Cell PRESS

Structure
Perspective

# Conformational States of Macromolecular Assemblies Explored by Integrative Structure Calculation

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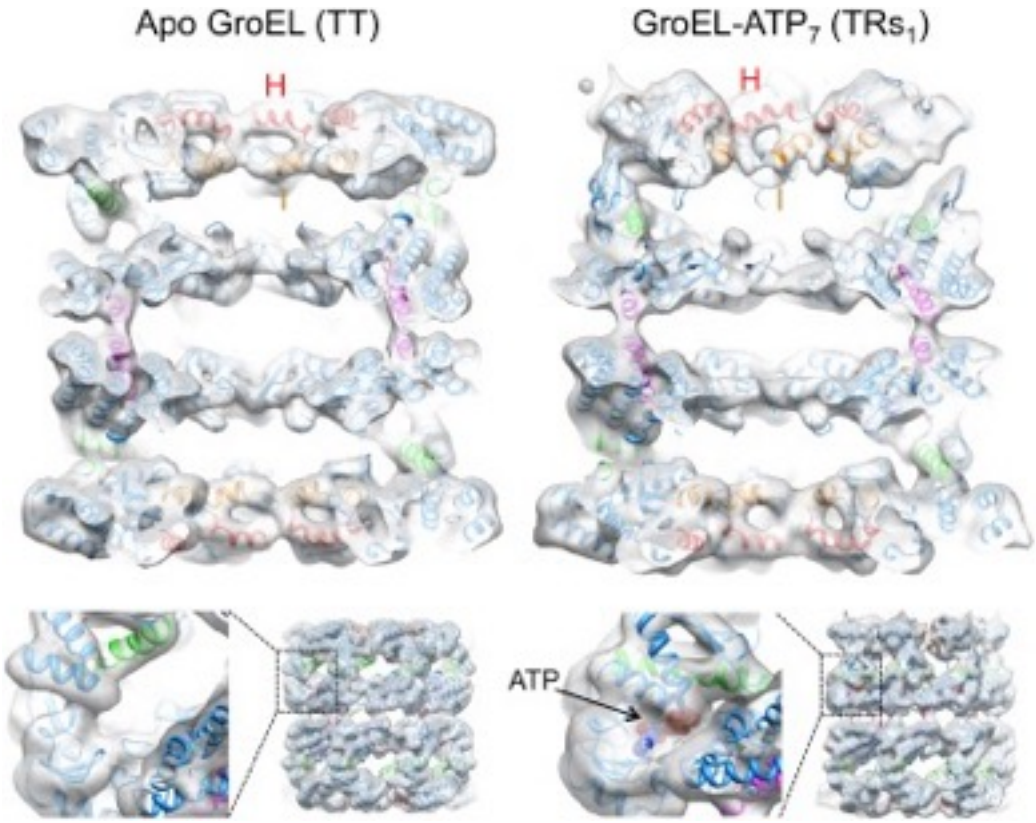
http://dx.doi.org/10.1016/j.str.2013.08.006

**Jenifer Brown**

# Why Study Protein Complexes?

- Oligomerization state
- Number of bound ligands
- Subunit stoichiometry
- Dynamics




Clare, D.K., et al. ATP-triggered conformational changes delineate... Cell 2012.

How can we use multiple techniques to probe the composition and dynamics of molecular complexes?

