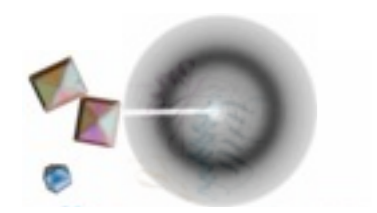
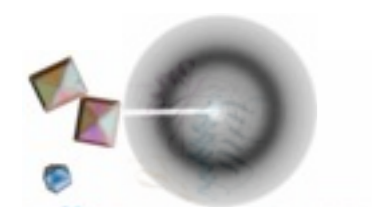


# Crystallization of Macromolecular complexes: use of limited proteolysis as a rational tool in construct design.





**"It has happened to all of us: after months/years of hard work dedicated to crystallizing a particular protein ; the drops stay stubbornly clear or opaque with precipitate, with no hint of a crystal."**



**Engineering proteins with enhanced solubility**

**Optimization of target constructs**

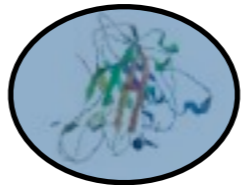
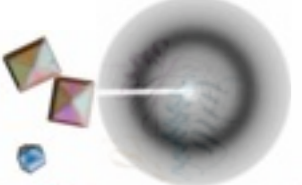
**Use of fusion proteins for crystallization**

**Non covalent crystallization chaperone**

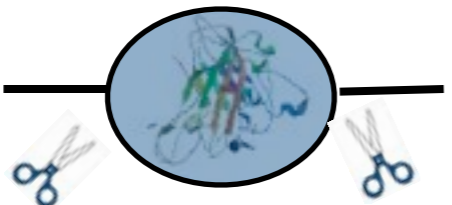
**Removal of post-translation modification**

**Surface entropy reduction** (<http://services.mbi.ucla.edu/SER/>)  $\approx$  100 pdb entries

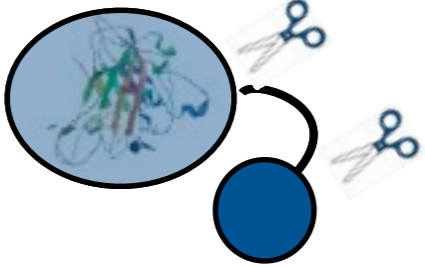
**Change species**



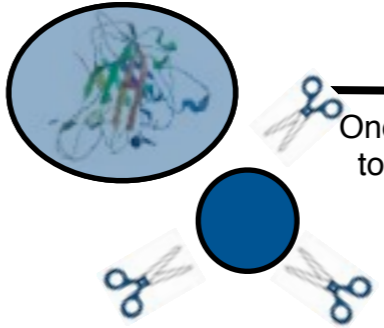
Stable, compact / globular structure  
Does not require protein engineering



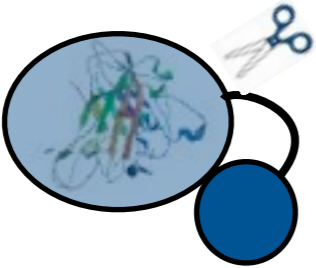
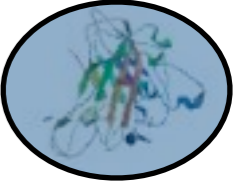
Flexible N and / or C -Termini  
Needs removal in order to crystallize



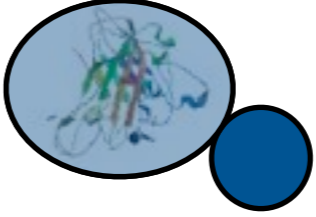
Internal loop needs  
to be removed



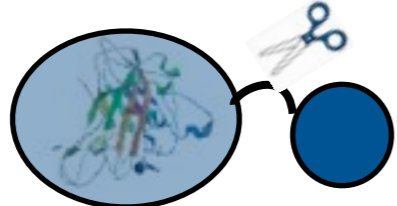
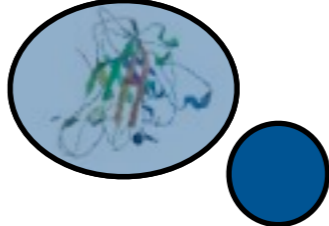
One domain needs  
to be completely  
digested



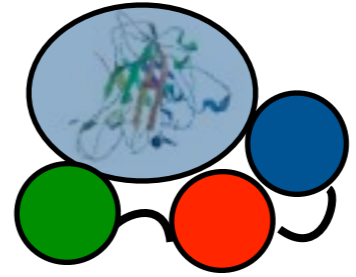
Internal loop needs  
to be removed



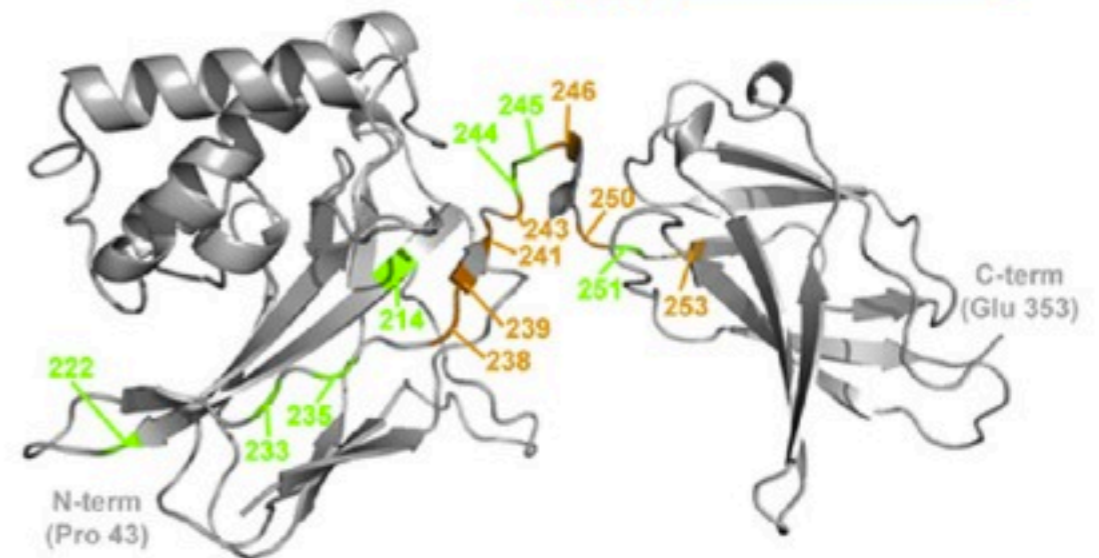
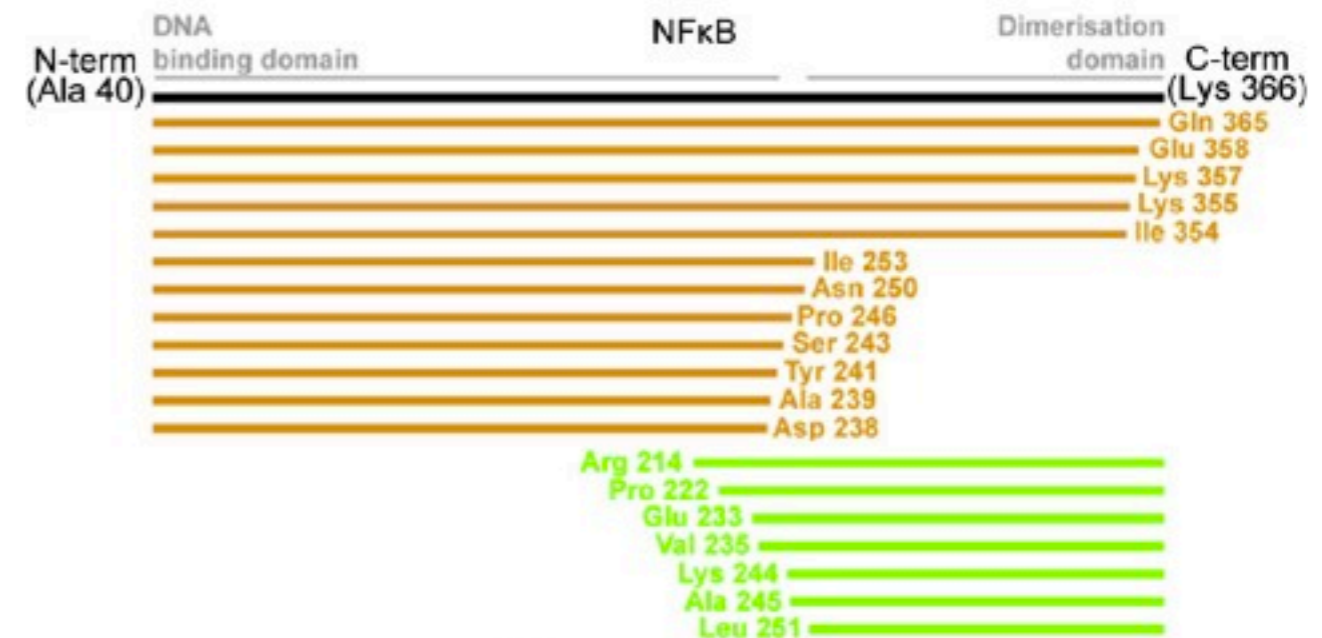
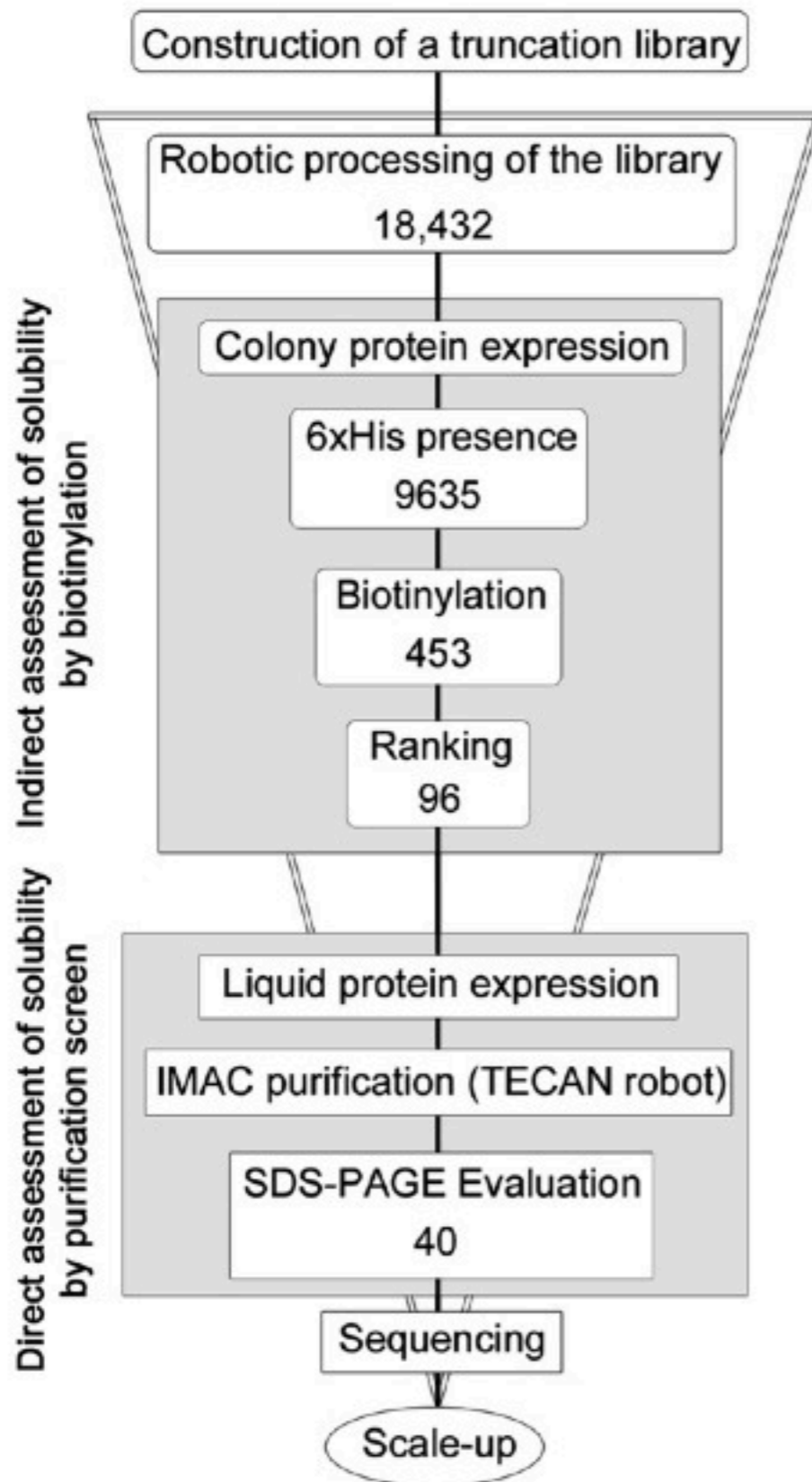
Re cloning / purification is required  
to select the domain of interest

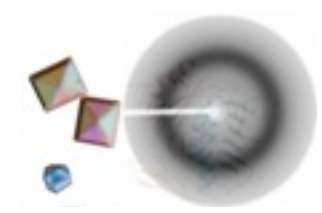


Biological understanding  
leading to crystallization



# ESPRIT : An automated , library based method for mapping and soluble expression of protein domains





# Optimization of target constructs

## Bioinformatics

### [PipeAlign](#)

Searches for similar protein sequences, generates a clustered alignment

### [PHYRE](#)

Protein Homology/analogY Recognition Engine

### [FFAS](#)

Fold and Function Assignment System

### [PSIPred](#)

Protein structure prediction server

### [IUPred](#)

Predict intrinsic disorder and globularity in polypeptide chains

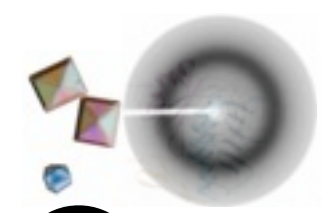
### [GlobPlot](#)

Predict intrinsic disorder and globularity in polypeptide chains

### [SER](#)

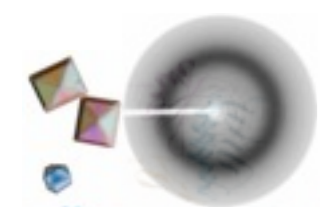
Identification of sites that are most suitable for mutation designed to enhance crystallizability; using a Surface Entropy Reduction approach

