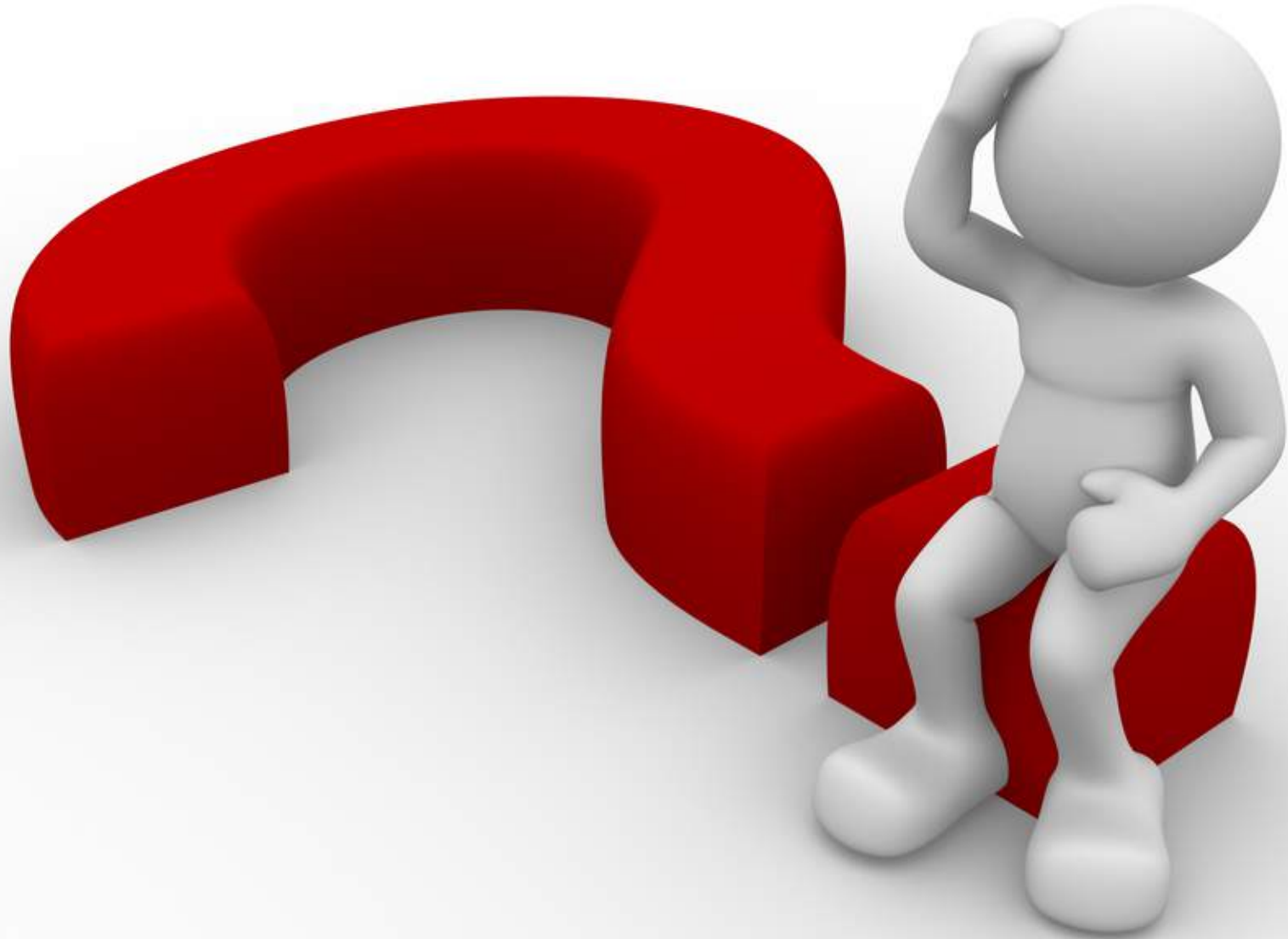


How would YOU study a 3.3MDa complex implicated in bacterial acid stress response?

Hélène Malet

Aknowledgements

Grenoble

Irina Gutsche

Guy Schoehn

Benoit Arragain

Maria Bacia

Toronto

Walid Houry

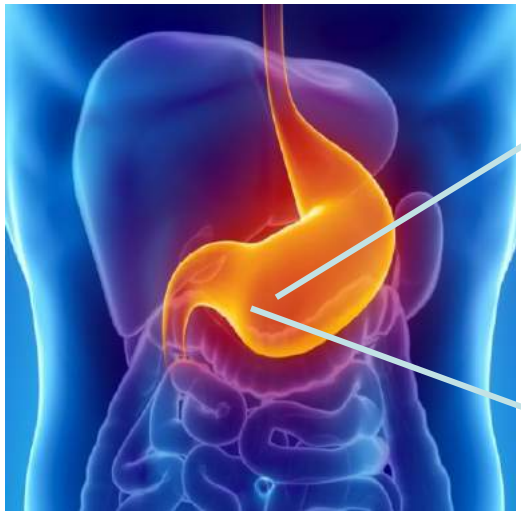
Acid stress response in *Escherichia coli*

Acid stress response

Amino Acid decarboxylases

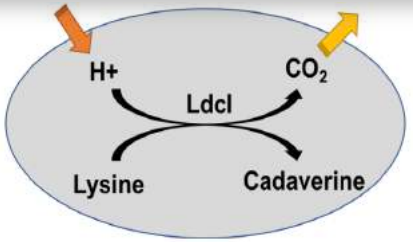
Acid stress

Enzymes which increase the internal pH



E. coli

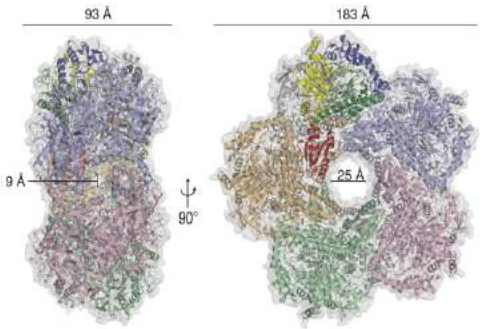
Inducible Lysine decarboxylase (LdcI)



Acidic environment (pH 2.5)

Natural antibiotic barrier

800 kDa

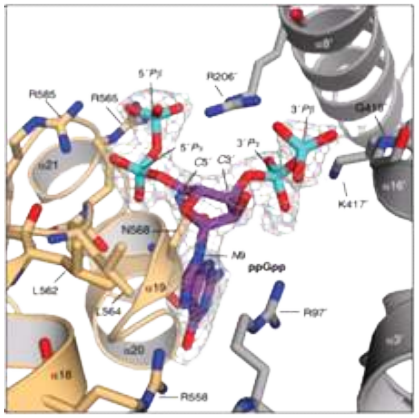


Double ring decamer under acidic conditions

LdcI regulation in nutrient and acidic stress condition

Nutrient stress

Binding of LdcI to the alarmone ppGpp



Inhibition of LdcI activity to maintain lysine pool

No acid stress response

Nutrient and acid stress

Binding of LdcI to an AAA+ ATPase: RavA

RavA antagonizes LdcI inhibition by the alarmone

Acid and nutrient stress response

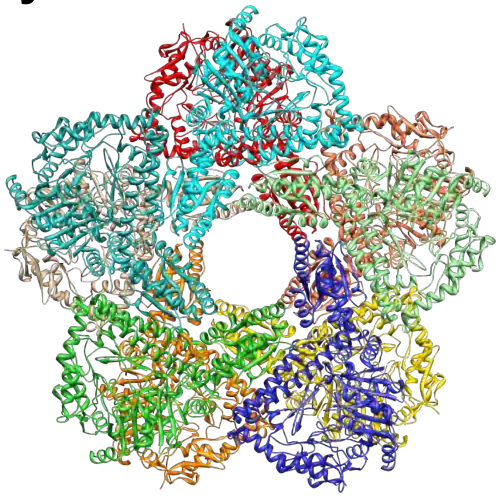
Alarmone and AAA+ ATPase RavA: Key regulators of LdcI activity

El Bakkouri et al., PNAS 2010

Interaction of LdcI decarboxylase with its ATPase regulator

RavA hexamer (330kDa)

+

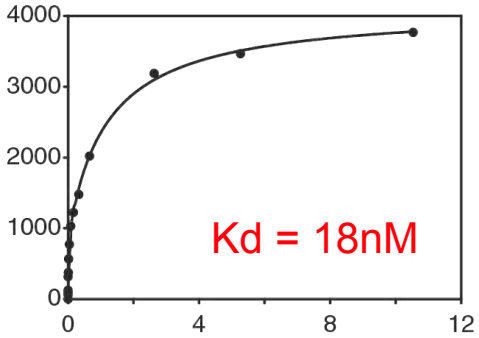


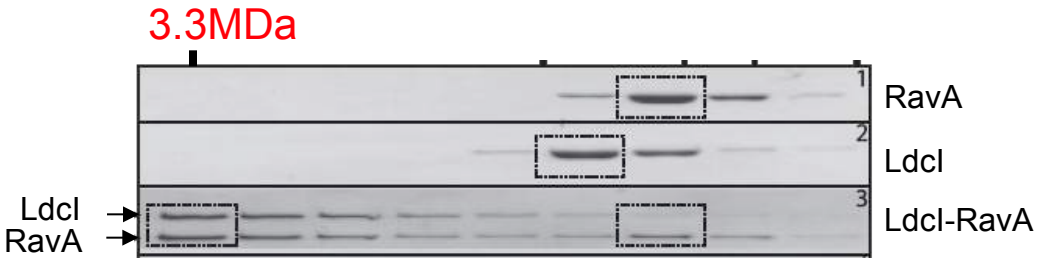
LdcI double pentamer (800kDa)

=?

How can a double pentameric lysine decarboxylase ring form a complex with a hexameric RavA AAA+ ATPase?

