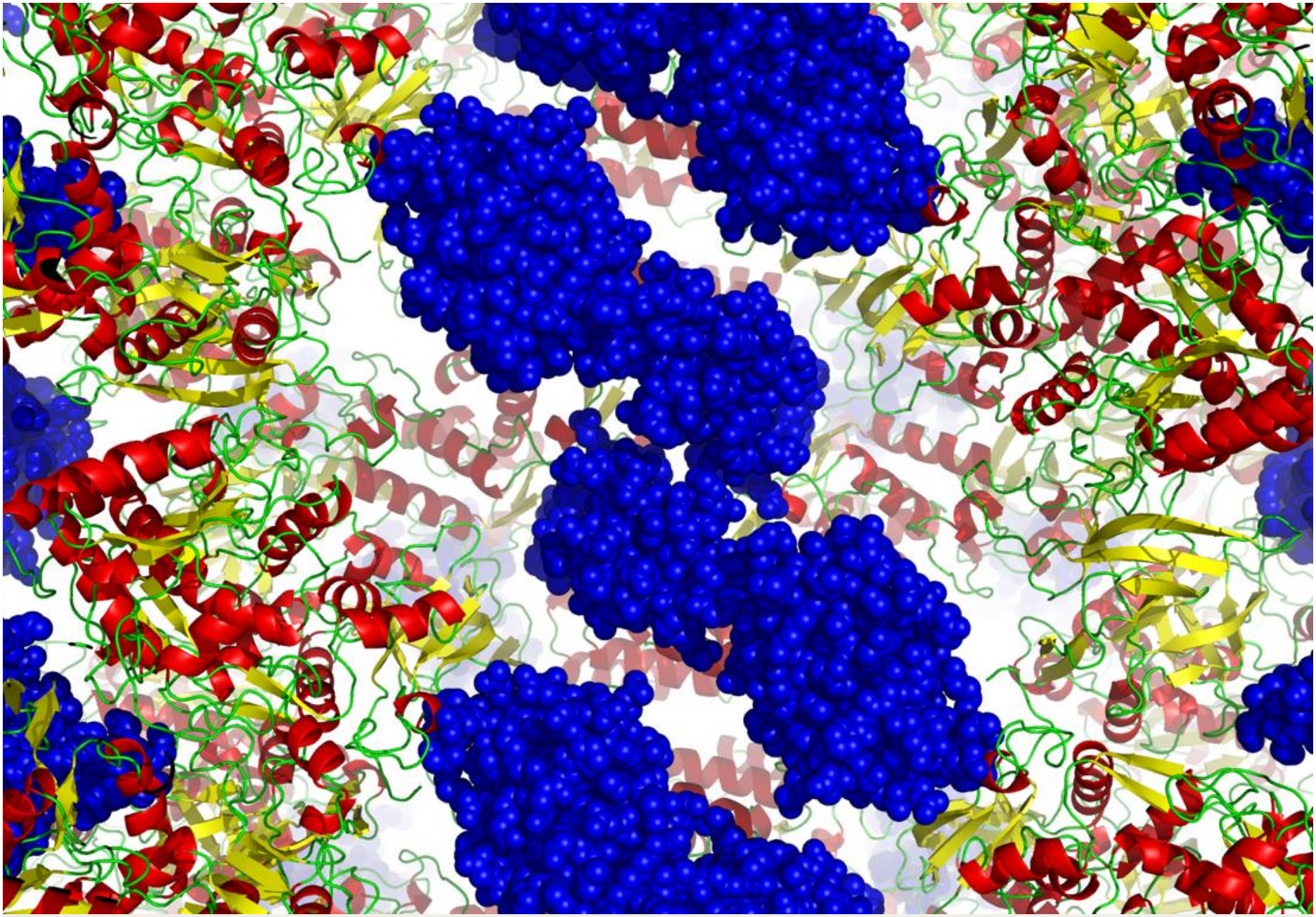

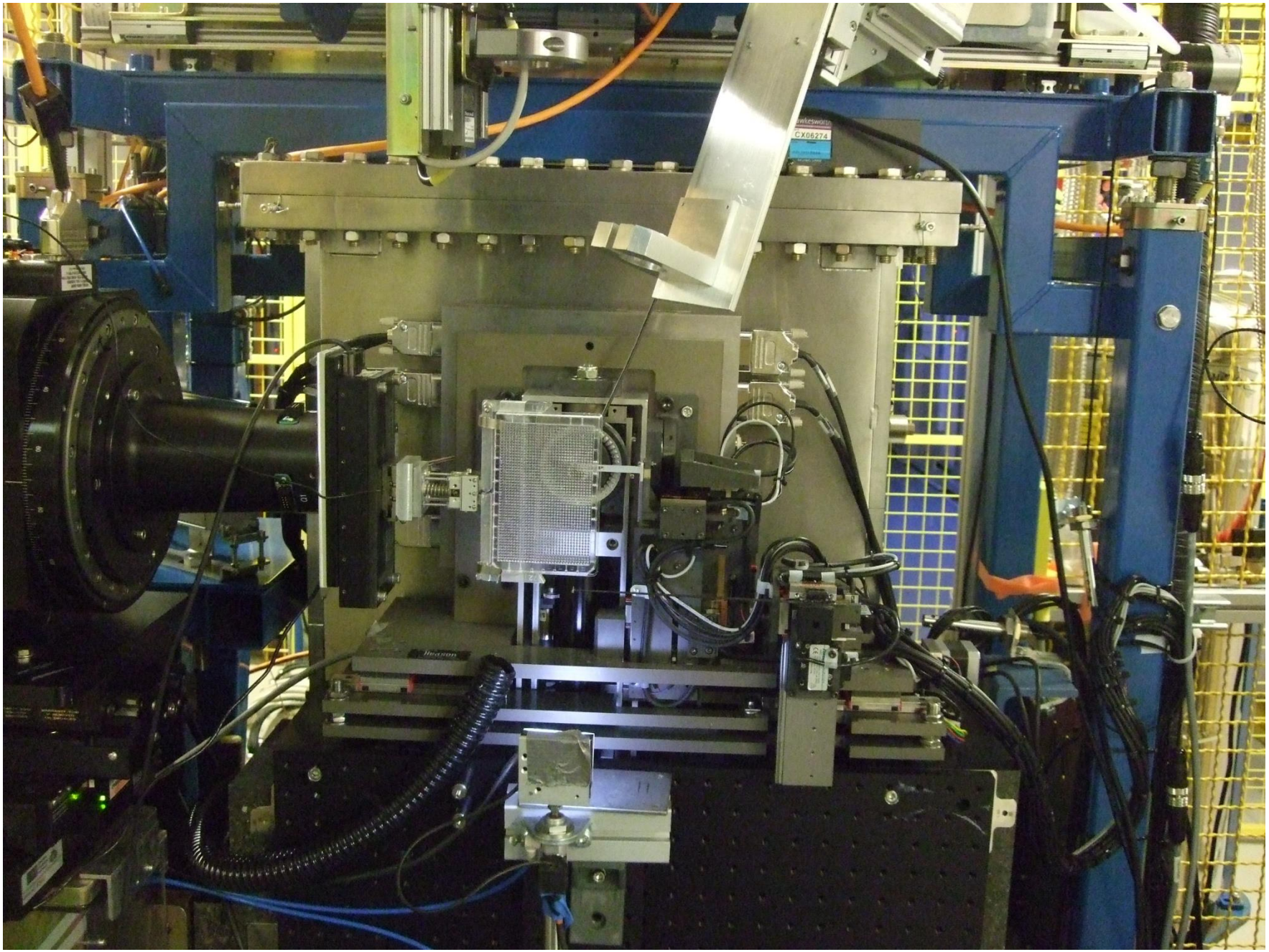
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