# Crystal construct designer software



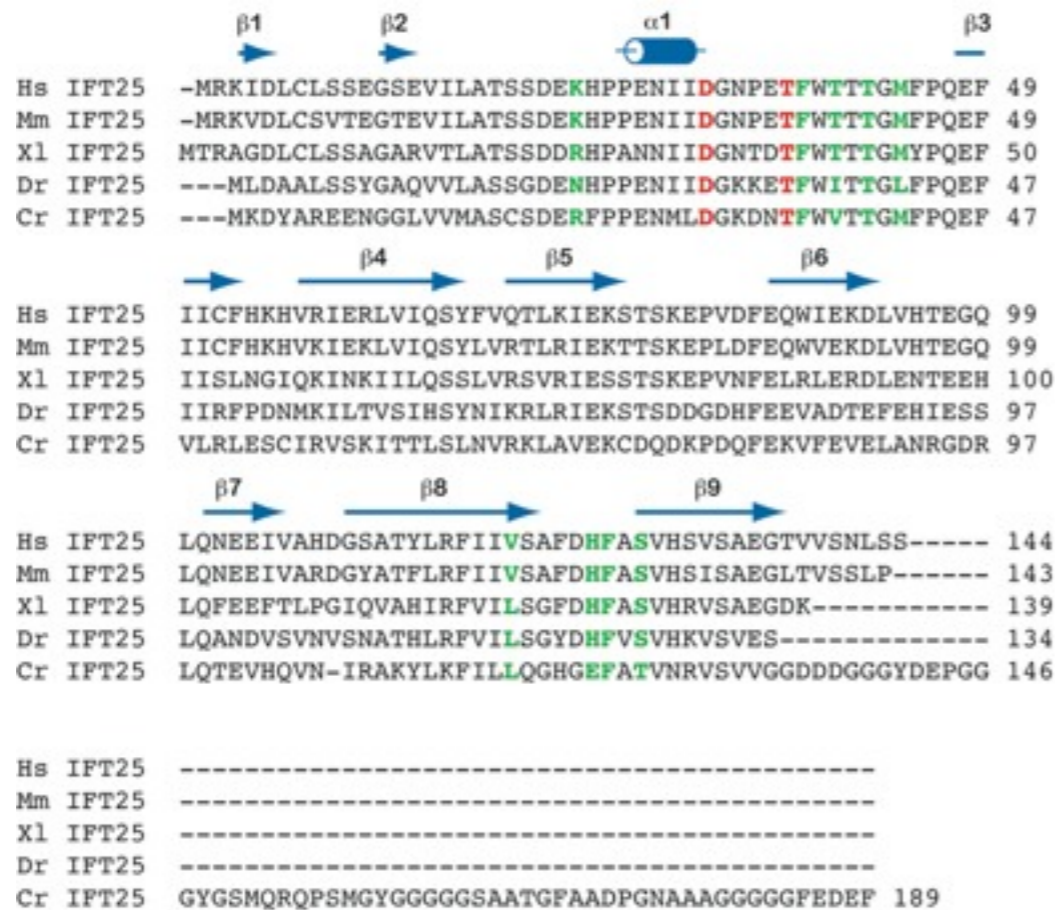
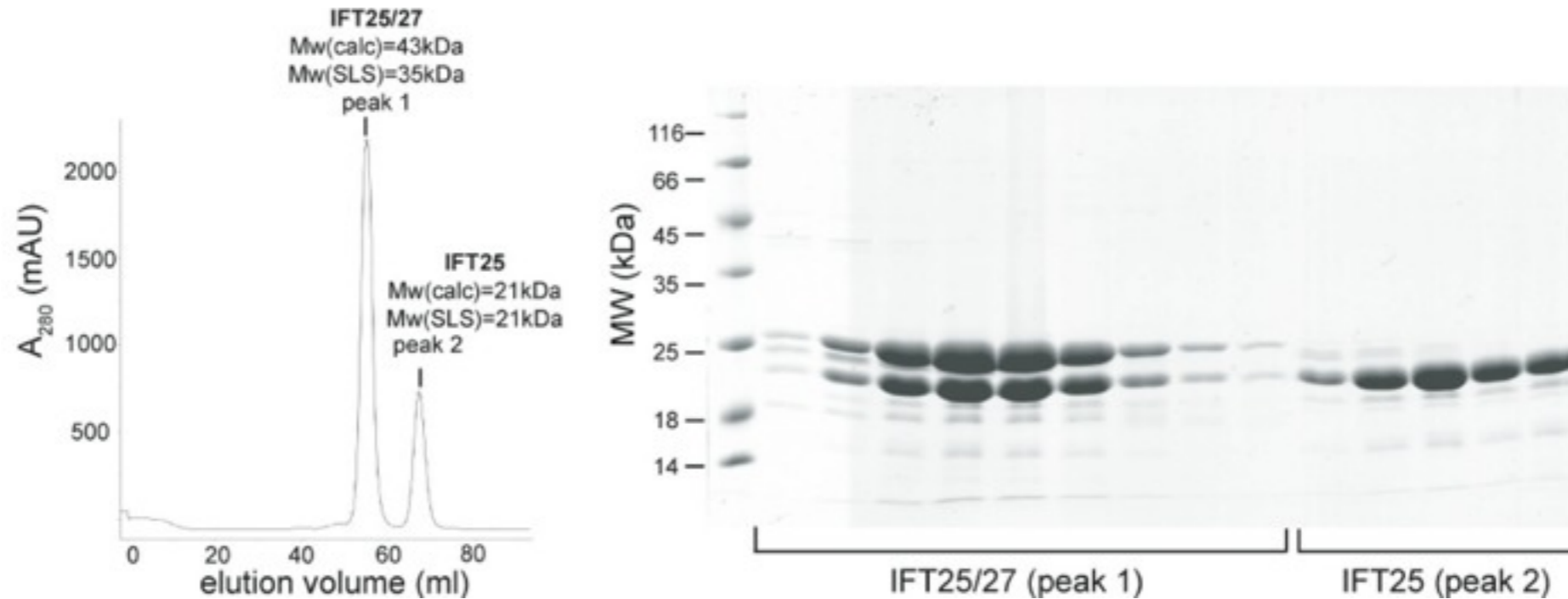
The screenshot shows the Crystalisation Construct Designer software interface. It includes a top navigation bar, a main input area for DNA sequences, and a large output area for predictions and primers. Annotations with magnifying glasses point to specific features:

- Check prediction server:** Points to the 'Predictions' tab in the top left.
- choose your LIC vecteur:** Points to the 'N:' field containing the number '20'.
- spot your start and stop amino acid:** Points to the 'Output DNA sequence' field, which contains a long DNA sequence with a green start codon (ATG) and a red stop codon (TAA).
- you get your primers designed:** Points to the 'Oligos' section, which lists three primer sequences: 17W, 26FW, and 1001RV.
- and your target sequences:** Points to the 'Sequences' section at the bottom, which displays the amino acid sequence of the protein construct.

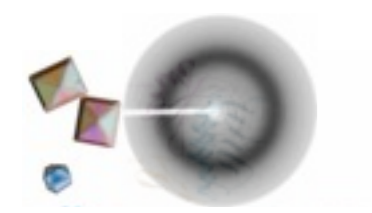


# Crystal construct designer software

Crystal structure of the intraflagellar transport complex 25/27

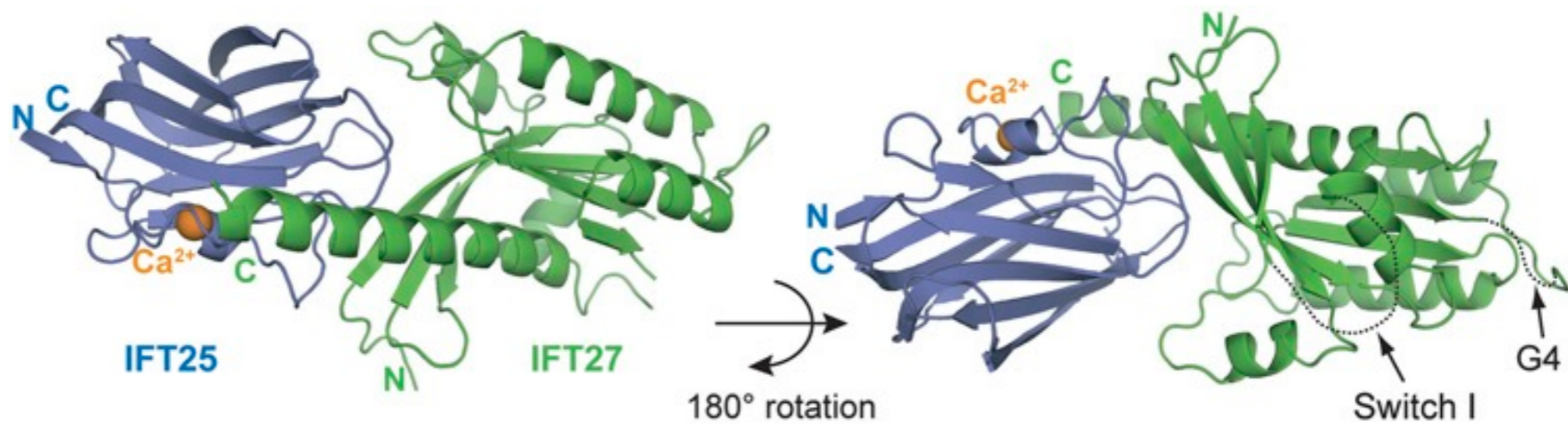
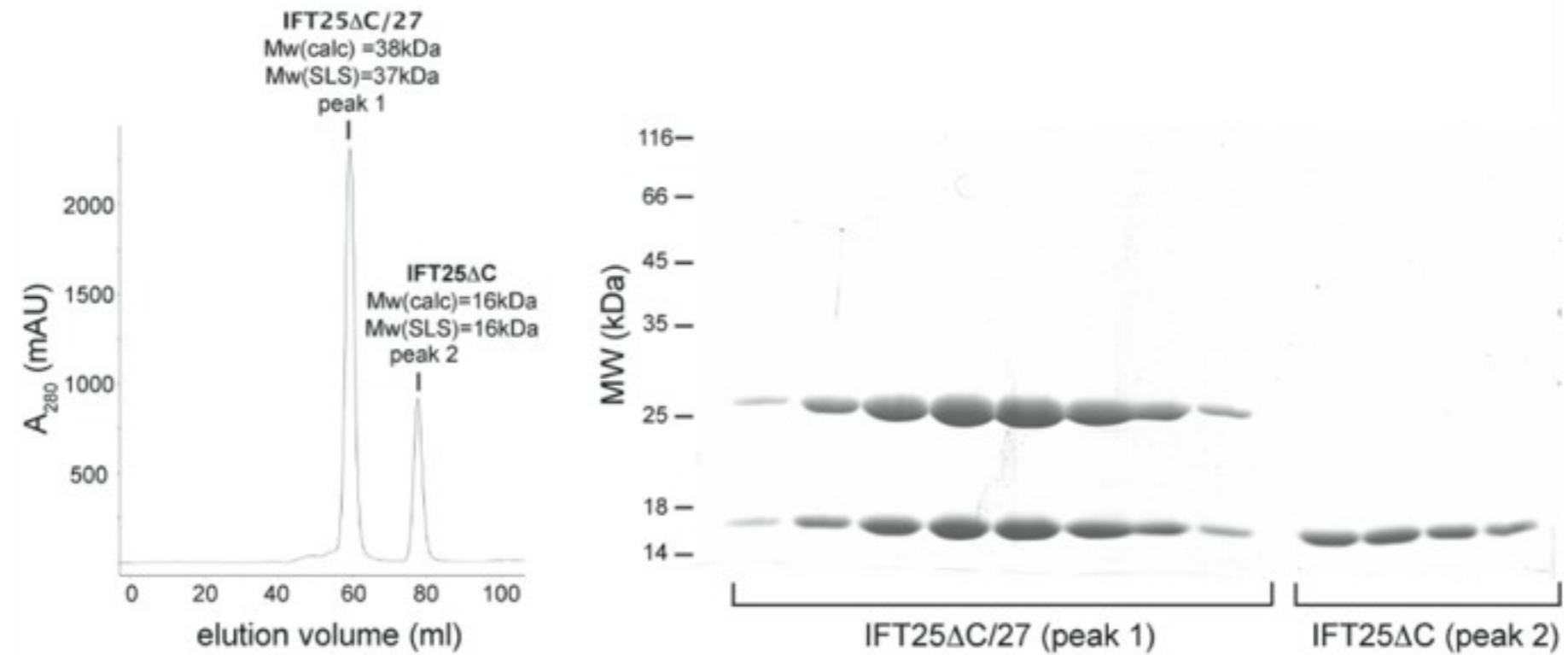


55 residue long C-terminal glycine-rich tail that is predicted to be disordered (DRIPPRED server, <http://www.sbc.su.se/~maccallr>)

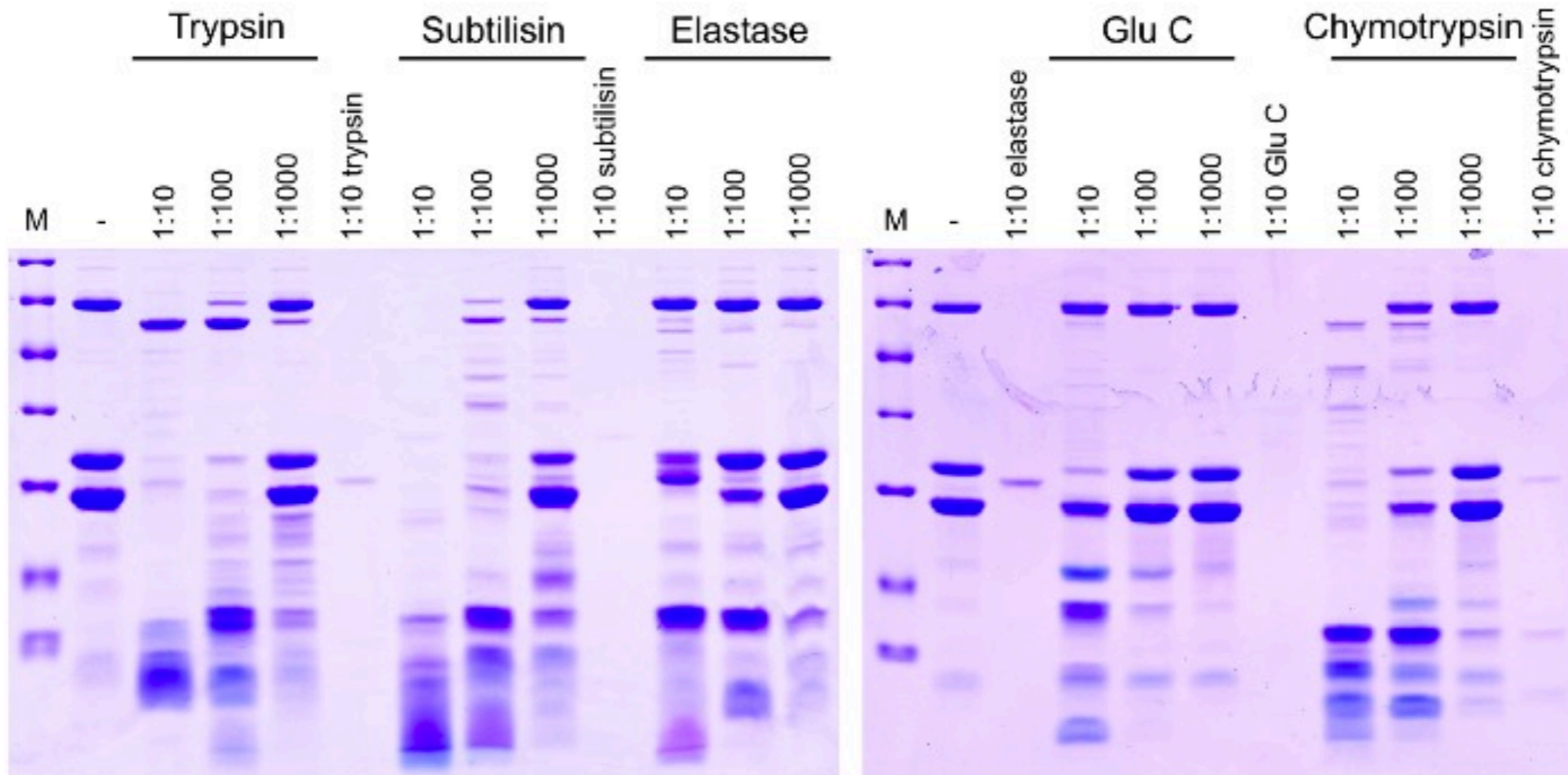


# Crystal construct designer software

## IFT25/27 complex formation



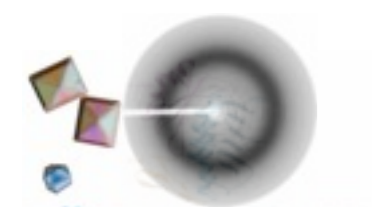
# Limited proteolysis : practical considerations