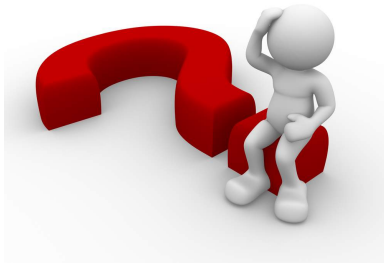
LdcI and RavA interact tightly and form a complex bigger than the ribosome



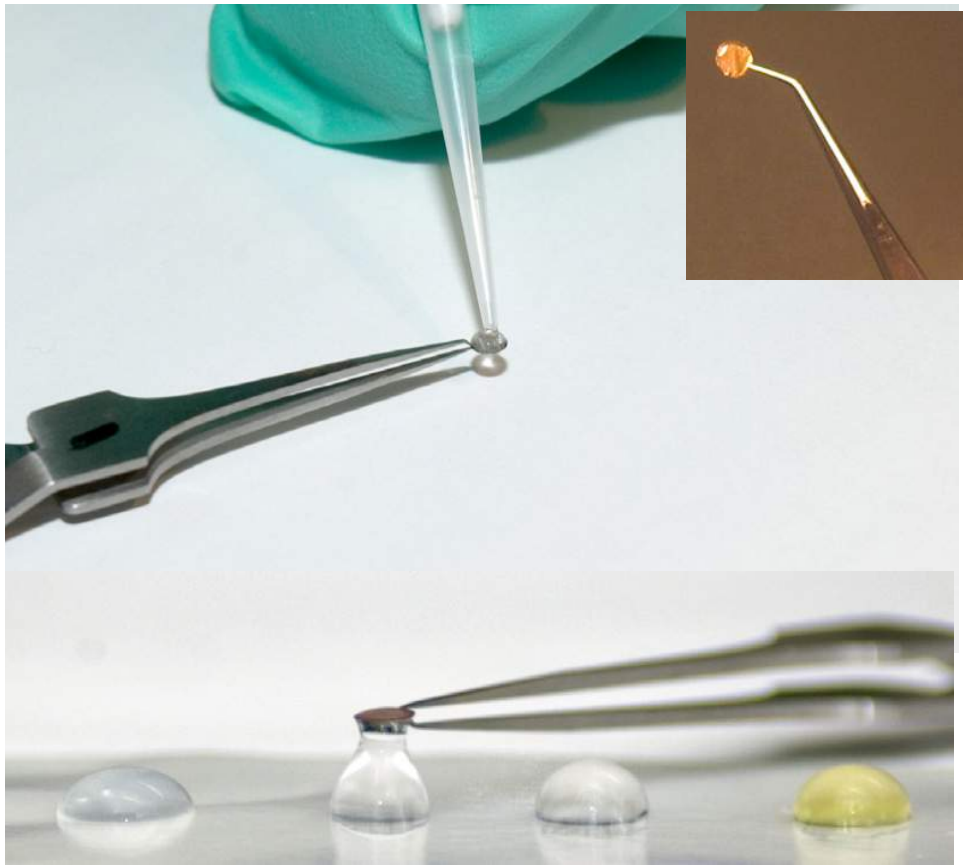


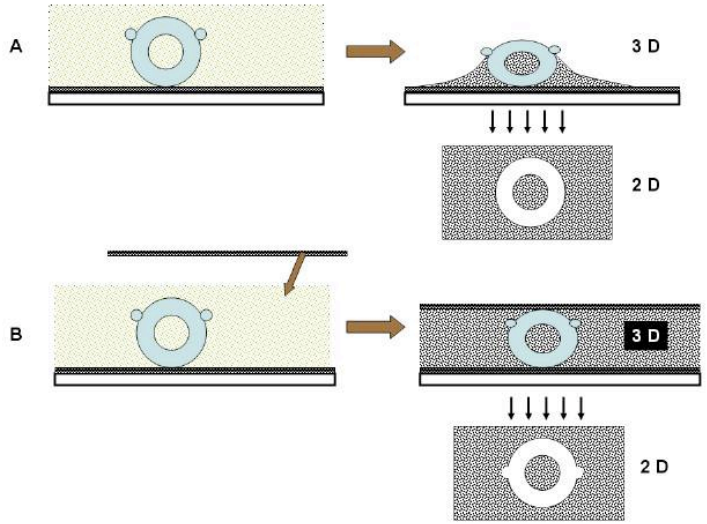
Snider et al, JBC, 2006
El Bakkouri et al., PNAS 2010

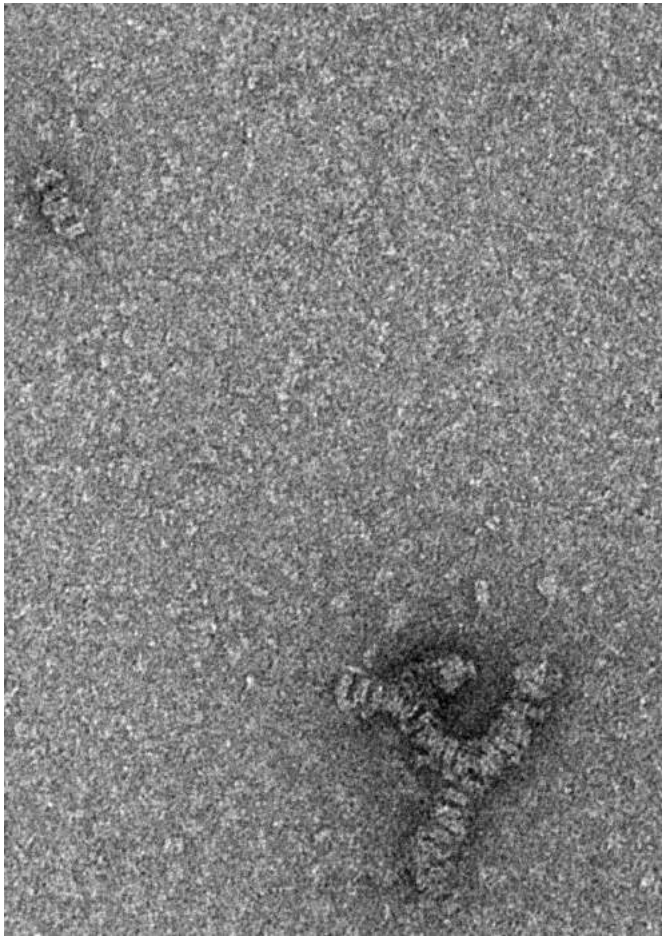
Which experiment would you do to quickly find out about this structure ?



- Negative stain electron microscopy!!







Never forget :

Rubbish in

Whatever expensive microscope

Rubbish out

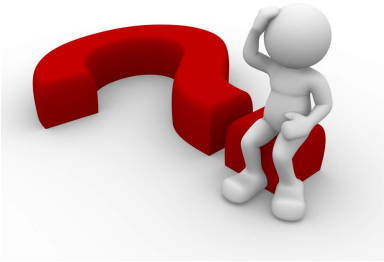
It is worth spending time getting a good sample !!!

How would you improve your sample?

- 1st strategy chosen (they are many others): use negative stai..
  EM to check different conditions for complex formation

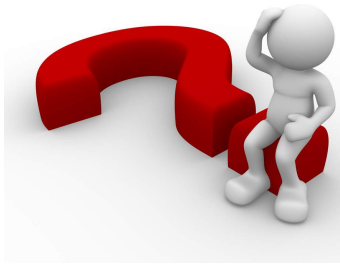
The sample is sensitive to pH, but uranyle acetate is at pH 4…

What would you change?

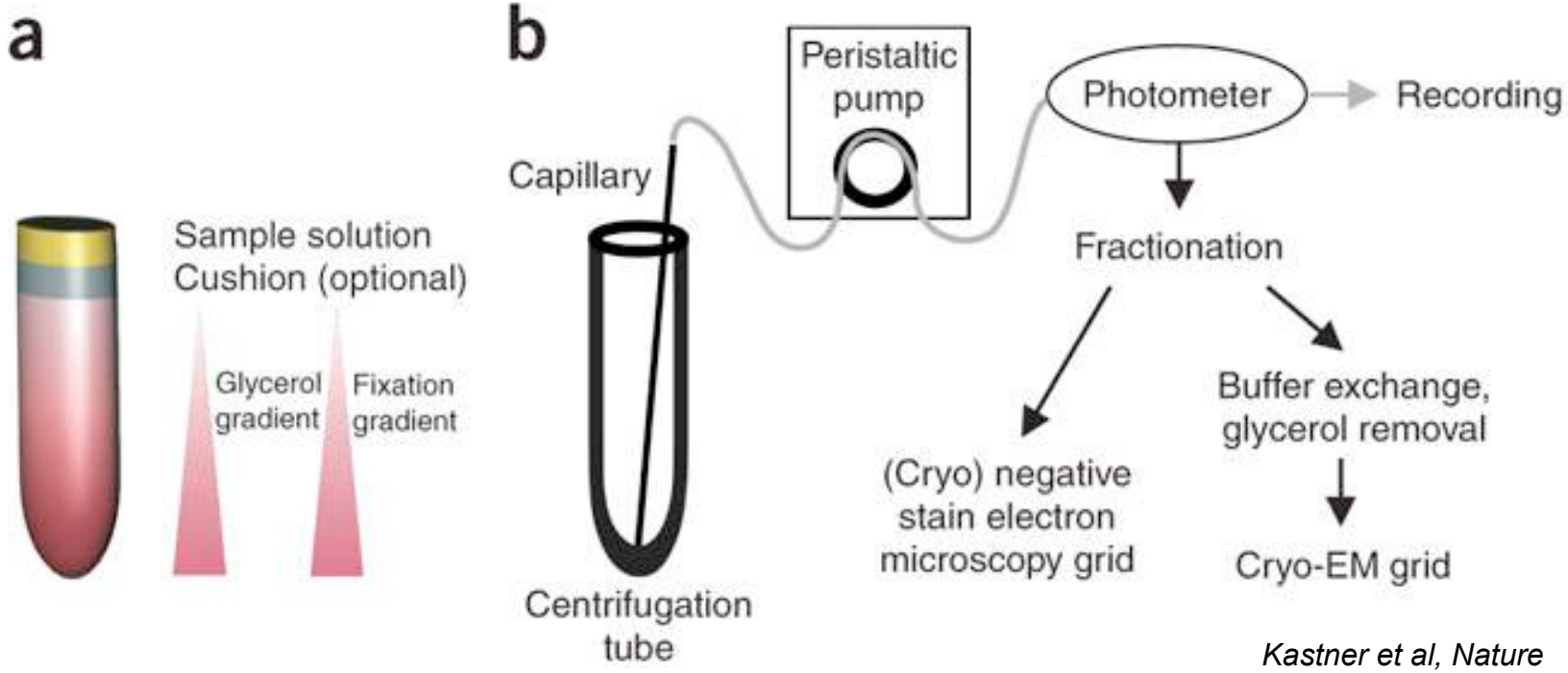


- Uranyle acetate is acidic but there are many other stains:
- SST (sodium silico tungstate)
- nanoW (methylamine tungstate)
- nanoVan (Vandanate)
- Ammonium molybdate…

How would you improve your sample?



- 2nd strategy (in this case, done after the 1st):
- Purify the complex.
- Hints of the problems for this complex: not stable at high concentration without glycerol
- Strategy used: Grafix



Kastner et al, Nature methods, 2008

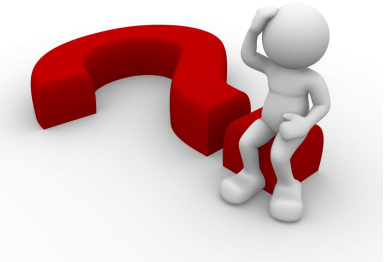
Is there any problem about grafix results for cryo-EM?

- Glycerol concentration needs to be as low as possible to have the best contrast possible:
  - 1% glycerol corresponds to 110mM glycerol
  - glycerol is viscuous: it can prevent nice ice formation on cryo-EM grids.

- More generally, the less you have in your buffer, the best it is for cryo-EM (as long as the protein is happy…)

How would you solve the glycerol problem?

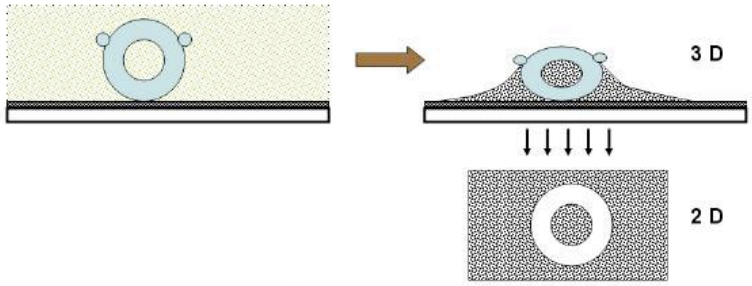
- Desalting columns
- Dialysis



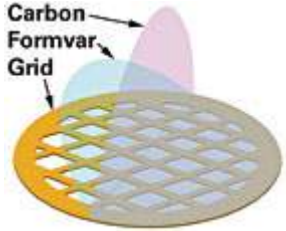
Other potential problem: the concentration

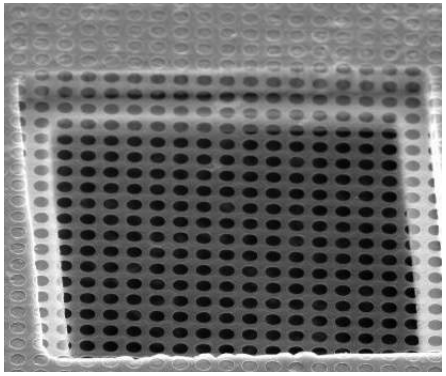
- To do a cryo-EM grid, you need 5 to 10 times more concentrated samples than for negative stain EM.
- Do you know why?

Negative stain EM grid

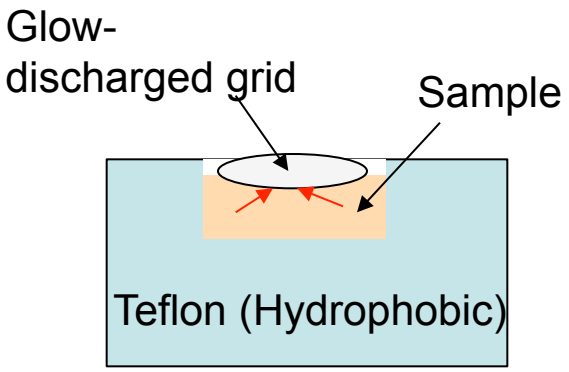


Cryo EM grid



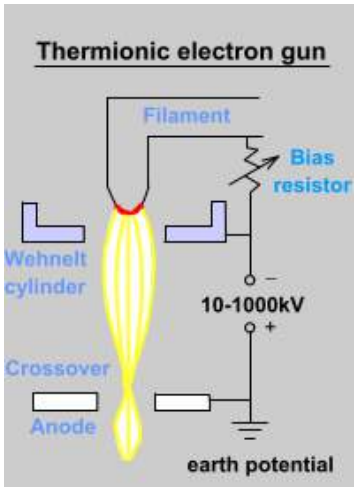


- How would you solve the problem?
  - Concentrator…
  - Teflon block

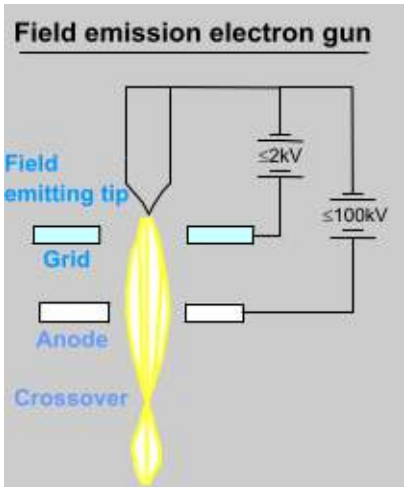


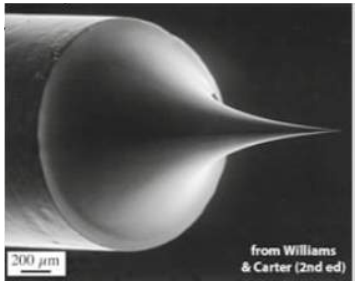
You are lucky to have access to a FEG microscope equipped with a direct detector

- Why is it important to use a FEG microscope if you want high resolution?



VS

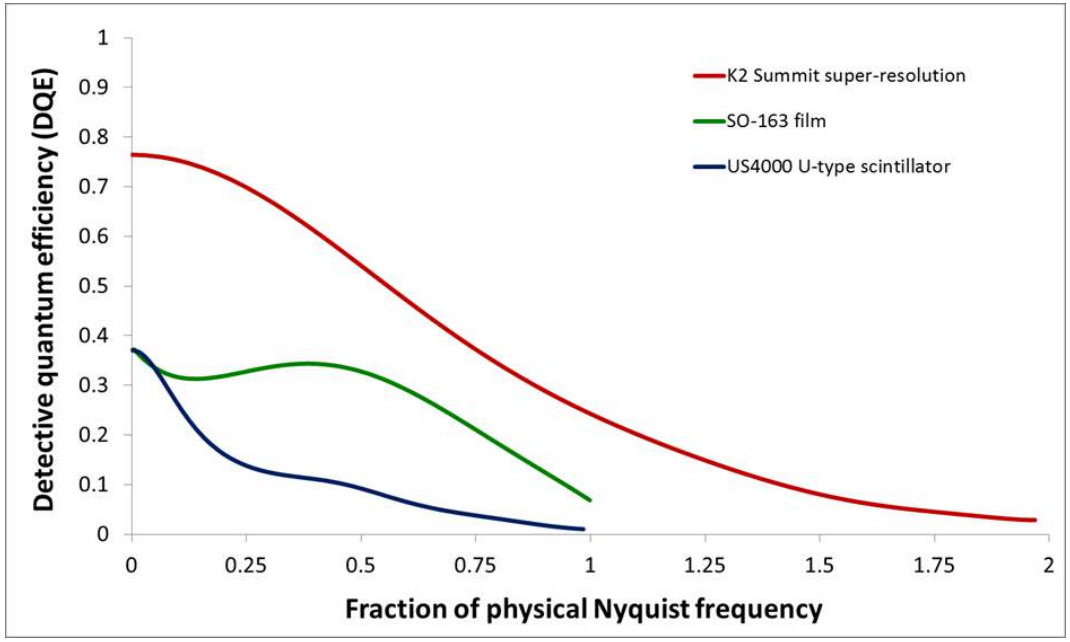


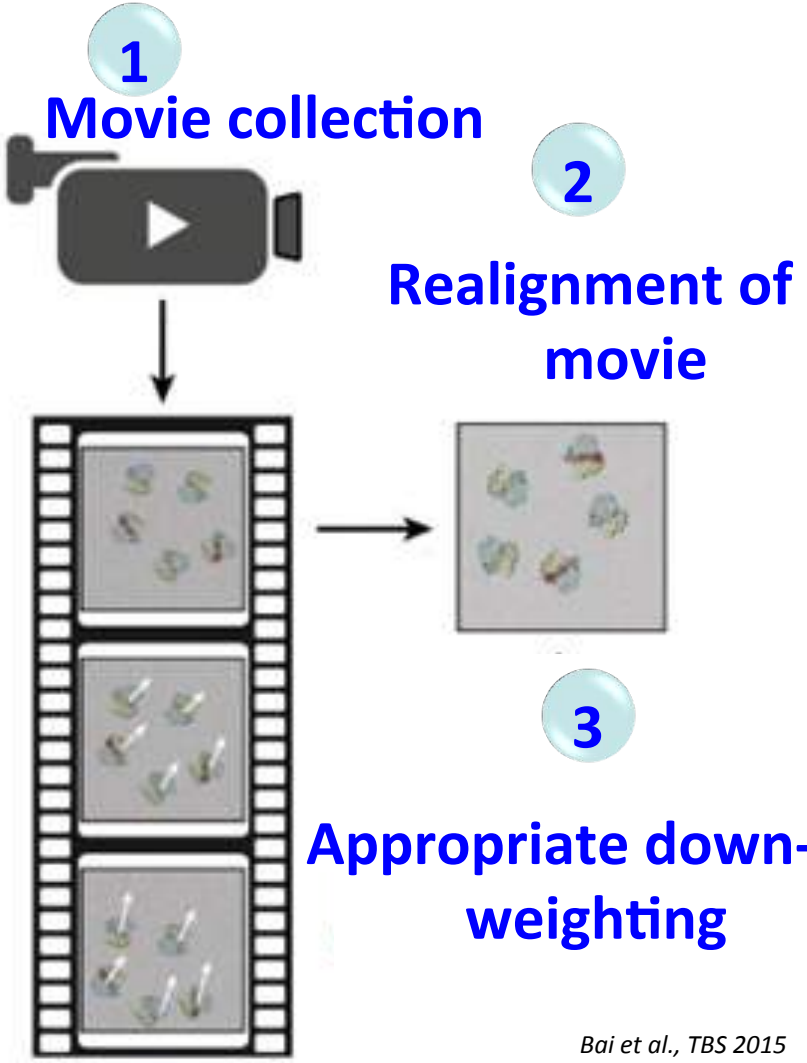


VS

You are lucky to have access to a FEG microscope equipped with a direct detector

- What is really great about direct detectors?