Followed by Mass spectrometry analysis

ESI-TOF (SEC) / MALDI (In gel)

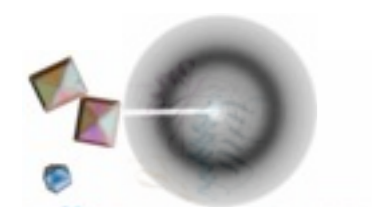
N-Terminal sequencing



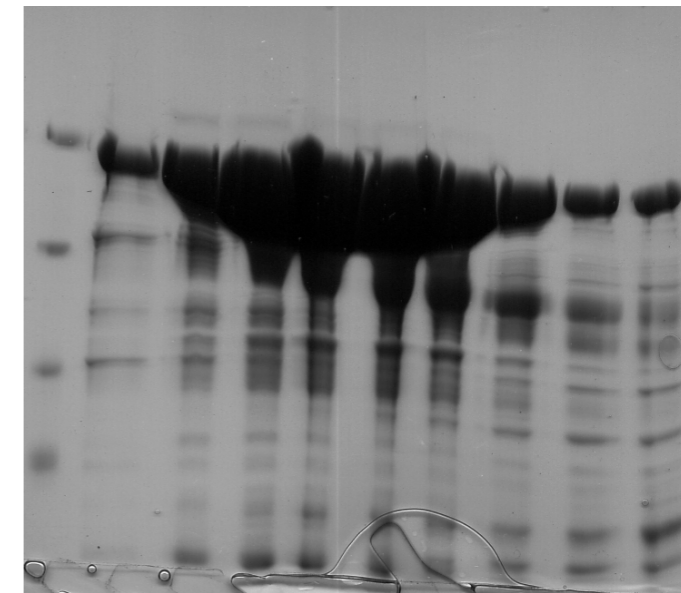
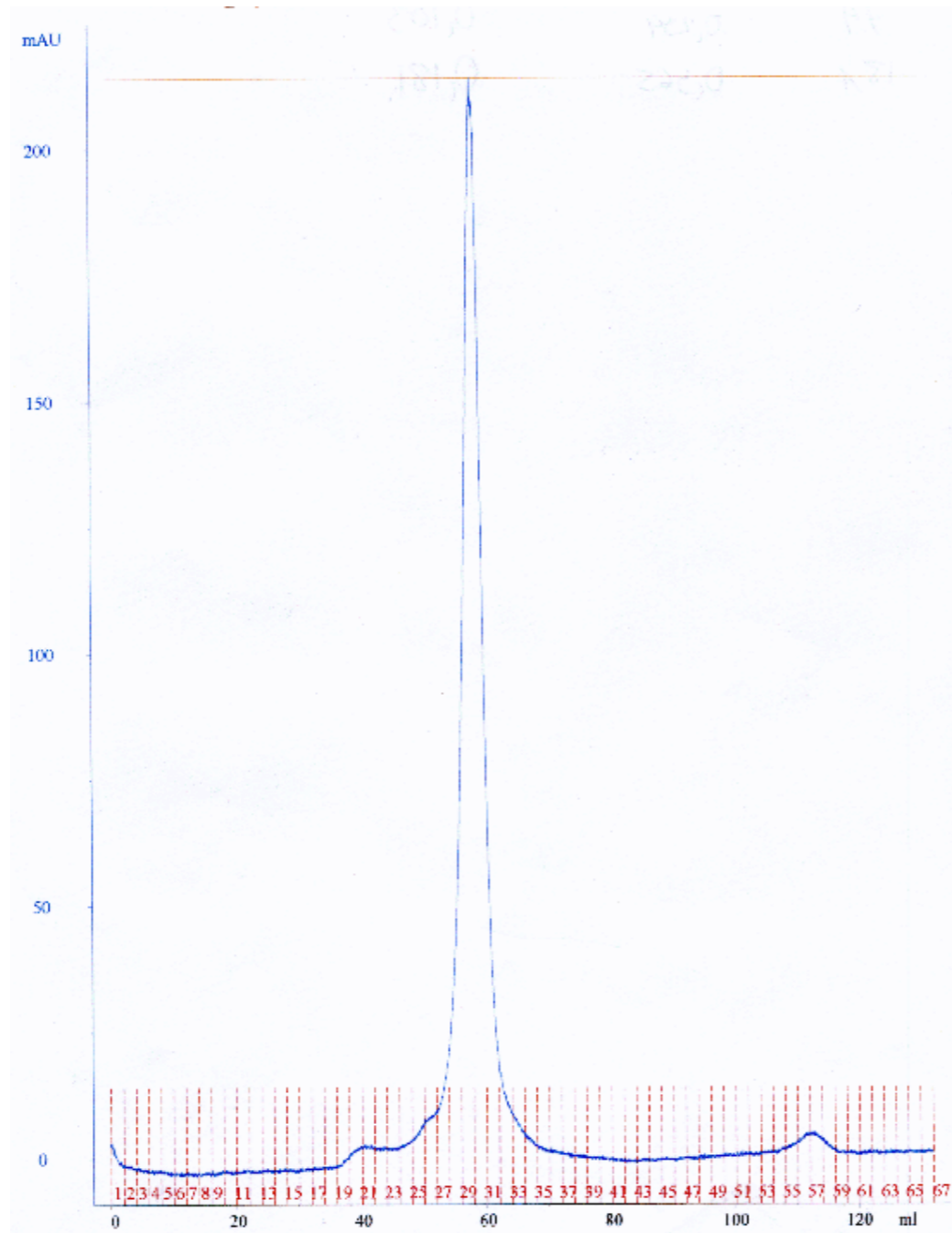
# Yeast Rrp44 structural studies

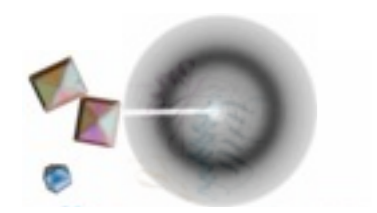
- Components of the eukaryotic exosome
- belongs to the RNase II/R superfamily of hydrolytic Rnases
- Rrp44 is the only subunit with catalytic activity of the yeast exosome





# Yeast Rrp44 overexpression





# Yeast Rrp44 limited proteolysis

**Elastase      Subtilisin      Glu-C**

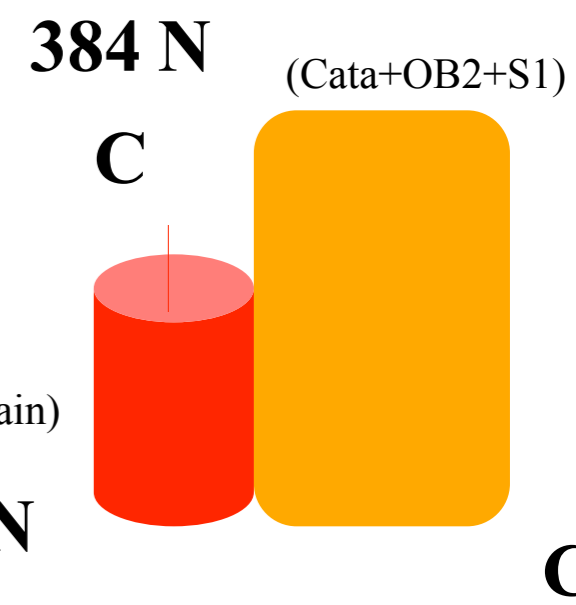
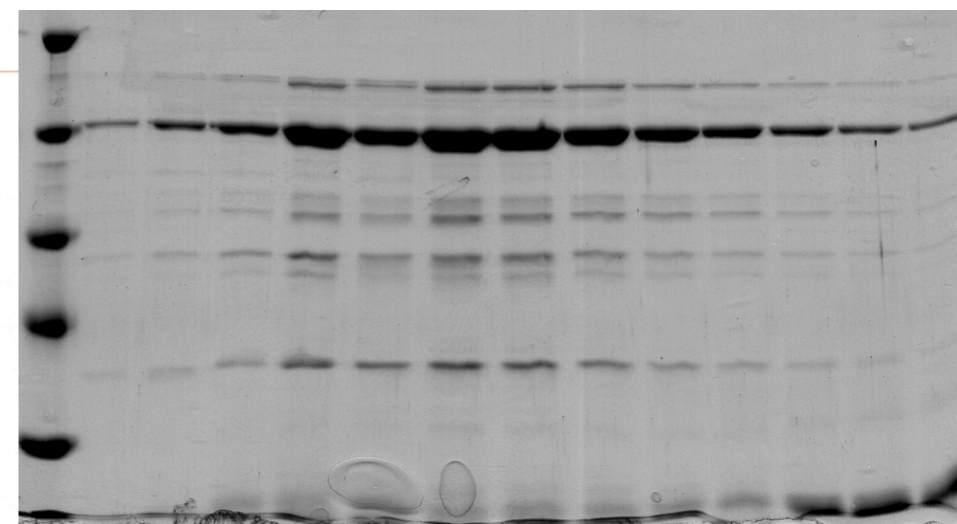
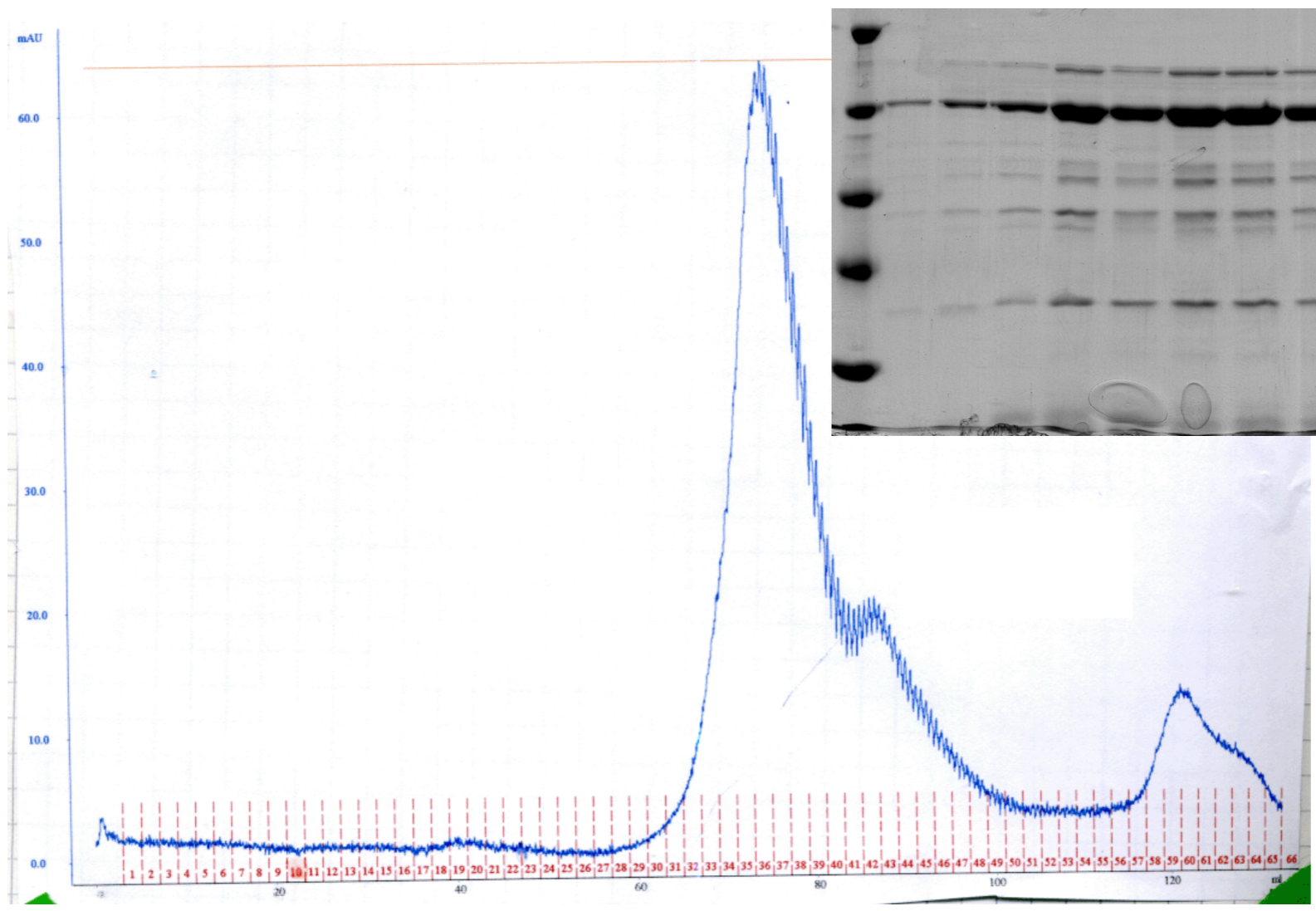
-    1/1000 1/100 1/10    1/1000 1/100 1/10    1/1000 1/100 1/10

**116 kDa**

**66 kDa**



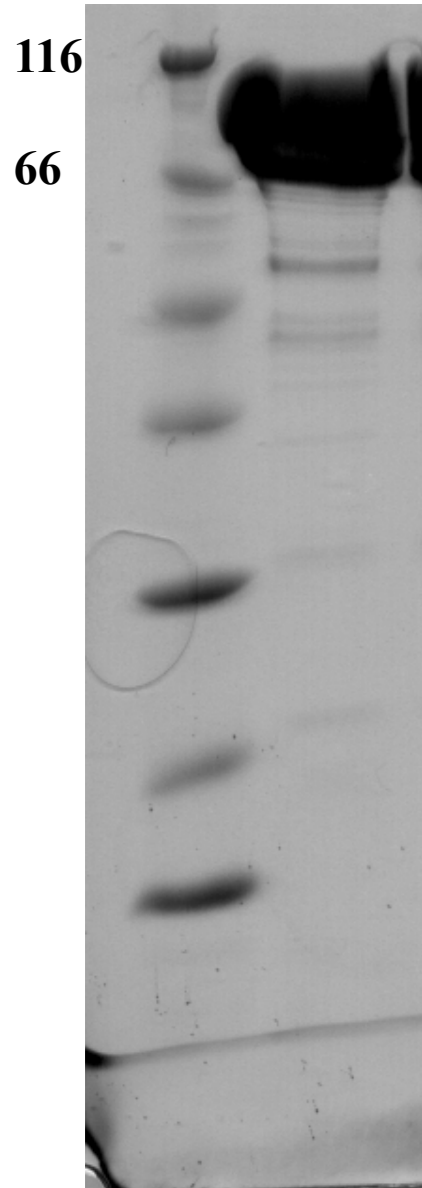
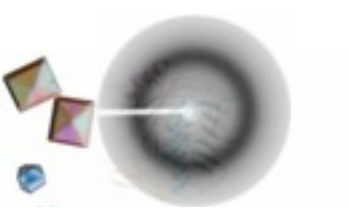
# Yeast Rrp44 preparative limited proteolysis



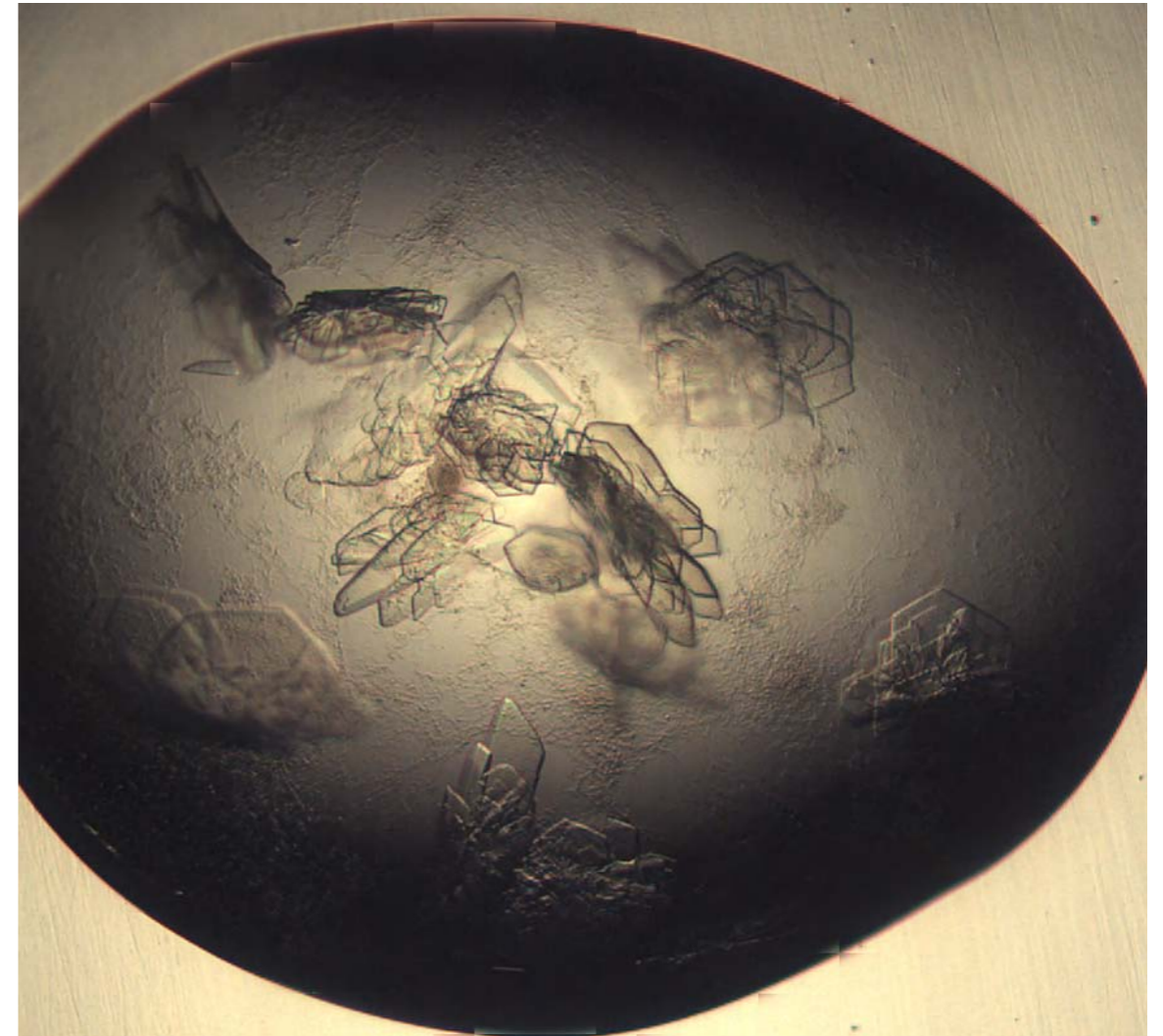
86 kDa , 242-1001, 1st N-term: DLEQDTFSDF

69 kDa , 384-1001, 2nd N-term: KKKRPLAKVVTFPRR

# Purification & crystallization of the construct (242-1000)



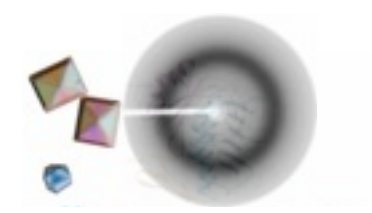
- 1) Ni-NTA
- 2) Q sepharose
- 3) Superdex 200



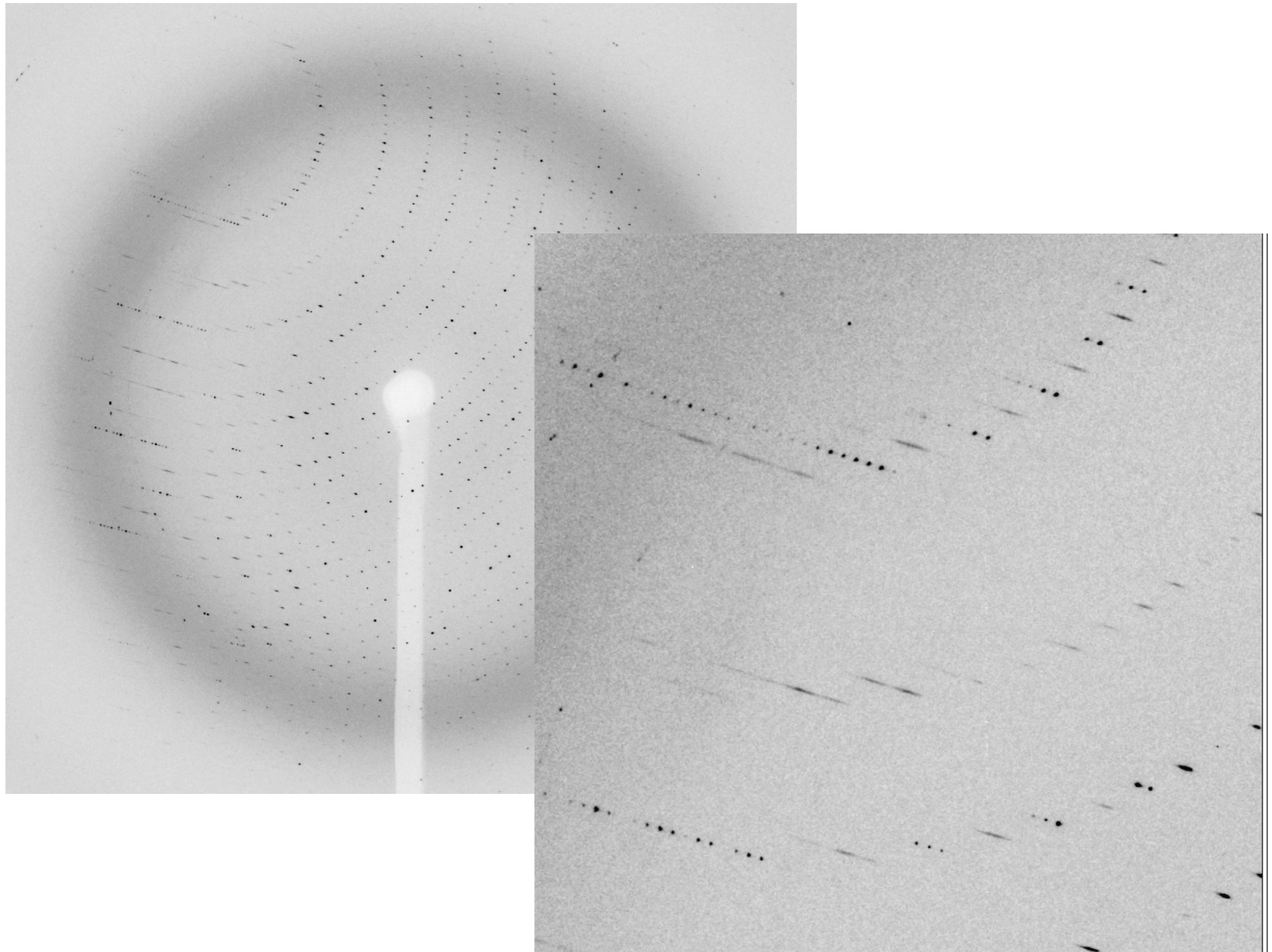
## Conditions:

8% PEG 3350  
6% MPD  
50mM Tris 8.0

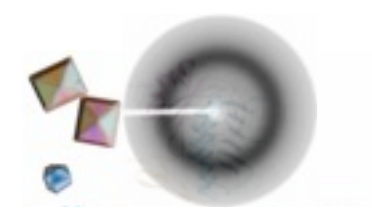
increase MPD to  
20% for cryo  
protection



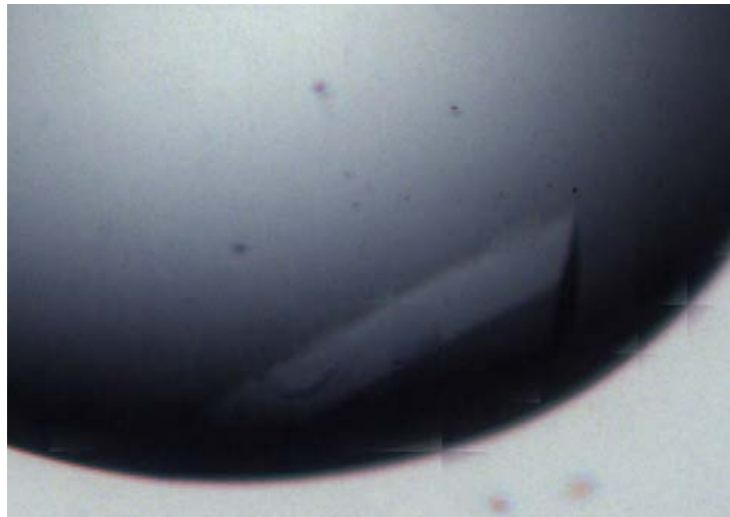
# Yeast Rrp44 diffraction quality



**2.3 Å but streaky**

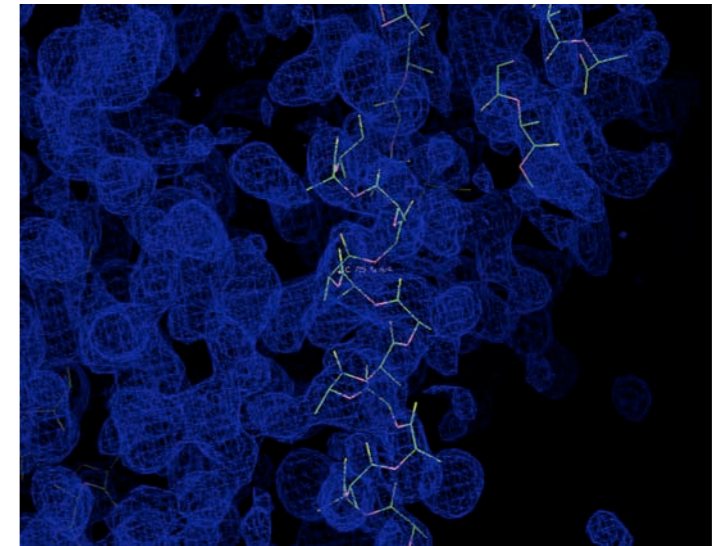


# Crystal of RNA bound Rrp44



aa 242-1001  
D551N mutant  
Se-Met protein  
Co-crystal with 13A RNA  
1:1.2 protein to RNA