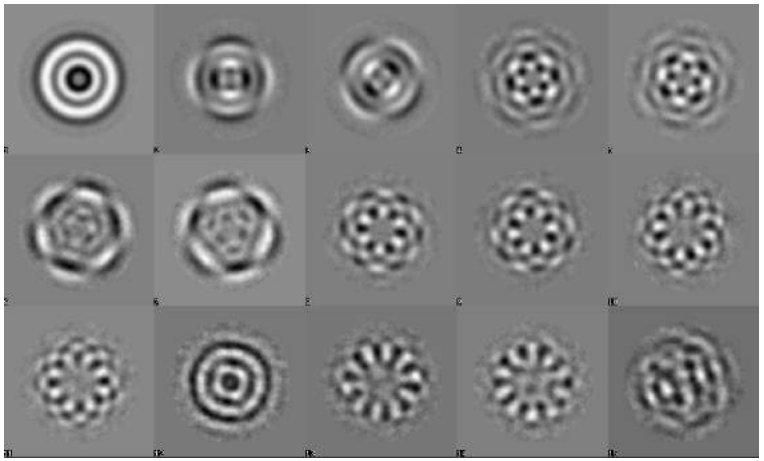
Bai et al., TBS 2015

What would you do to find out about the complex symmetry?

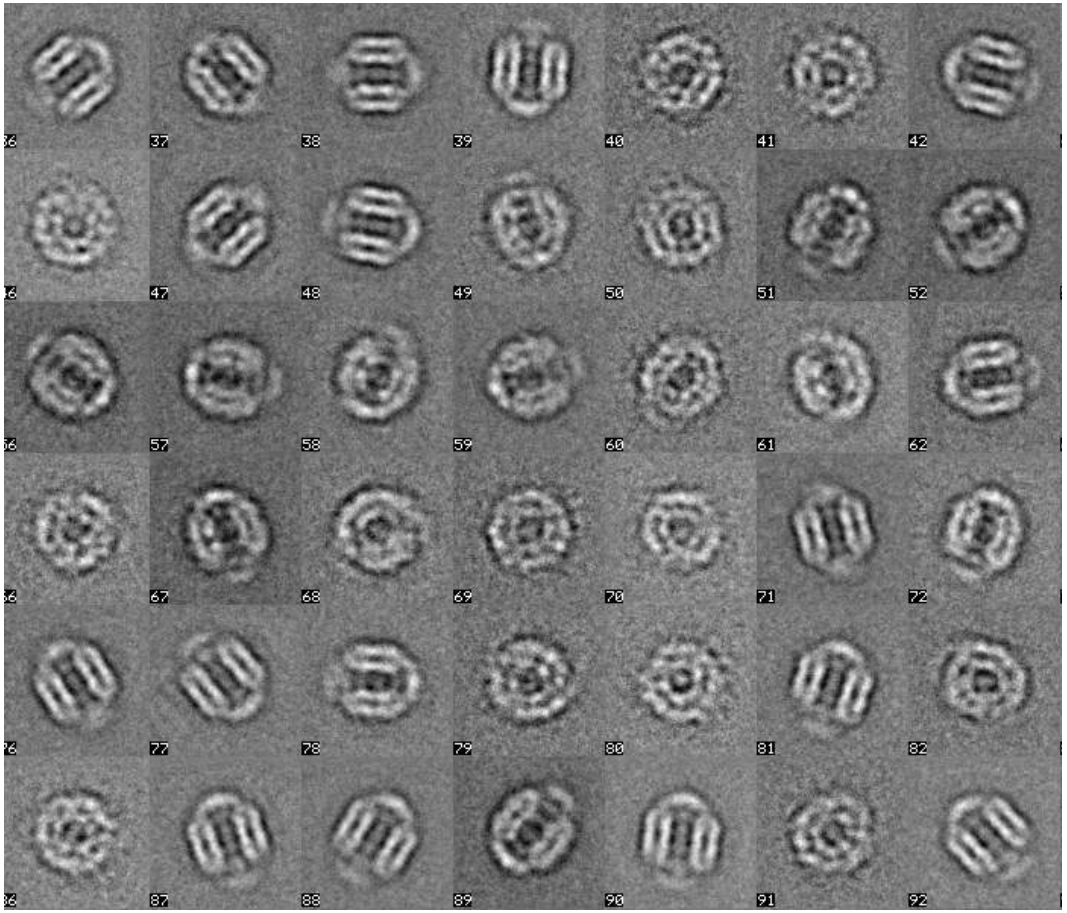
MSA analysis

1st MSA with all images (centered and band-pass filtered)

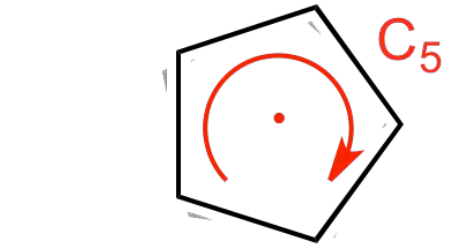
Eigenimages

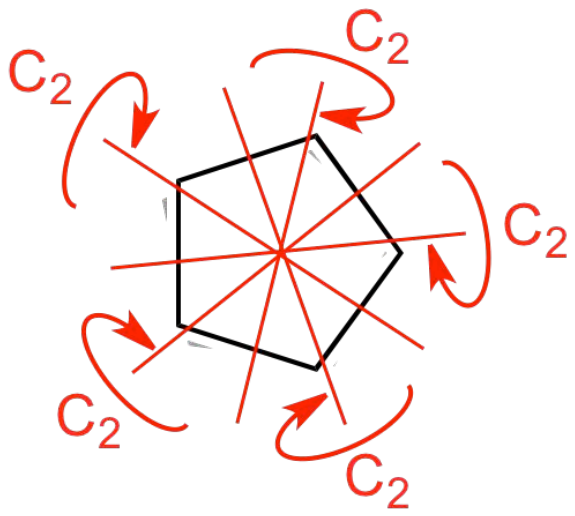


Class averages



Which symmetry would you like to impose for image processing?

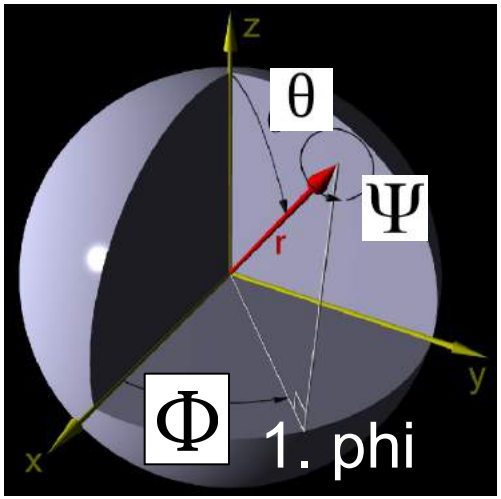




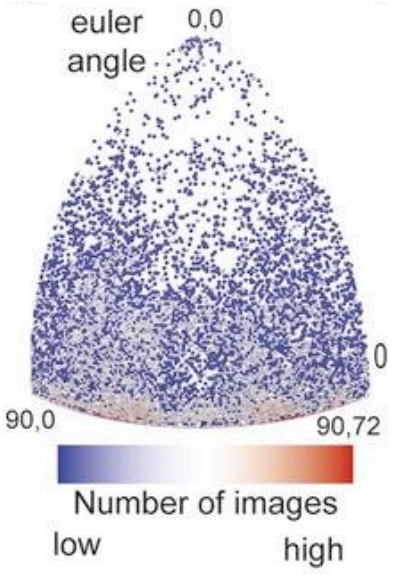
Why does symmetry help for 3D reconstruction?

For angle assignation:
Instead of looking for euler angles over the entire sphere, we will limit the search to the D5 asymetric unit.

For 3D reconstruction:
Each image will be back-projected 10 times for a D5 symmetry.

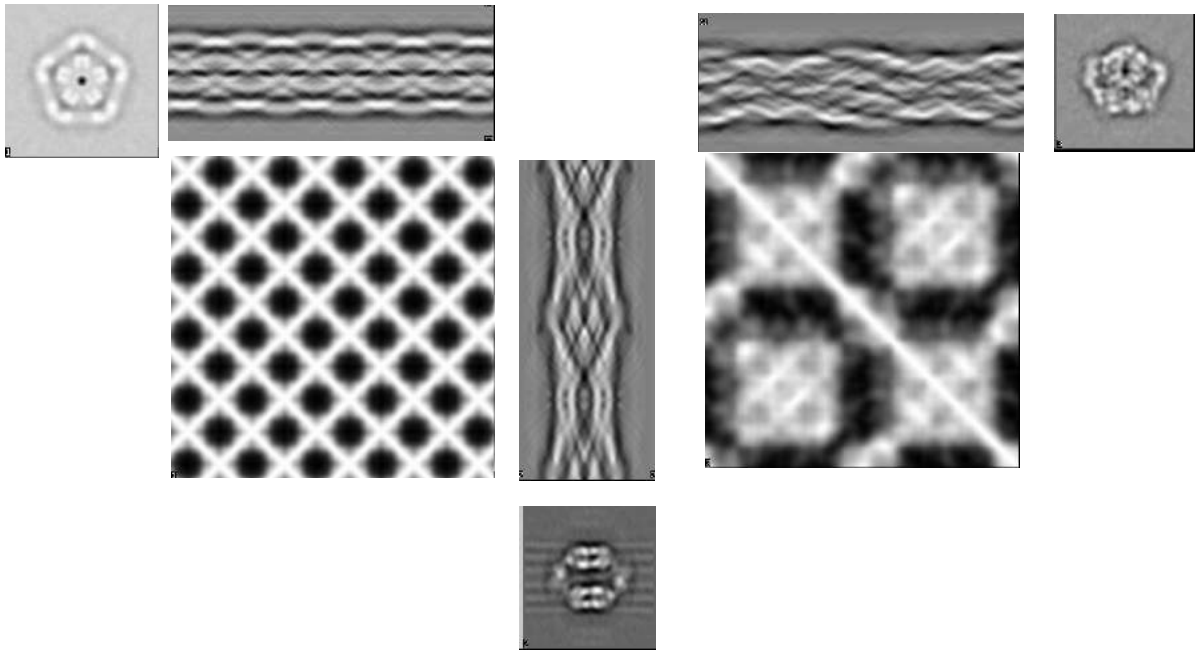


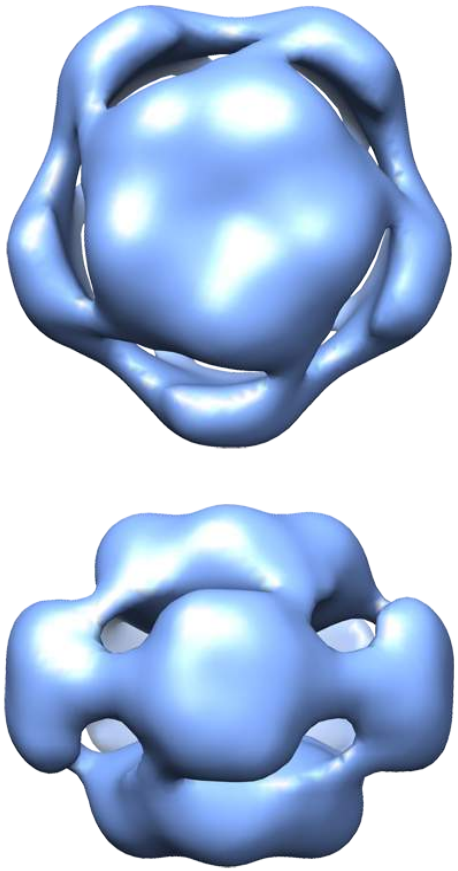
Asymmetric unit in D5



Which strategy would you choose for initial 3D reconstruction?