2A diffraction at SLS

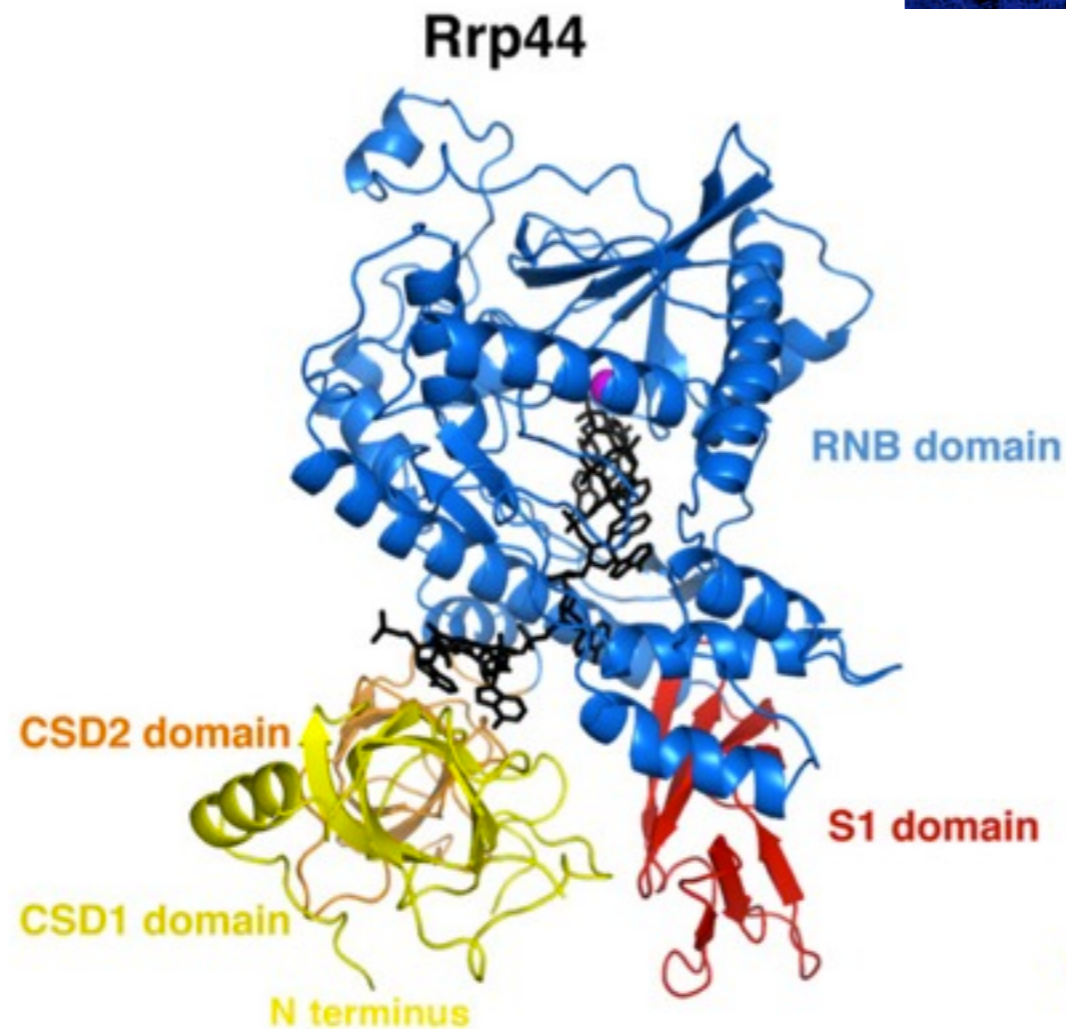


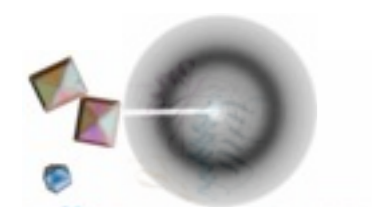
2.3A MAD density  
After Shelx D/E

18/20 Se sites

Anomalous signal  
to 3.5 A

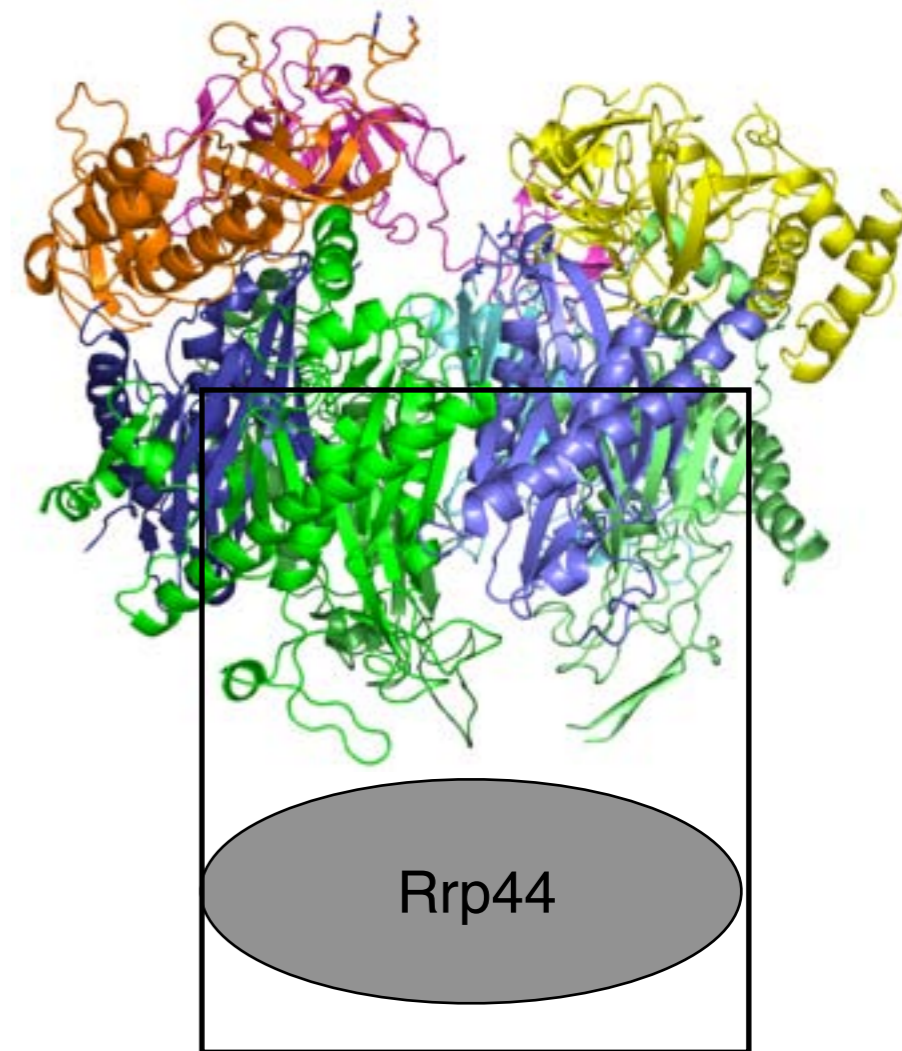
1 mol/AU



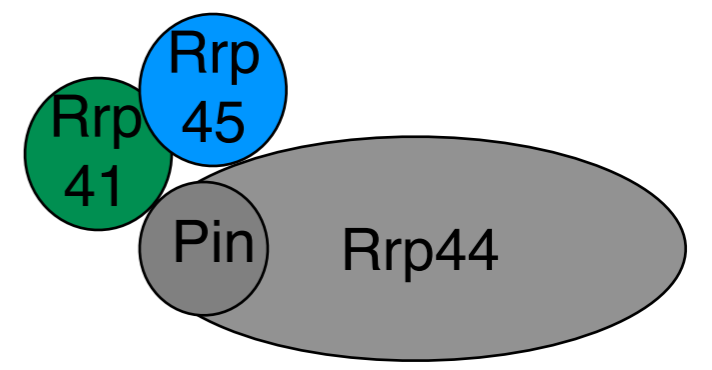
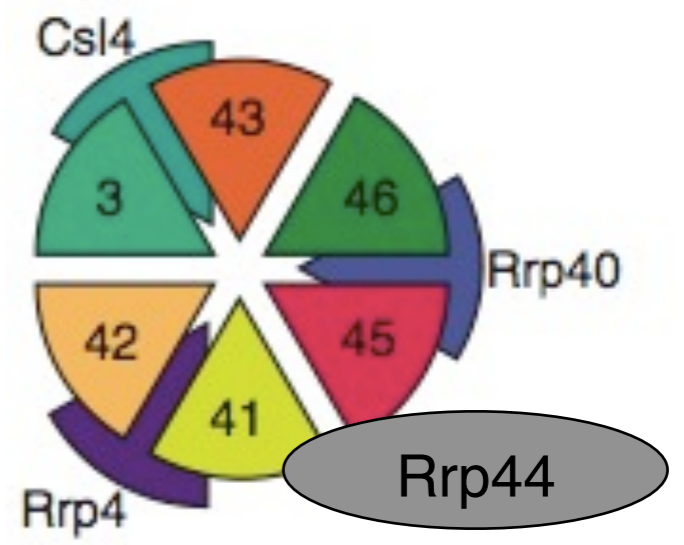
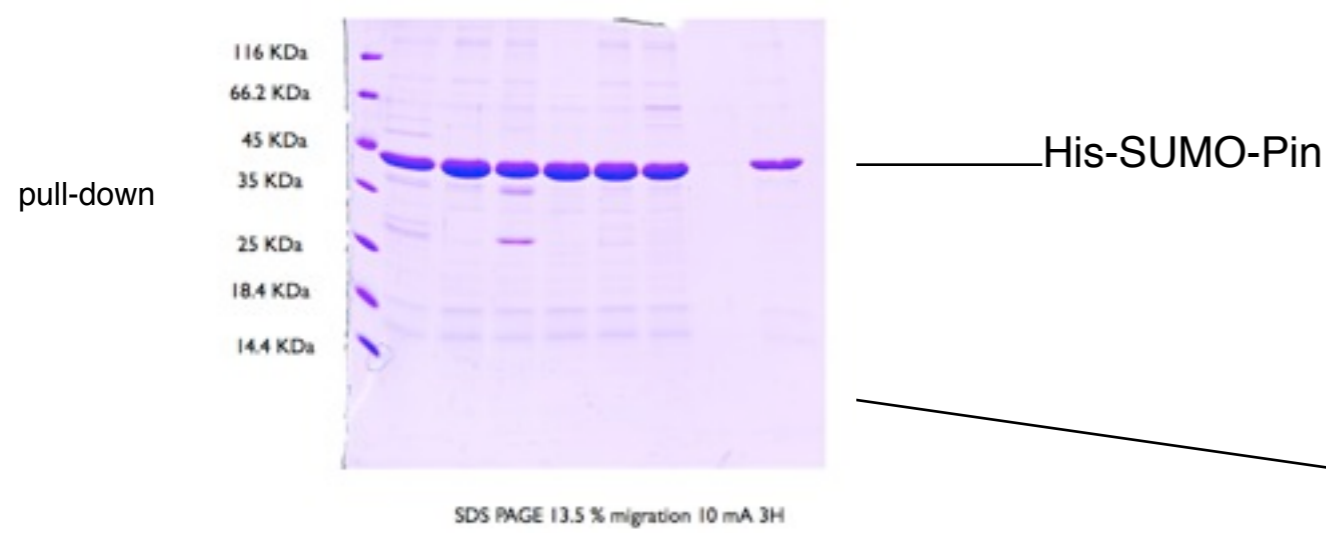
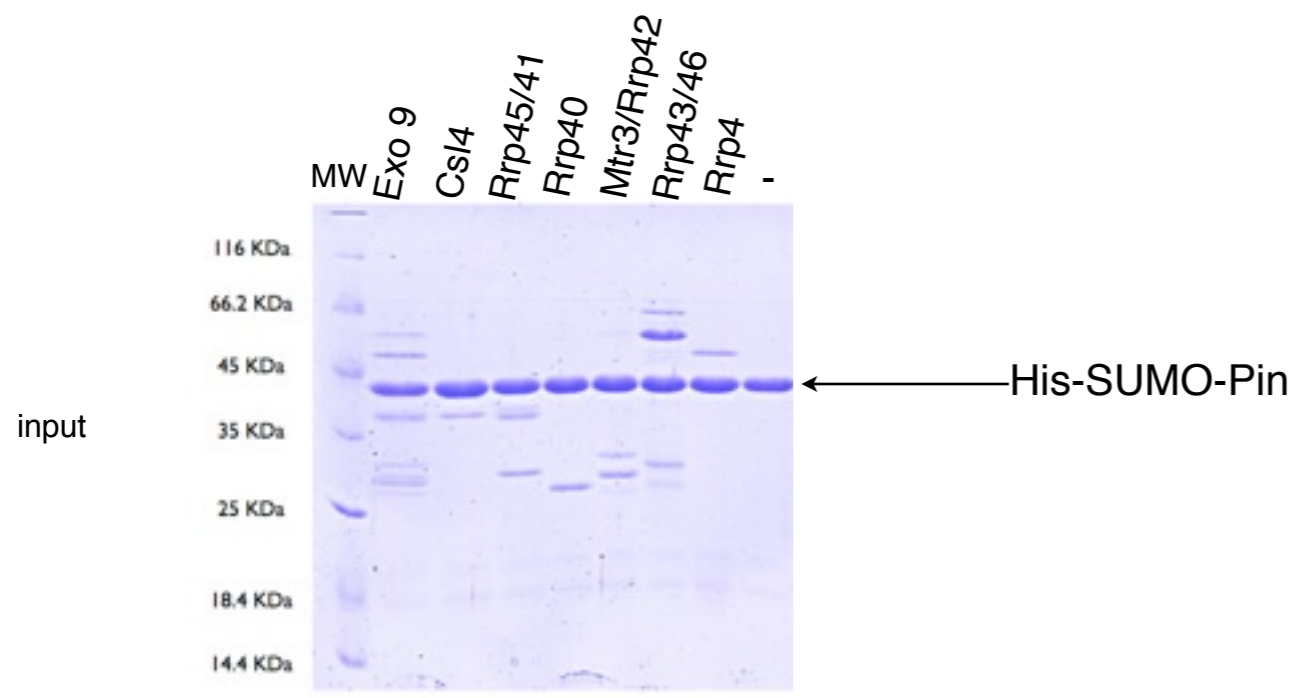
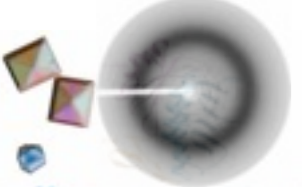


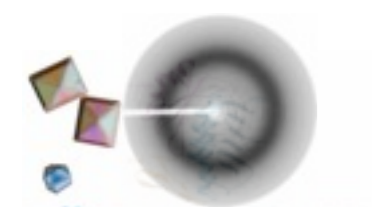
## How is Rrp44 bound to the exosome ?

- Biochemical mapping of the interacting regions
- Finding the right construct for crystallization
- crystal optimization