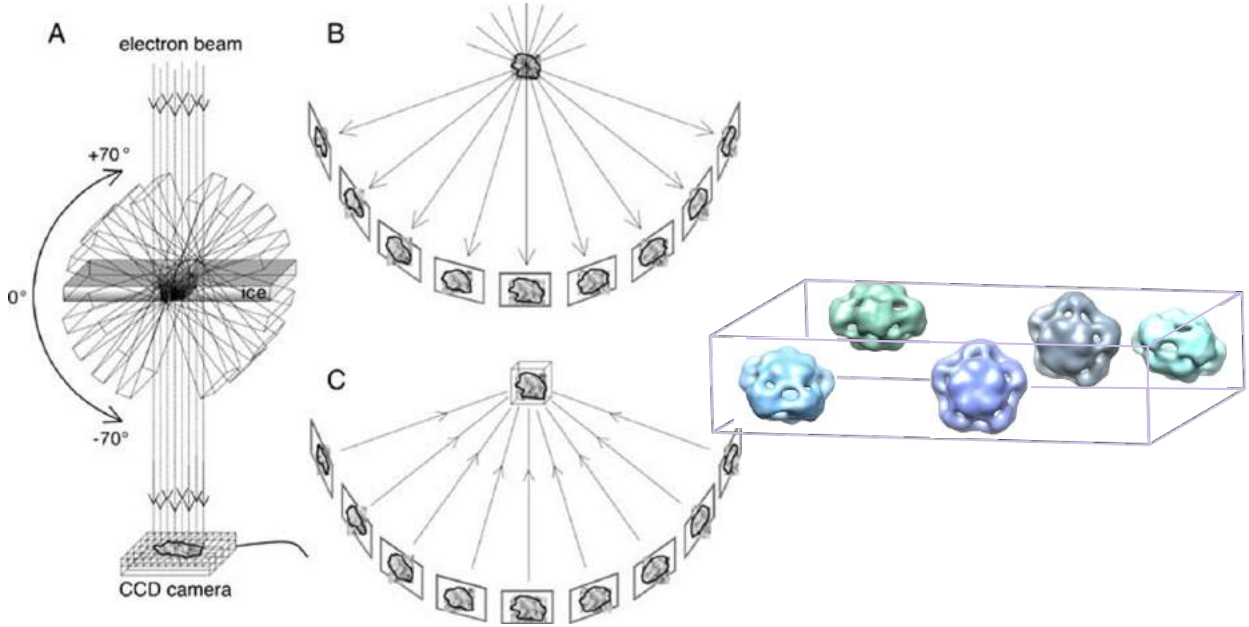
Strategy 1: Angular reconstitution

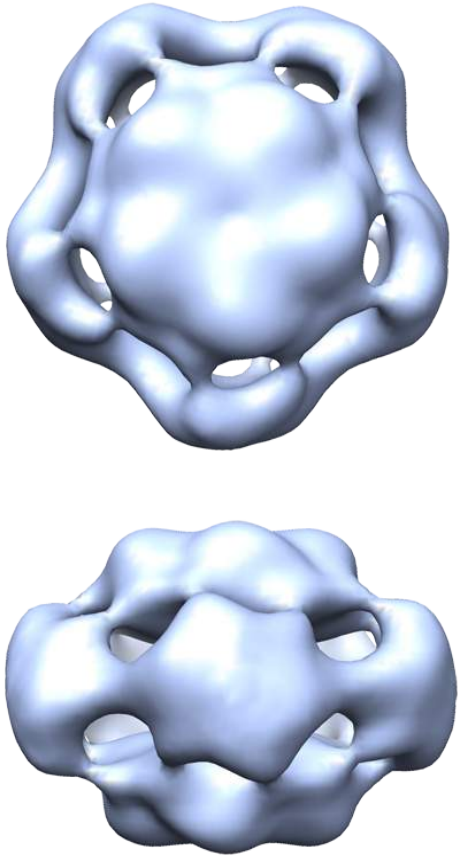




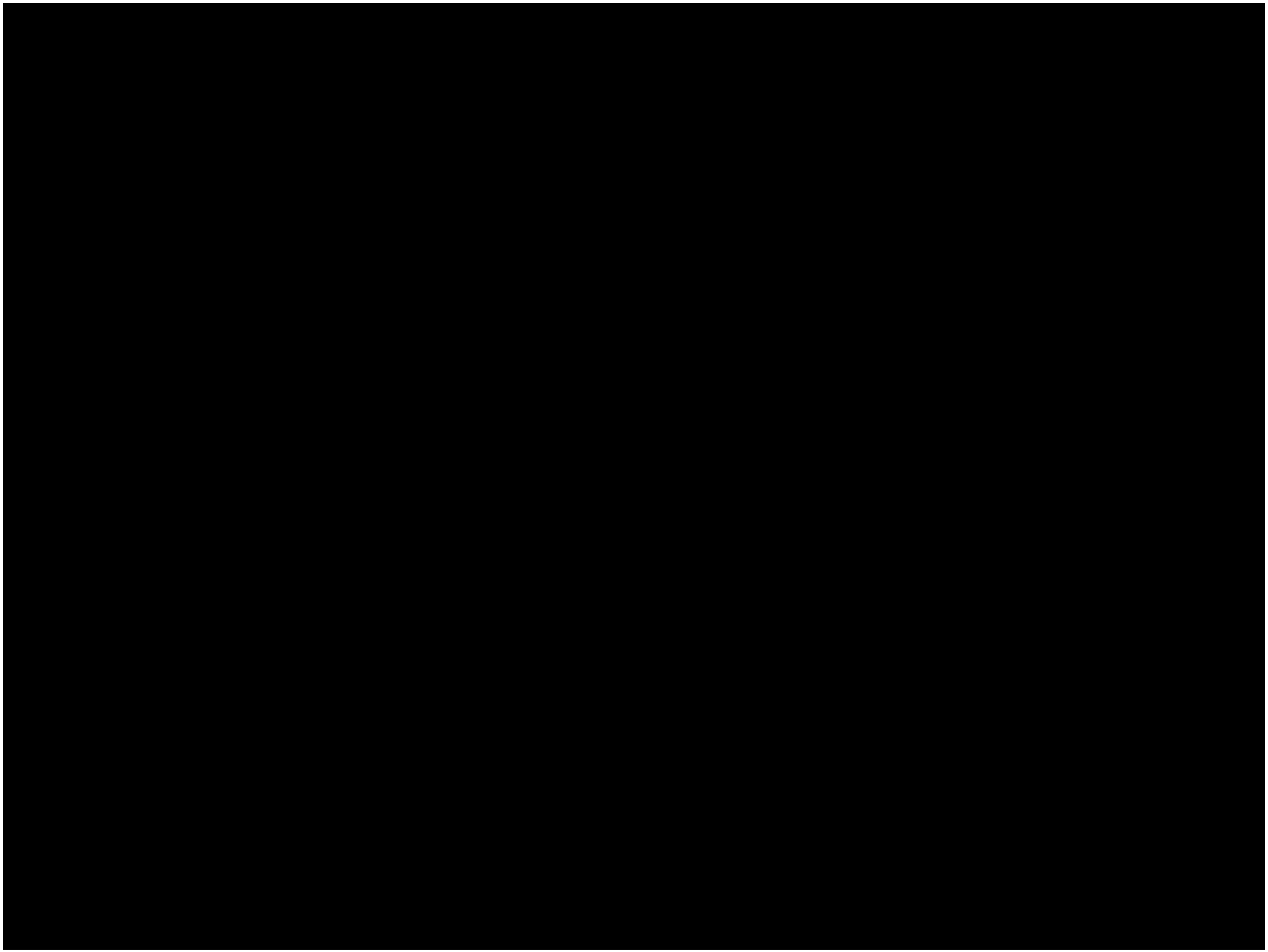
Would you like to verify your initial 3D reconstruction?

Strategy 2: tomography + subtomogram averaging

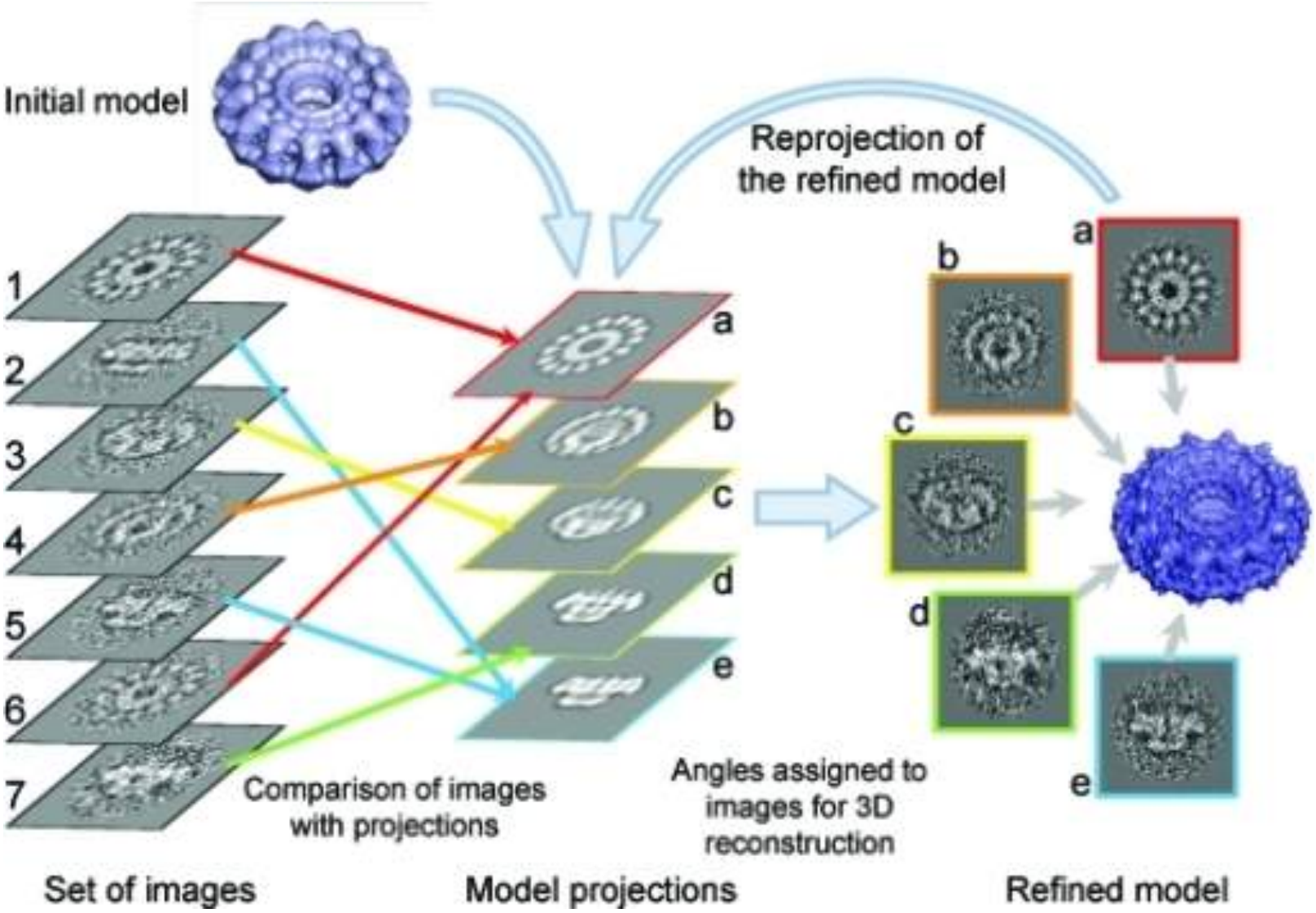




Cryo-tomography
Sub-tomogram
average map



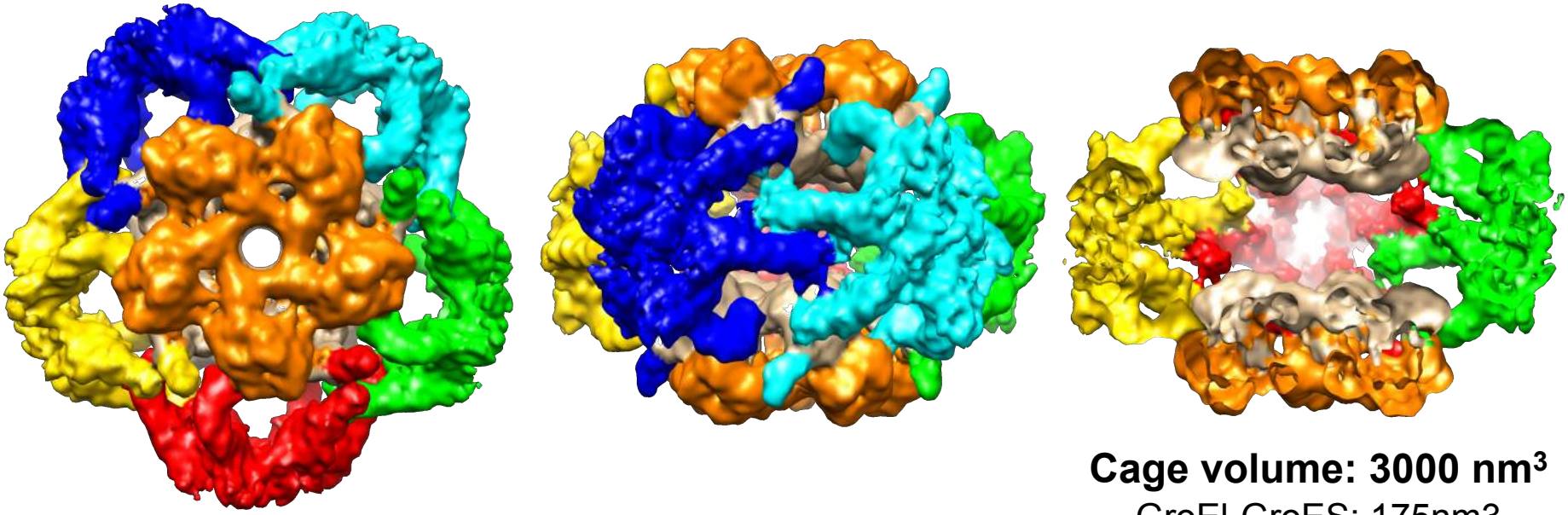
How would you do to improve the resolution?



Orlova EV, Saibil HR - Chem. Rev. (2011)

LdcI-RavA complex: a symmetrical floral design

A large cage formed by 5 hexamers of RavA and 2 double pentamers of LdcI



Cage volume: 3000 nm^3

GroEl-GroES: 175nm3

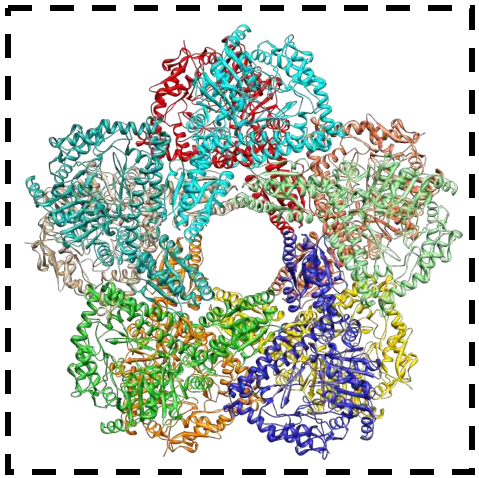
A unique architecture among all known AAA+ assemblies

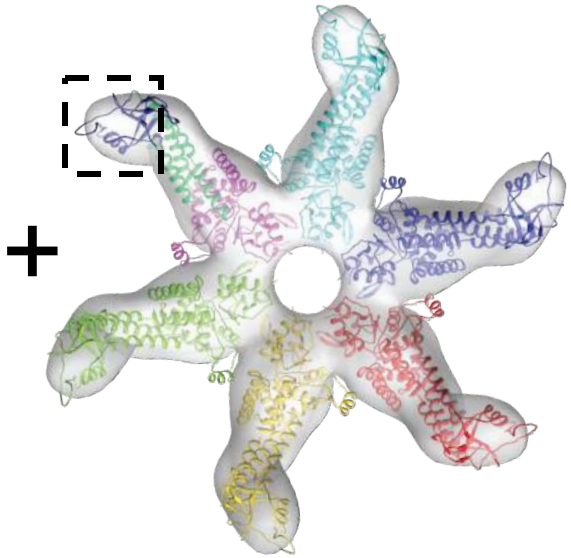
LdcI-RavA is the only AAA+ ATPase containing complex
✓composed of several interacting AAA+ ATPase rings
✓enclosing a central cavity other than the one in the center of the AAA+ ring

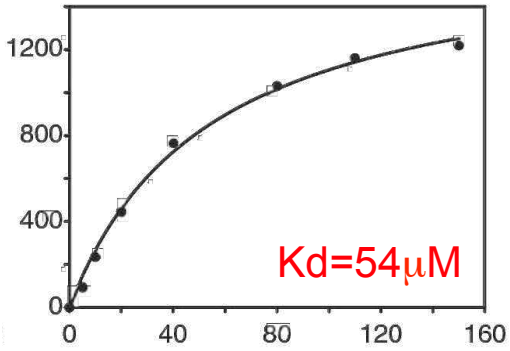
Insights into LdcI-RavA interaction: Structural study of the minimal complex LdcI-LARA

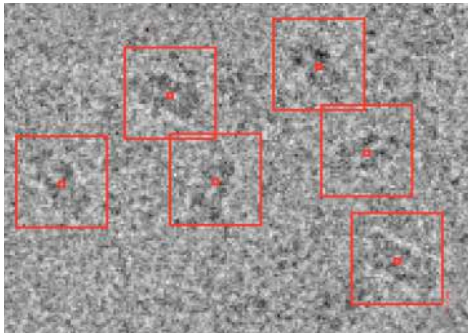
Pseudo-atomic model much more precise when secondary structures visible

Structural study of LdcI in complex the LdcI-binding domain of RavA









1 LdcI decamer

10 LARA monomers

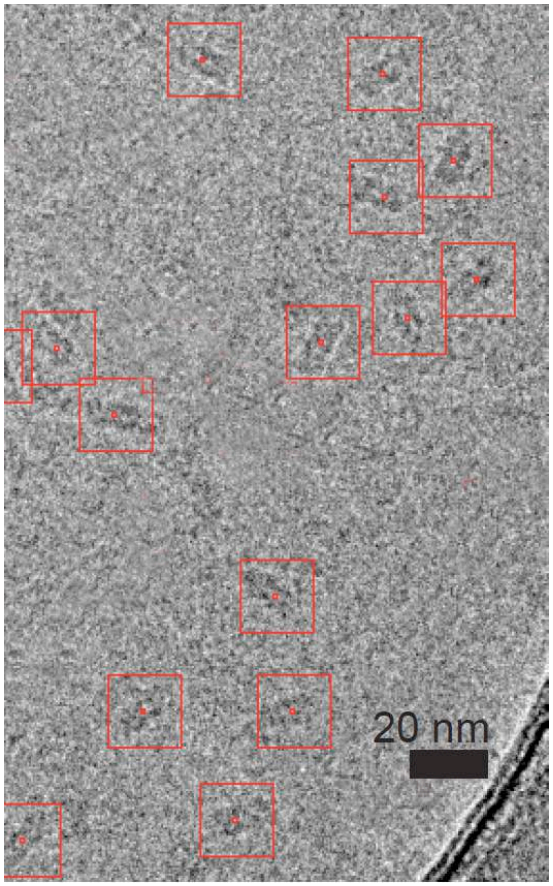
LdcI-LARA complex

LdcI **A**ssociating domain of **R**av**A**

Malet et al, eLife 2014
El Bakkouri et al., PNAS 2010

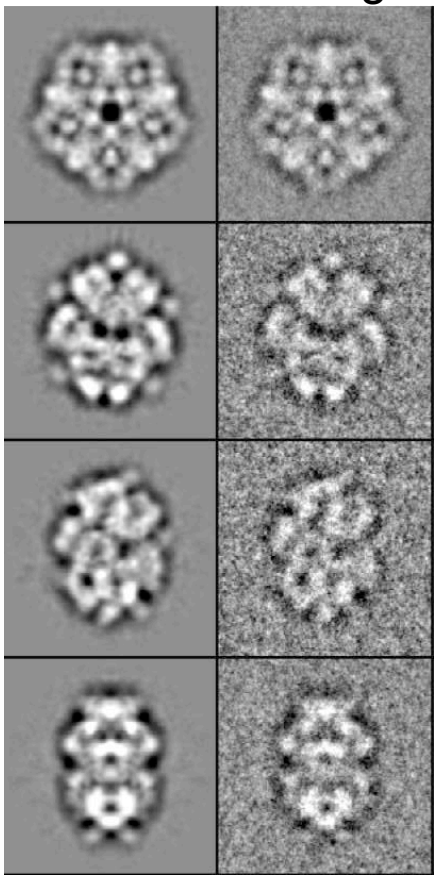
Cryo-EM analysis of the minimal LdcI-RavA complex