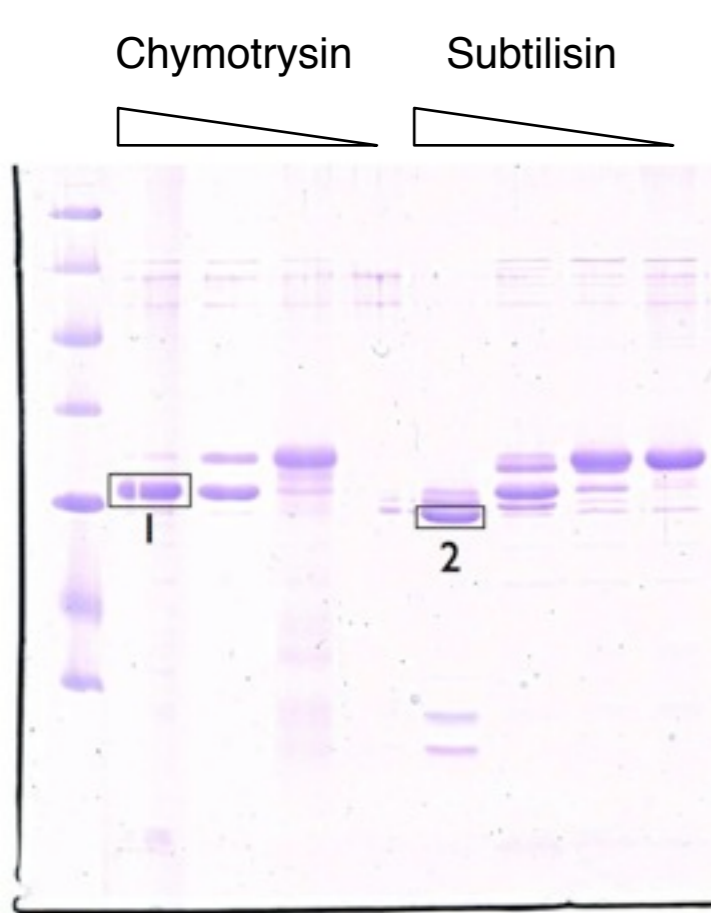


# Biochemical mapping of the interacting regions



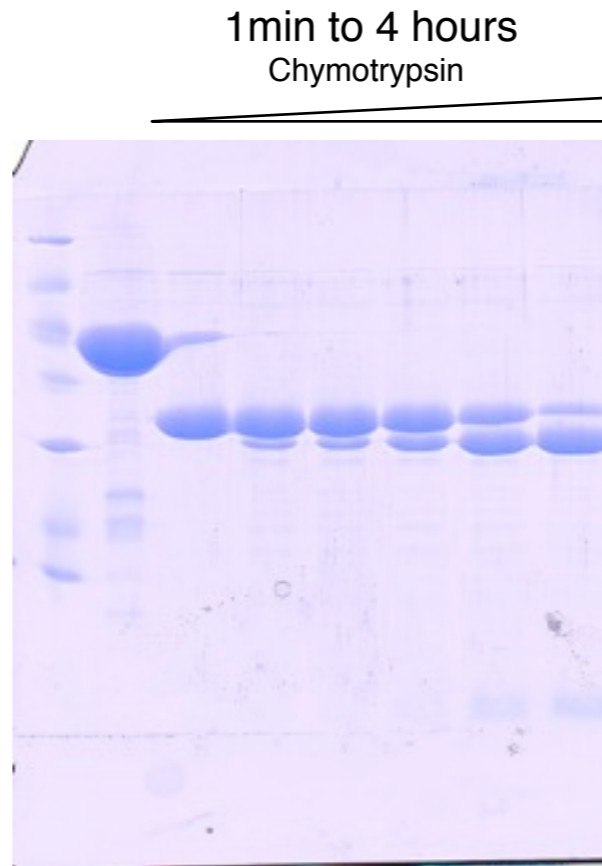


# Finding the right construct for crystallization



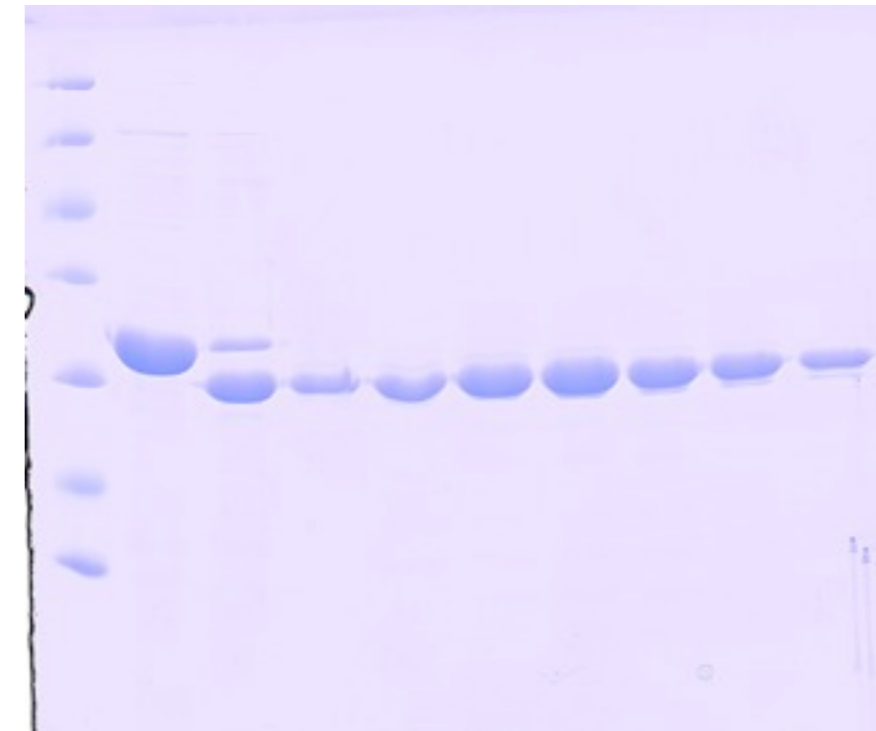
13.5 % SDS PAGE gel  
Coomassie staining

Protease screens



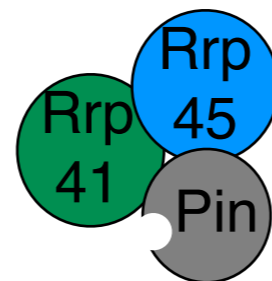
13.5 % SDS PAGE gel  
Coomassie staining

Time course

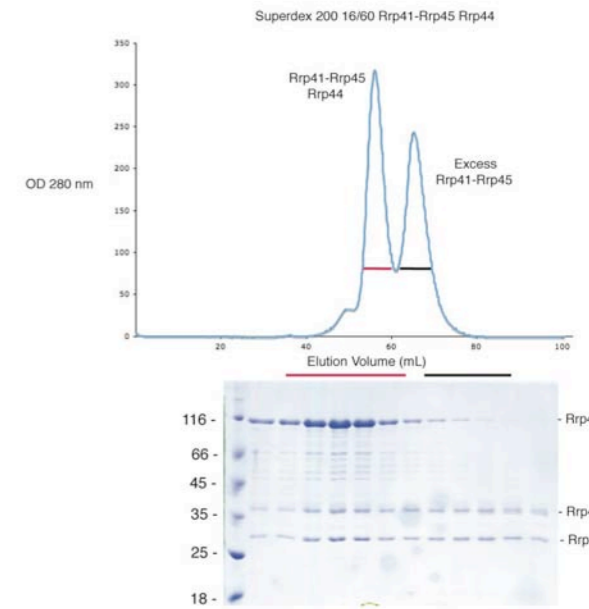
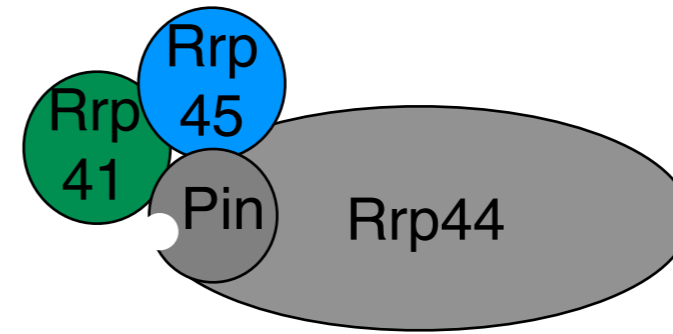
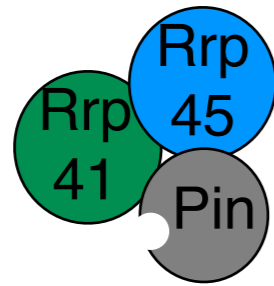


13.5 % SDS PAGE gel  
Coomassie staining

Preparative proteolysis +  
N-terminal sequencing



# Crystallization screens



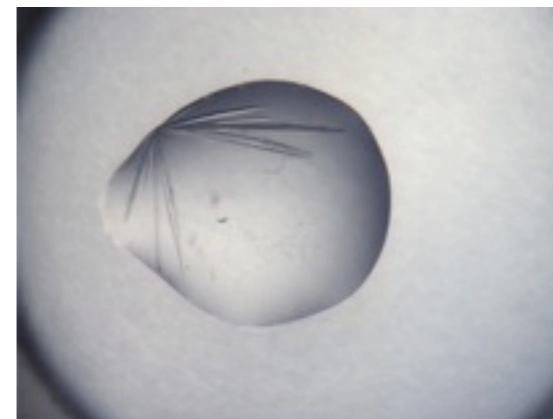
samples sent to crystallization facility

1200 trials RT/4C

1200 trials RT/4C



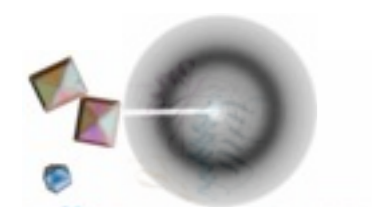
PEG condition



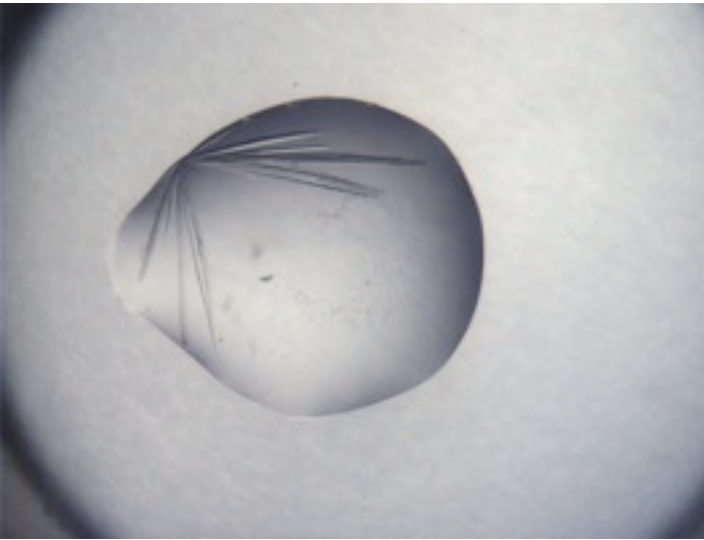
PEG condition



Ammonium sulfate condition



# Crystal optimization protocol of Rrp44-Rrp45-Rrp41 crystals



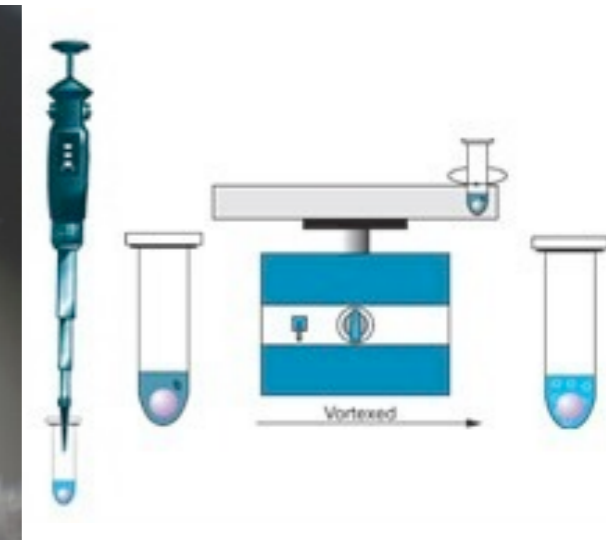
From initial hit



Use of my cat's whisker for streak seeding



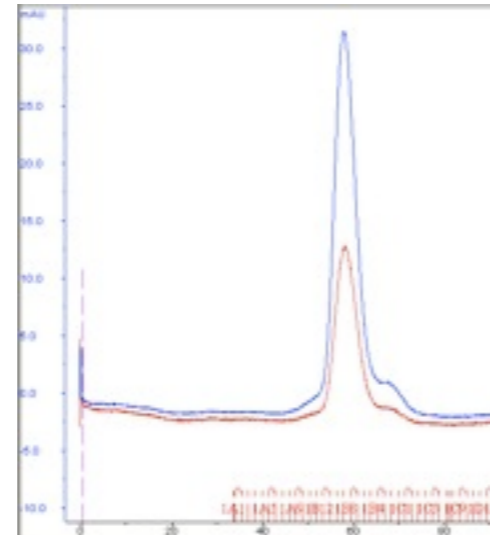
reproduce crystal with "Persilie" shape



seed beads



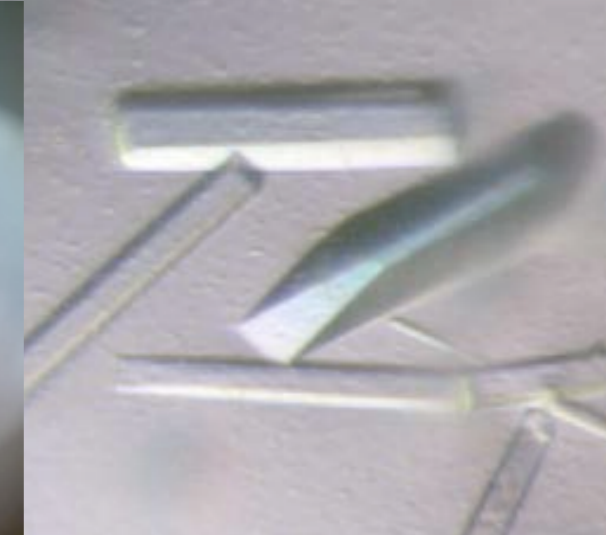
Preparative crystallization  
50 mg of protein crystallized in  
1 mL eppendorf tube



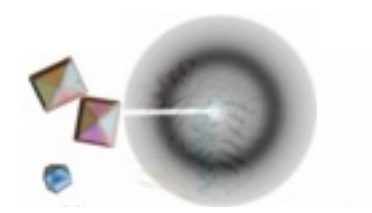
Re-purification  
centrifugation + S200



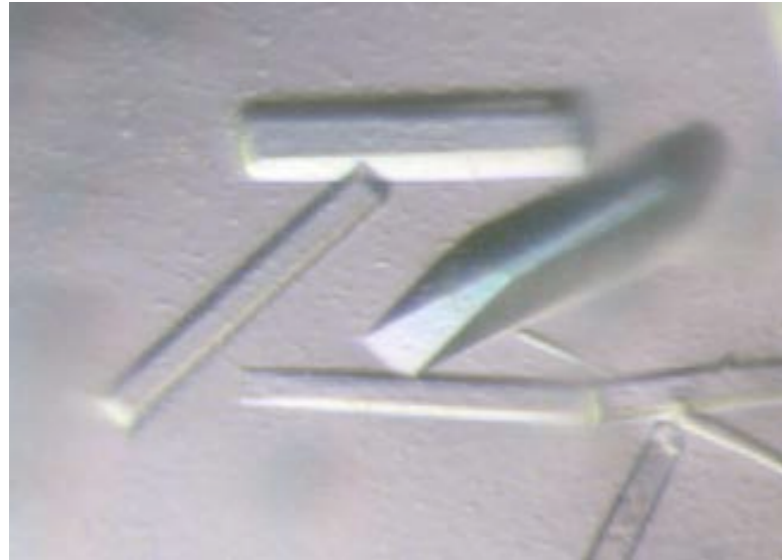
seed from the seed beads  
experiment + pH screens



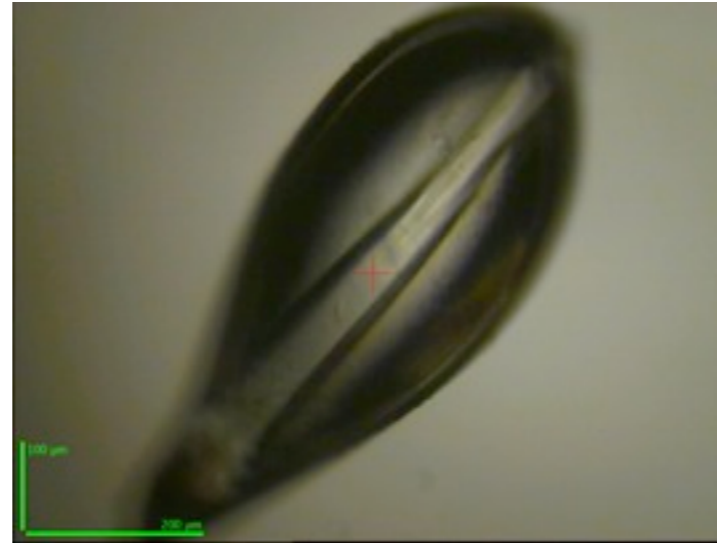
200 um crystals grown in  
cryoconditions



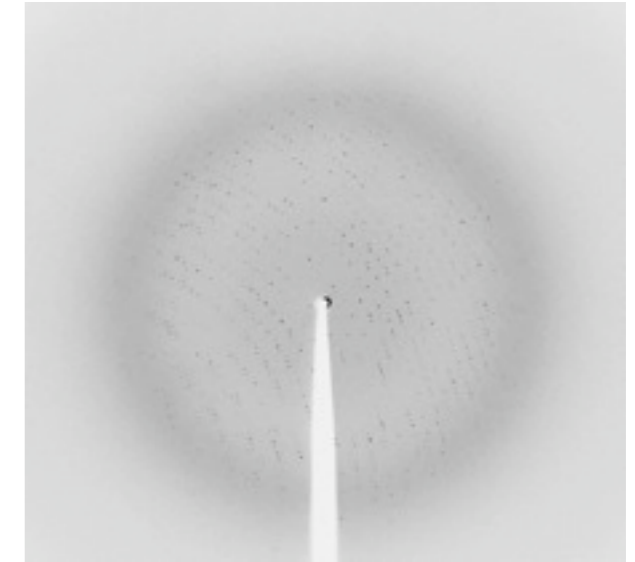
# Crystal optimization protocol of Rrp44-Rrp45-Rrp41 crystals



200 um crystals grown in cryocondition



1 single crystal mounted in a cryoloop for data collection



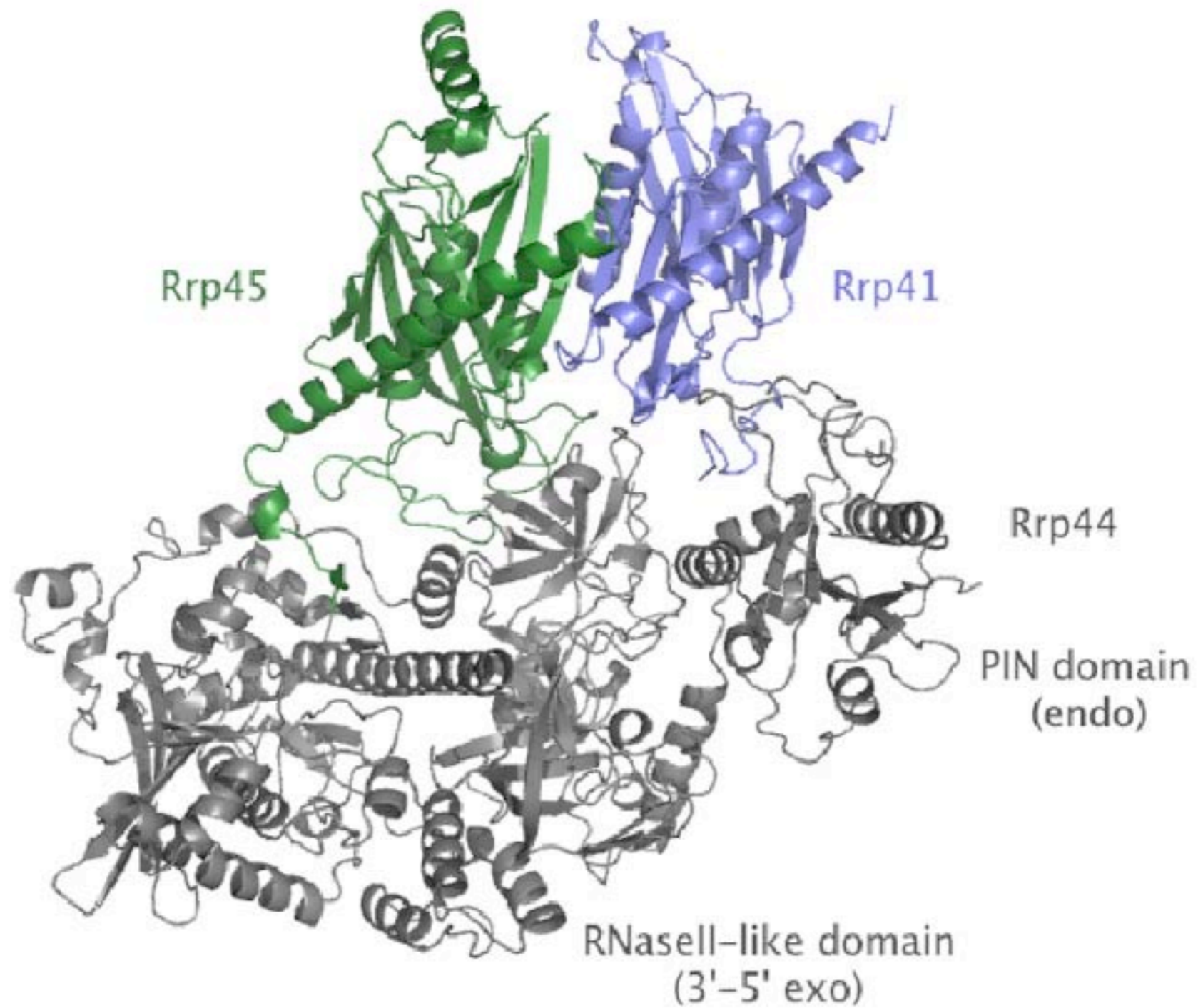
diffraction pattern at SLS beam-line SLS X10SA

Space group:  $P2_12_12_1$   
Unit cell (Å): a=110.9 b=125. c=139.4  
Resolution : 20-3.0 (Å)  
Rmerge(%) : 11.3 (52.2)  
 $I/\sigma(I)$  : 21.6 (5.7)  
Completeness(%) : 100 (100)

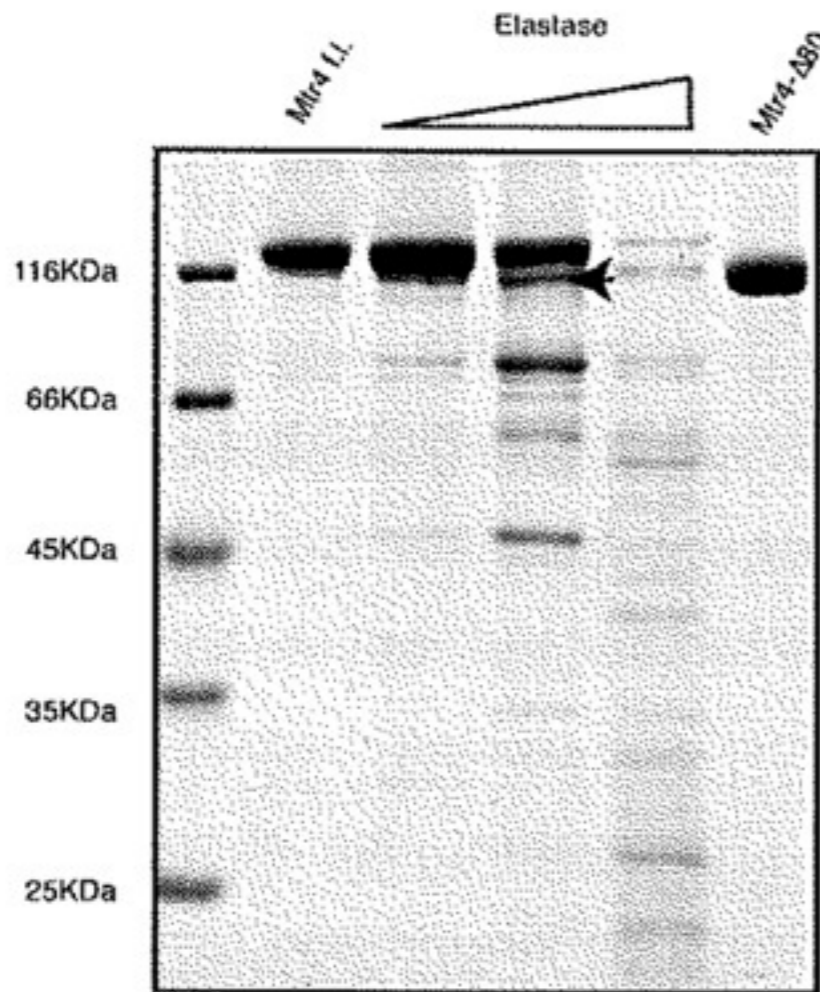
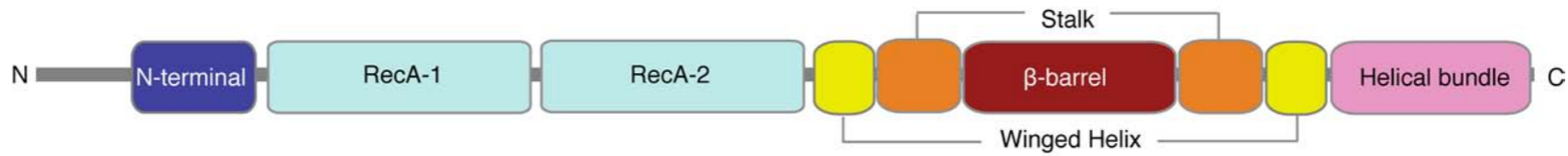
Solved by molecular replacement using the catalytic domain of Rrp44 and human Rrp45-41 as search models in the program Phaser.

deposited in the pdb :2WP8

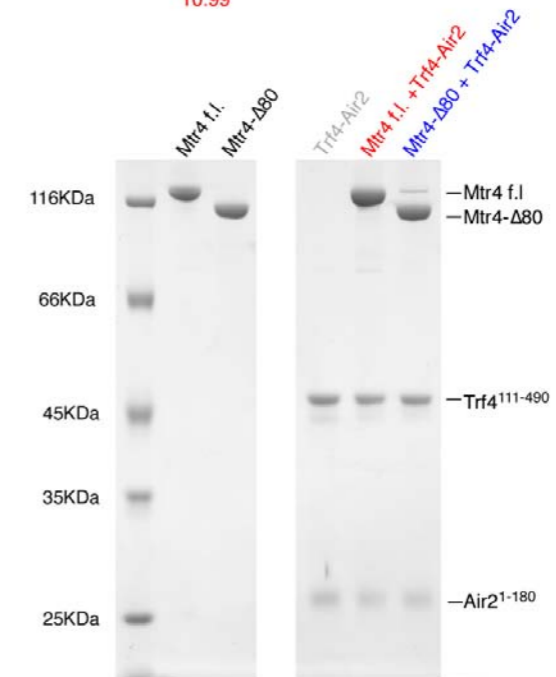
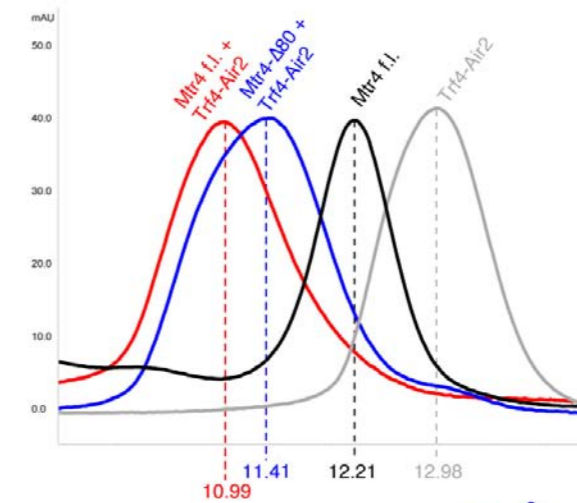
# Structure of yeast Rrp41-Rrp45-Rrp44



# Structural studies of the DExH helicase Mtr4

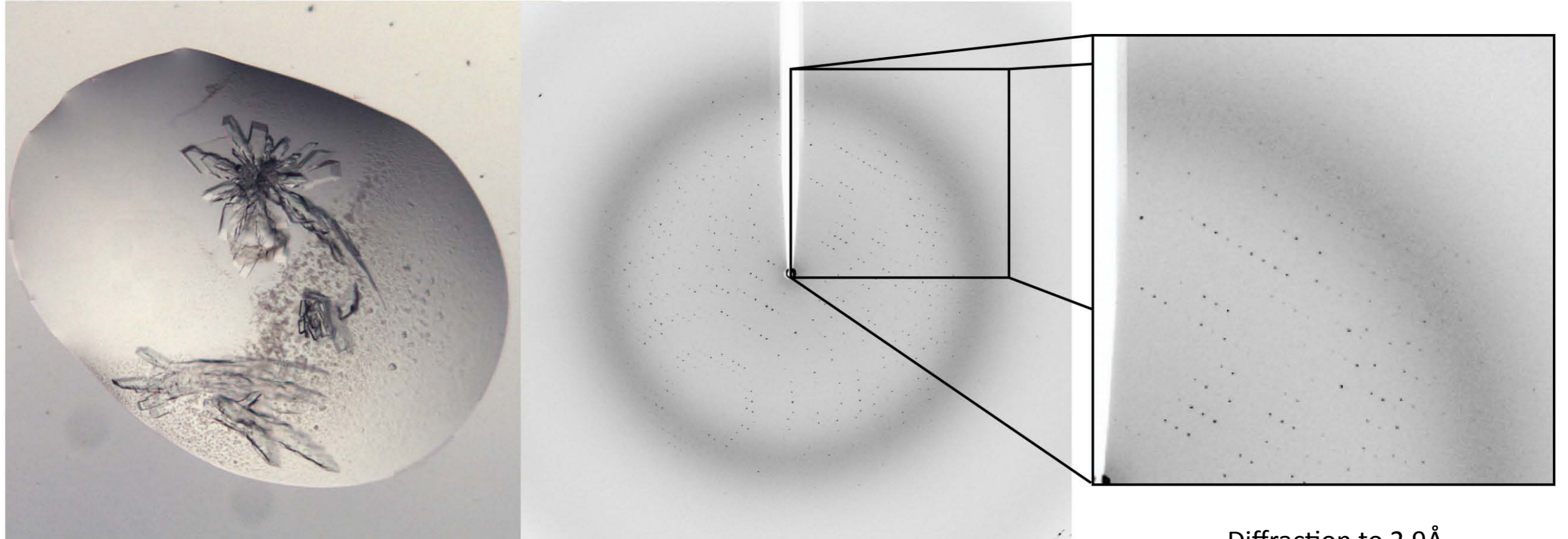


**Mtr4-Δ80 retains all functions of full length Mtr4**



Trf4-Air2 binding  
(TRAMP complex formation)