Cryo-EM image

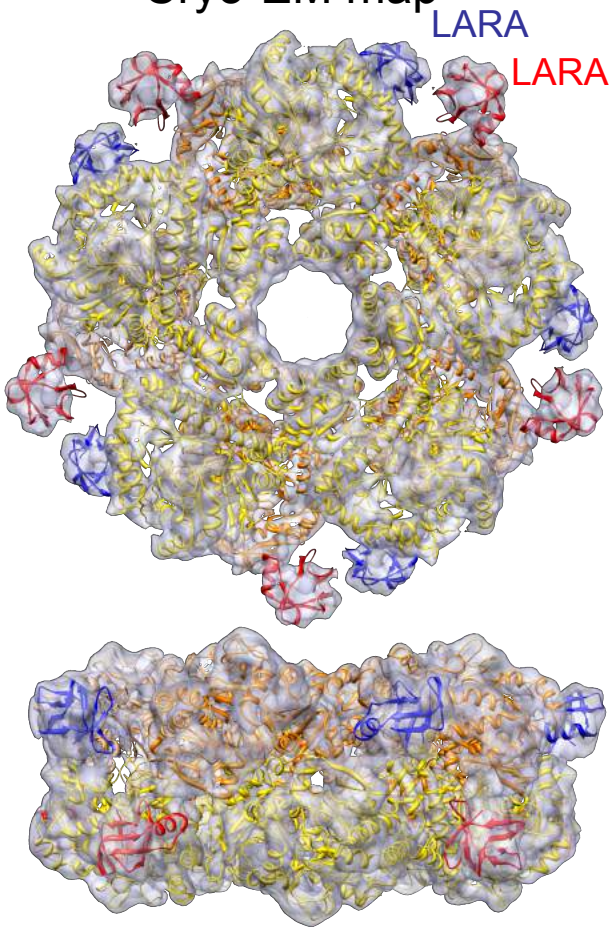


Projection

2D class average



Cryo-EM map

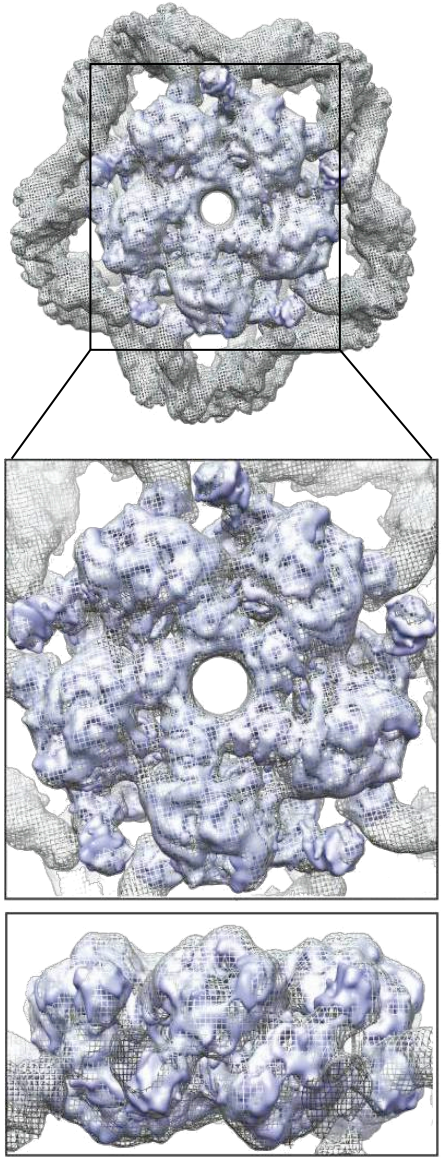


Resolution: 7.5 Å

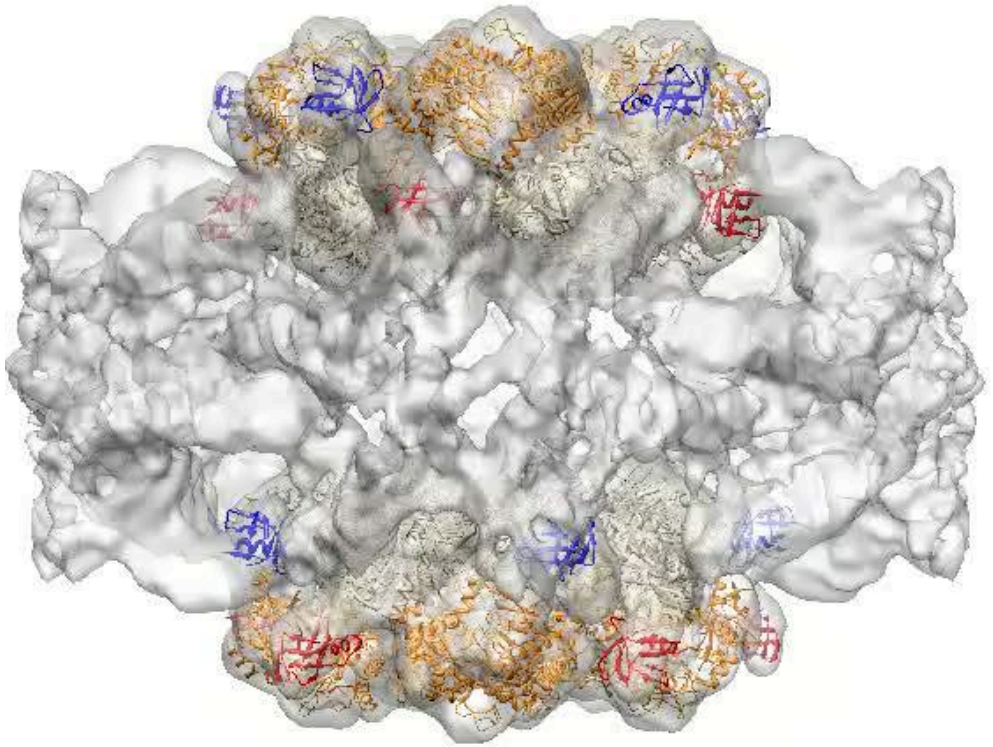
Malet et al, eLife 2014

Combination of LdcI-LARA and LdcI-RavA cryo-EM maps

Consistency between LdcI-LARA and LdcI-RavA maps



Building of a reliable LdcI-LARA pseudo-atomic model



Flexible fitting of the ATPase RavA

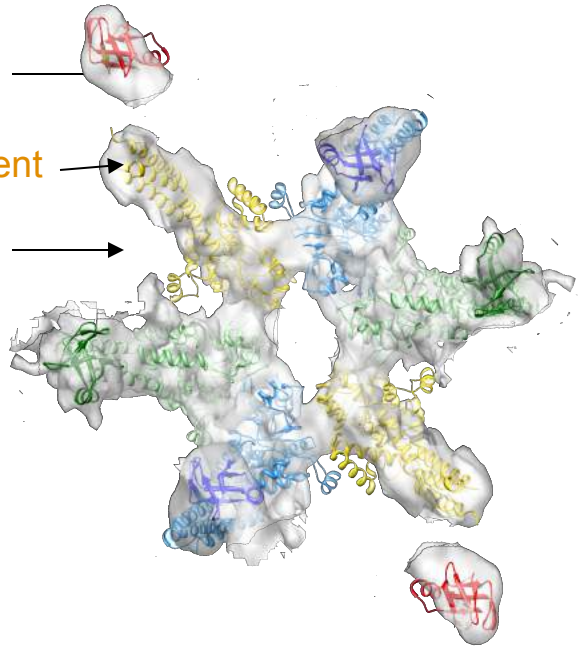
RavA ATPase alone

LARA domain: big movement

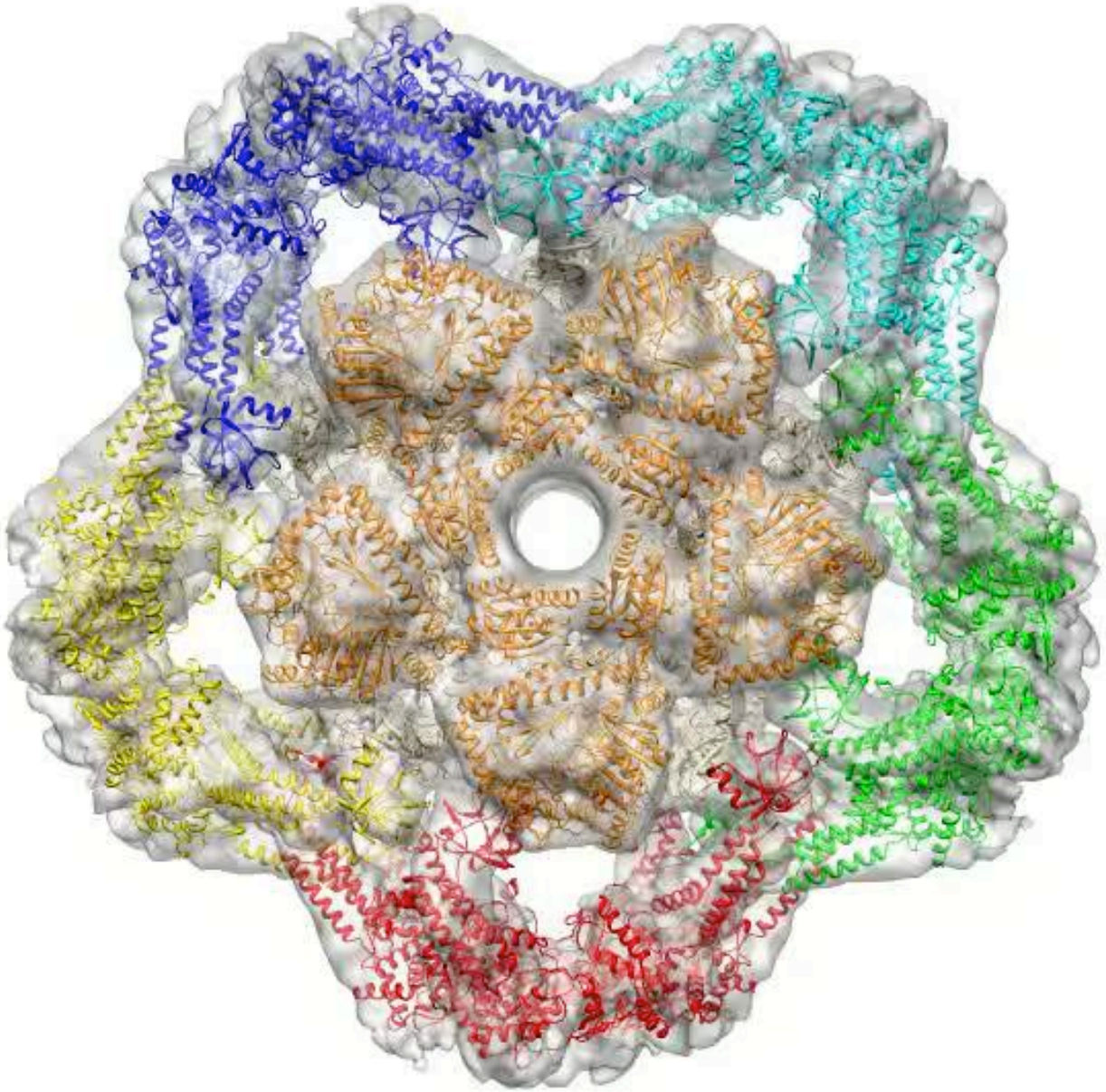
Triple helix domain: small movement