# Structural studies of the DExH helicase Mtr4

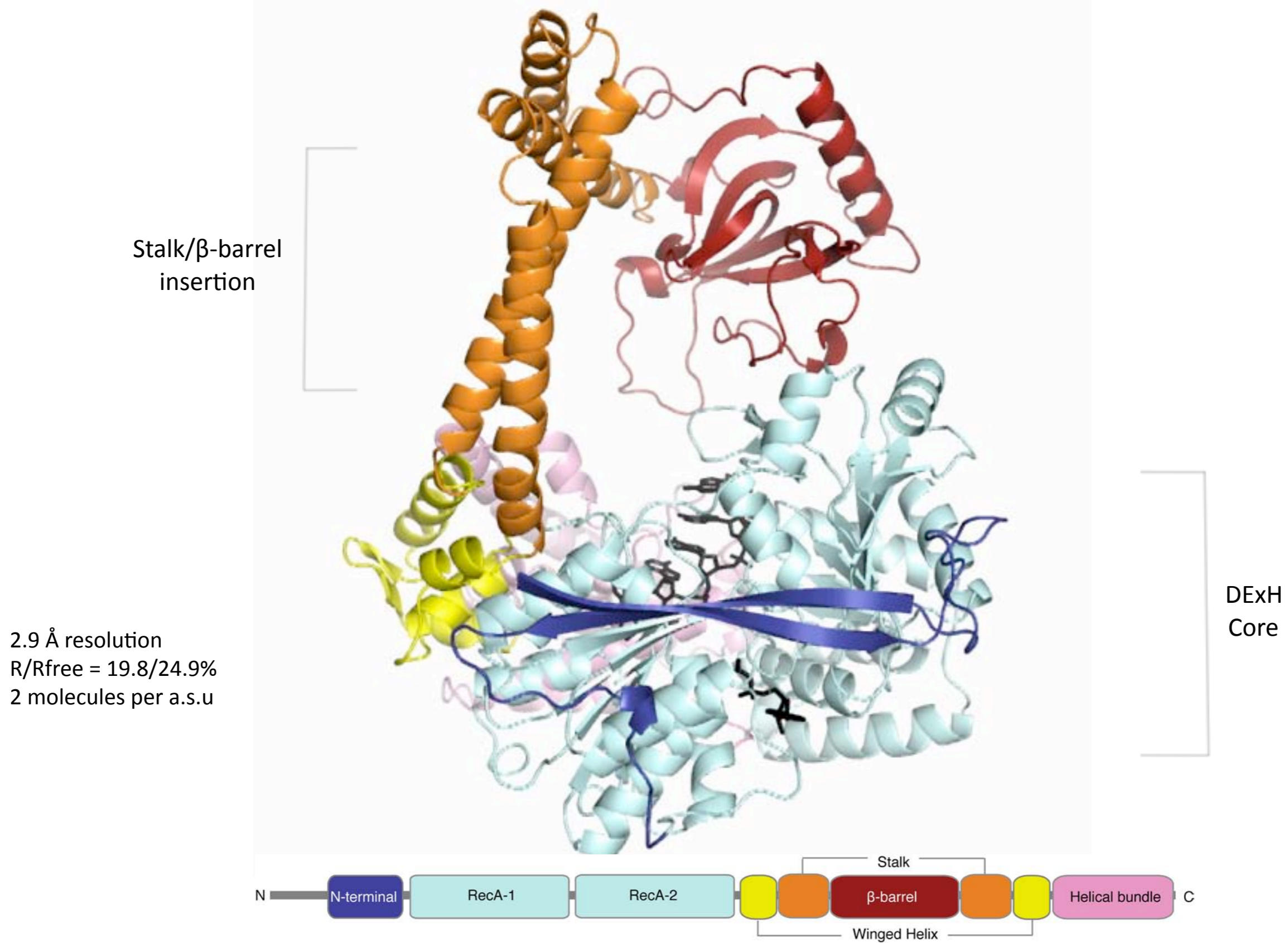


Mtr4-Δ80 crystals

Structure solved using 3 wavelength MAD from Se-Met  
derivitized protein

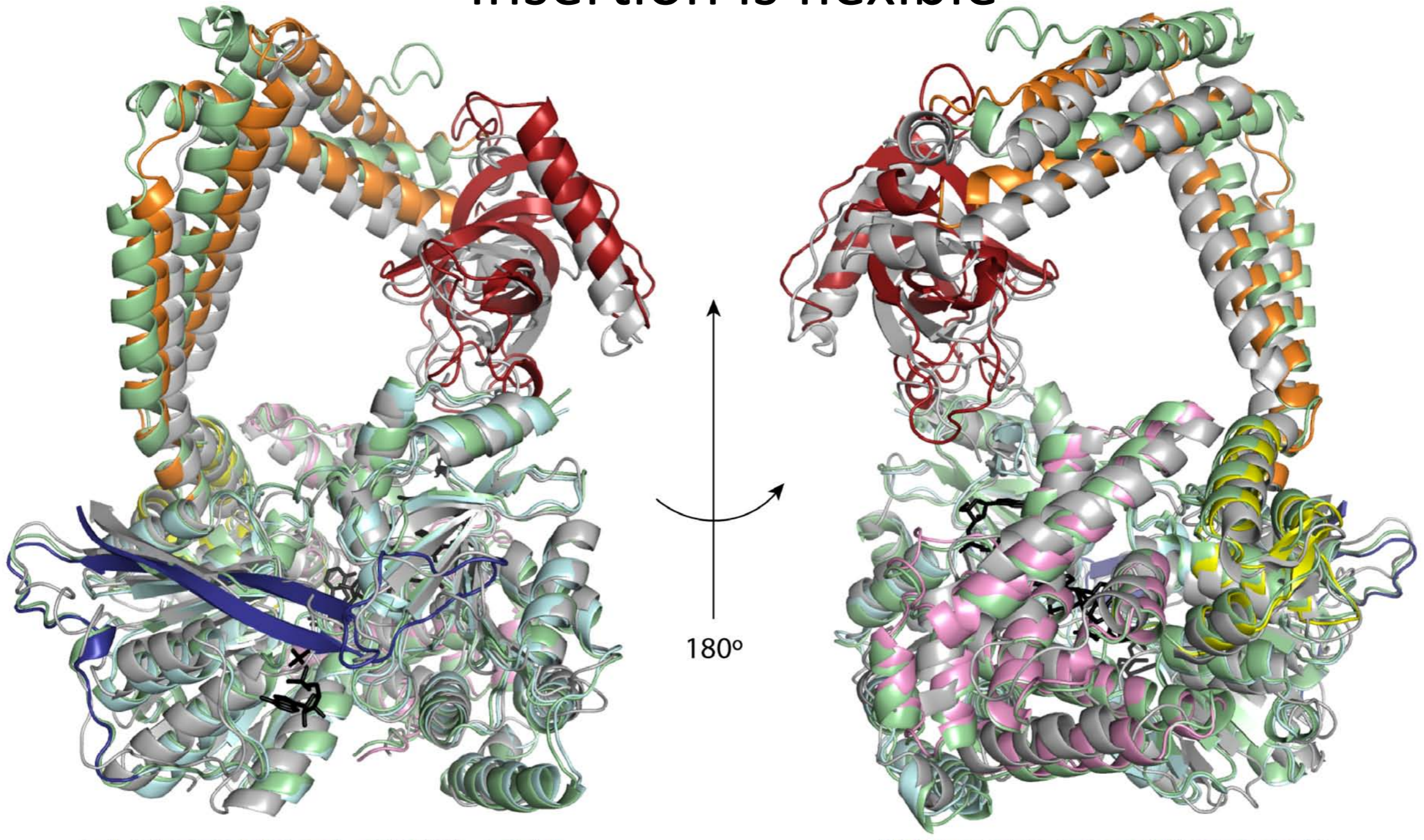
Diffraction to 2.9Å

# Structural studies of the DExH helicase Mtr4



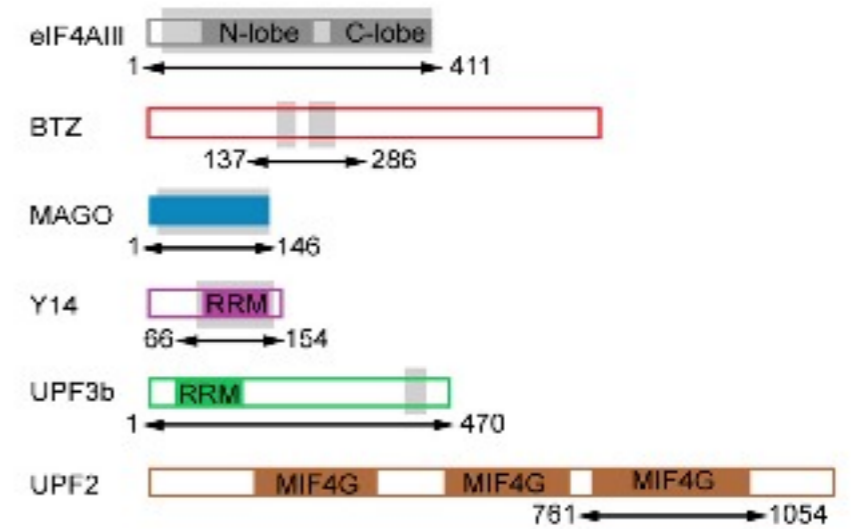
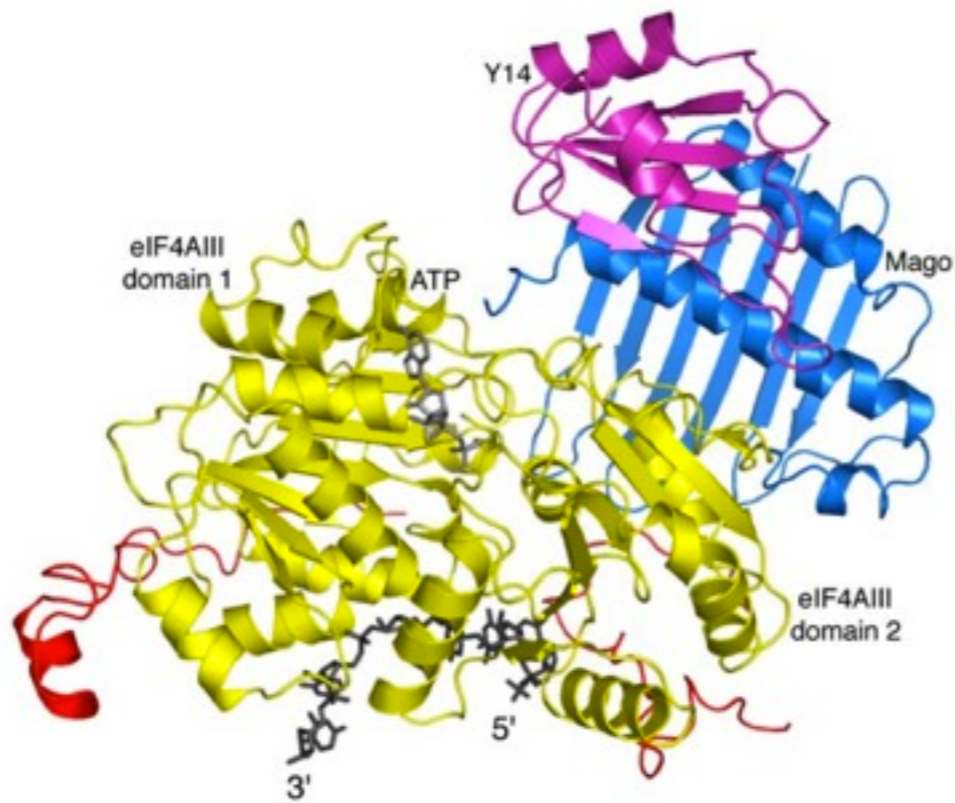
# A small cut can make a big difference

## Insertion is flexible

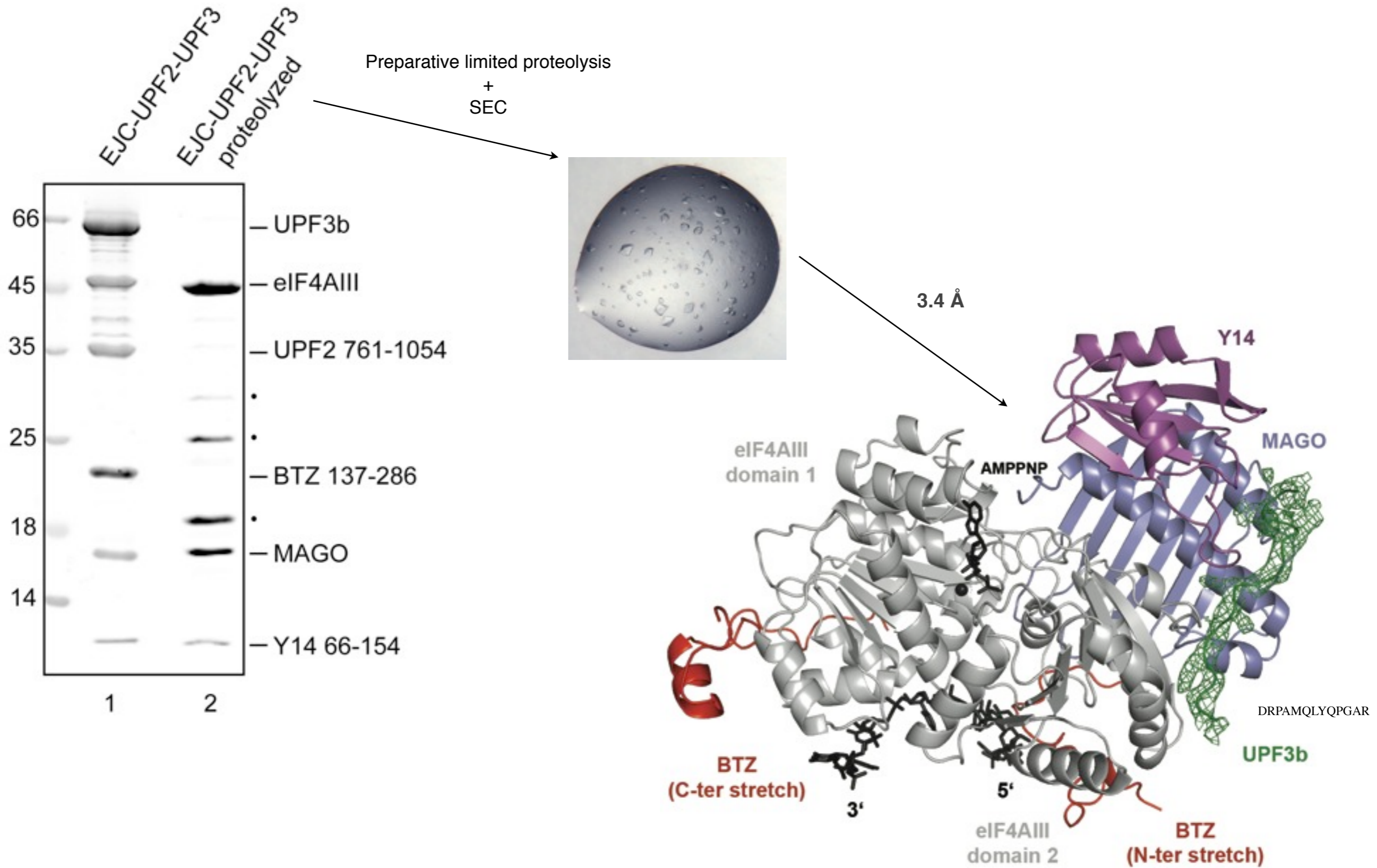


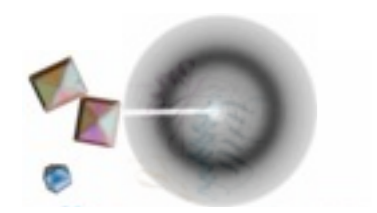
Second molecule in asymmetric unit  
3L90 – Jackson *et al.* EMBO J. May 2010

# Crystal structure of a core EJC-UPF3b complex



# Crystal structure of a core EJC-UPF3b complex





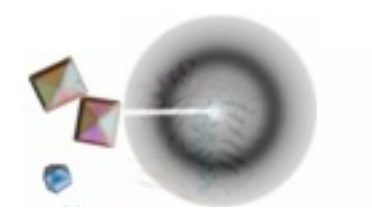
## **Conclusion & take home message**

**Characterization prior crystallization is crucial**

**limited proteolysis is a powerful method to identify useful constructs for crystallization**

**The method is easy to implement and to use**





# Acknowledgments



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