ATPase domain: no movement

RavA ATPase in complex with LdcI



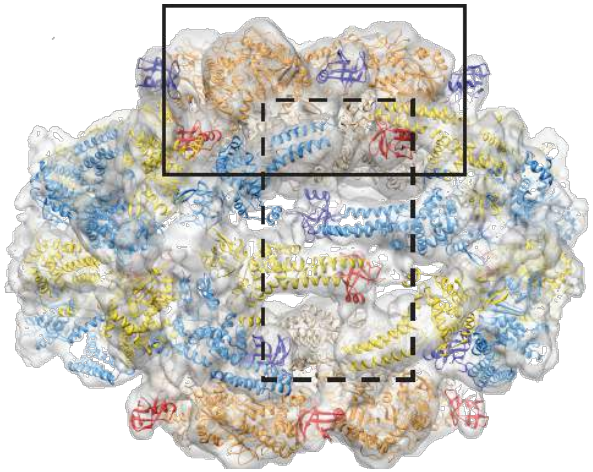
Malet et al, eLife 2014

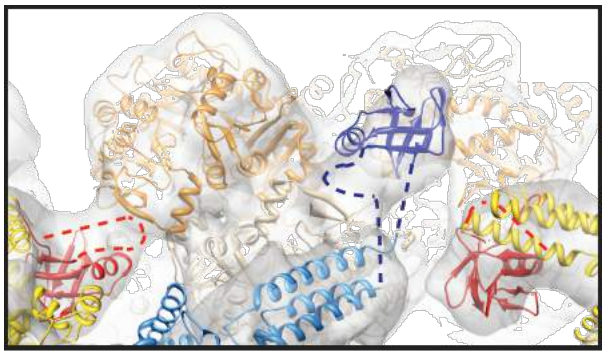


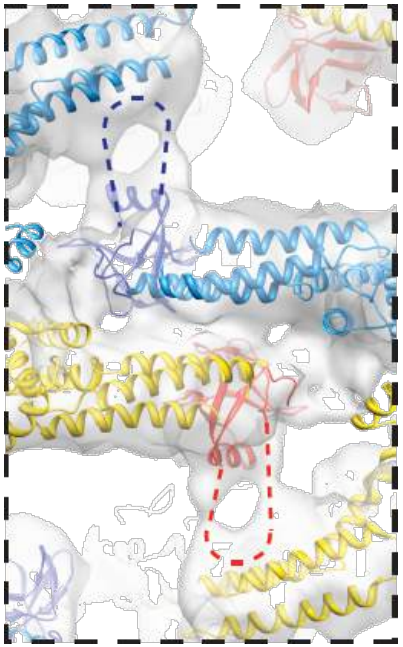
Malet et al, eLife 2014

A unique loop implicated in complex formation

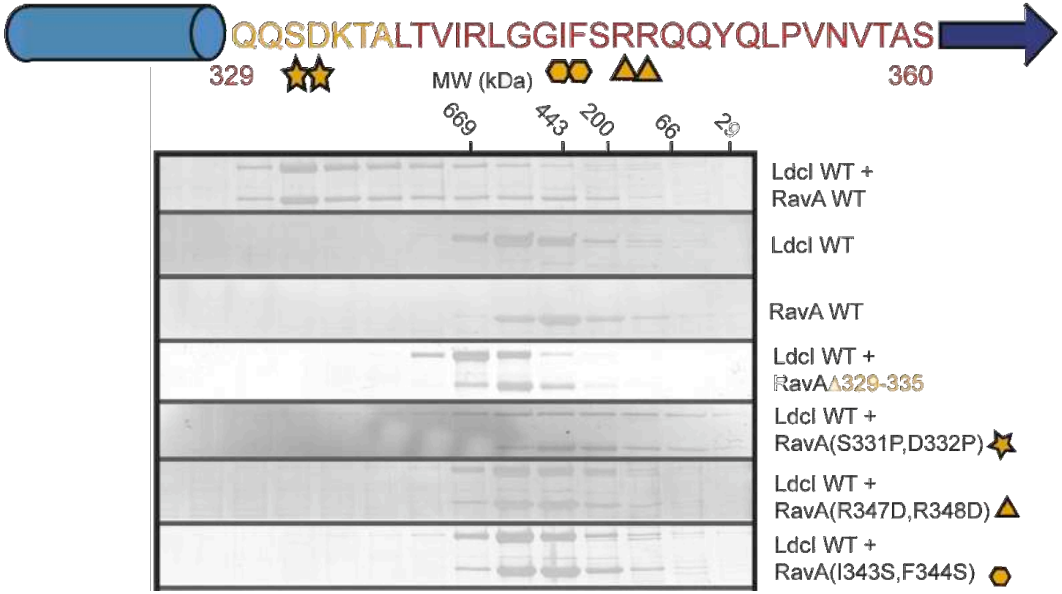
Interacting loop identified in the pseudo-atomic model





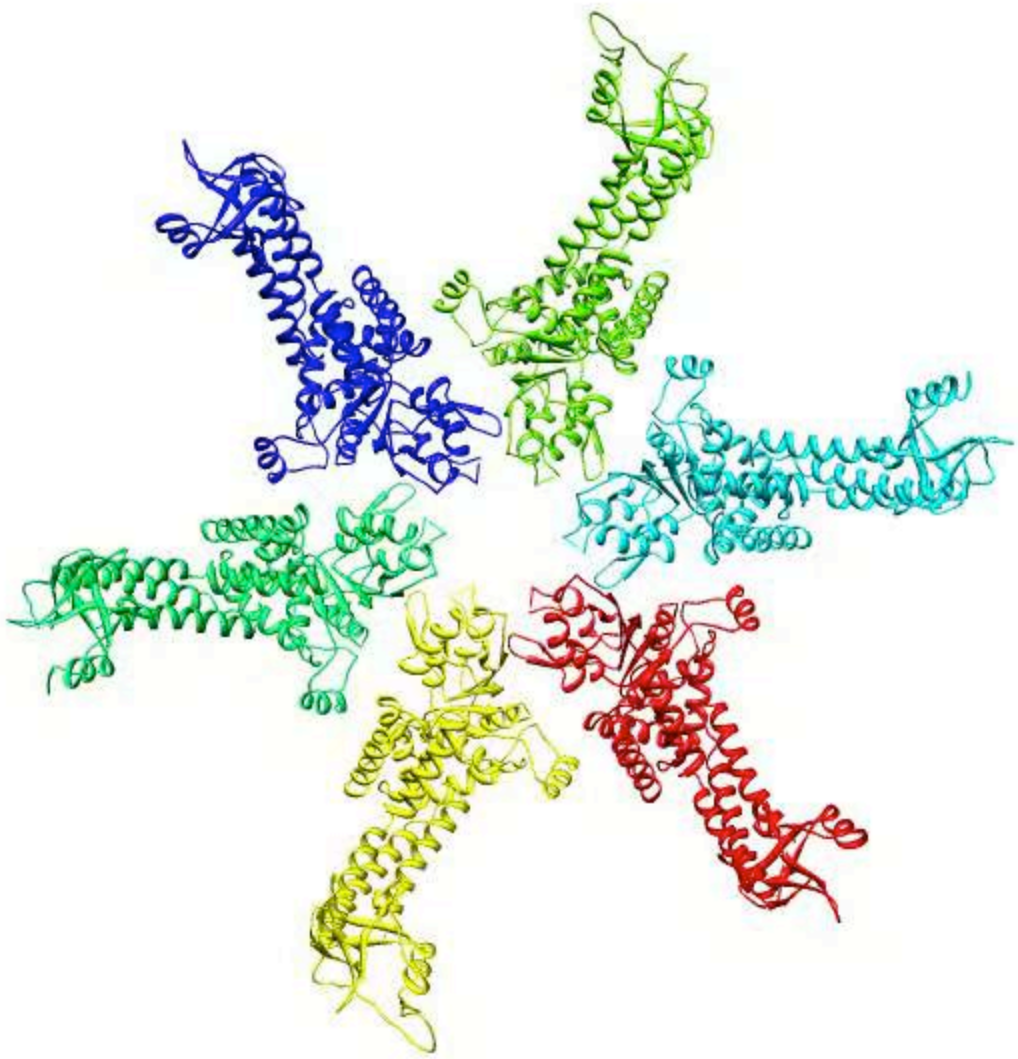


Importance of the loop verified by mutagenesis



Malet et al, eLife 2014

LdcI-RavA cage formation



Malet et al, eLife 2014

What would you do next?

Thank you !