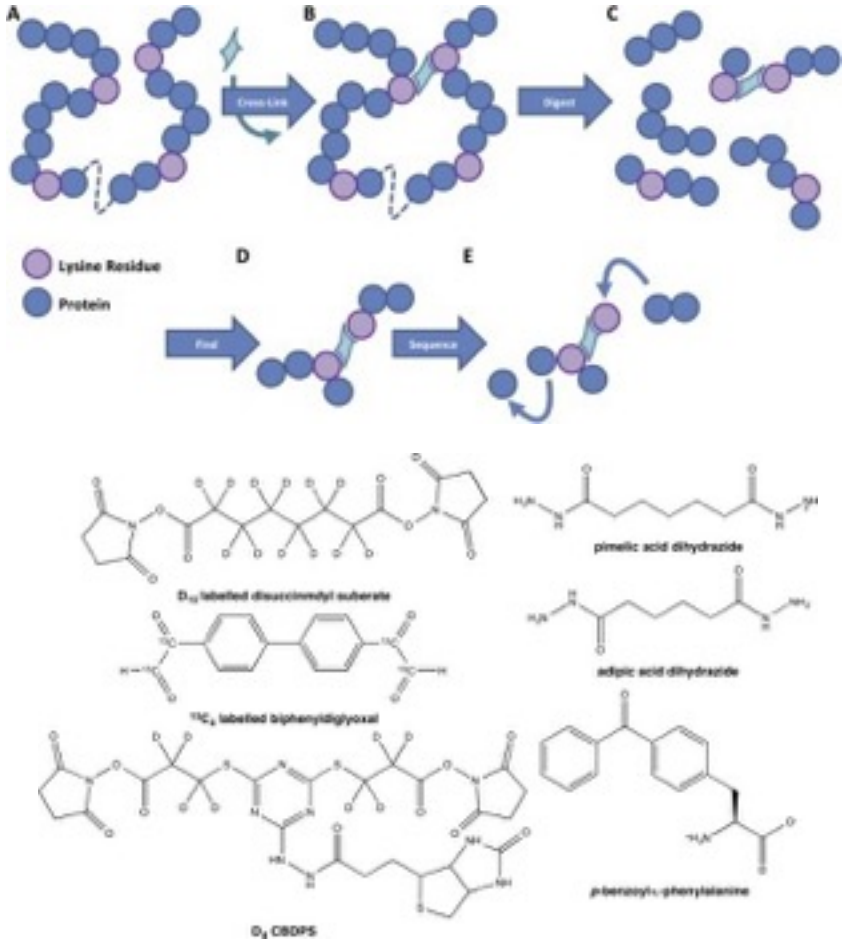
# Mass Spectrometry

- Measures mass-to-charge (*m/z*) ratio of ionized species
- Ion source induces formation of gas-phase peptide ions
- Peptides are separated based on *m/z* ratios
- Detector reads the number of ions at each *m/z* ratio
- Protein characteristics can be extrapolated by mapping peptide fragments to original protein

# Probing Dynamics with Mass Spectrometry

**Chemical Crosslinking + MS (XL-MS)**

- Examine non-covalent interactions between proteins or within a protein based on proximity
- Provides distance constraints for structural models
- Solution-based: natural fold, multiple conformations
- Challenge: high number of possible crosslinked peptides




Holding, A. XL-MS: Protein cross-linking coupled with mass spectrometry. Methods. 2015

# Ion-mobility MS (IM-MS)

- Separate different conformations of the same protein-protein complex
- The rotationally averaged collision cross-section (CCS; shape) of complex affects ion mobility

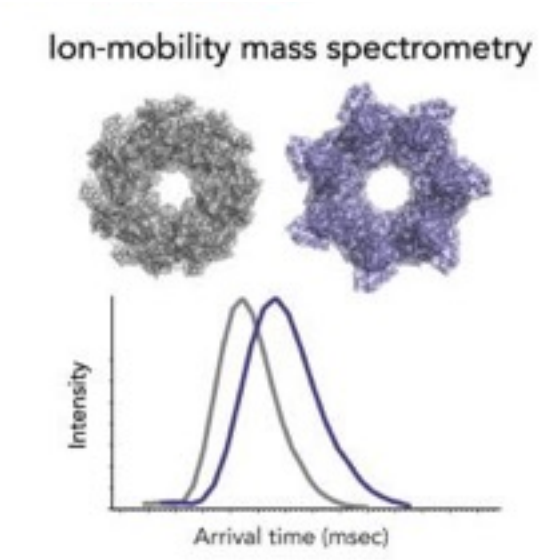
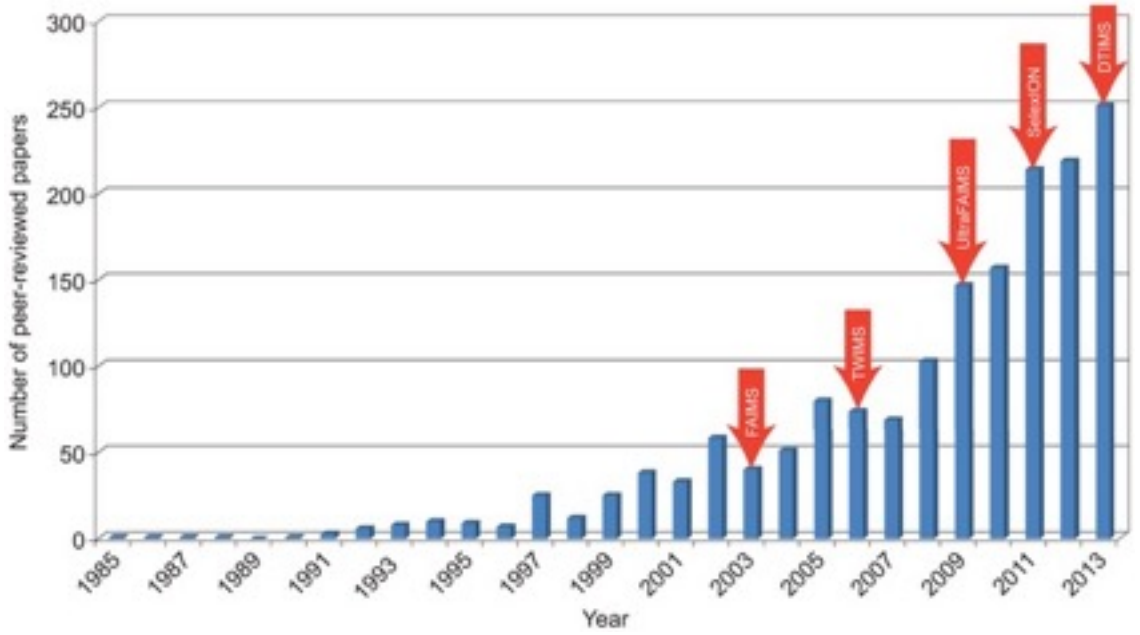
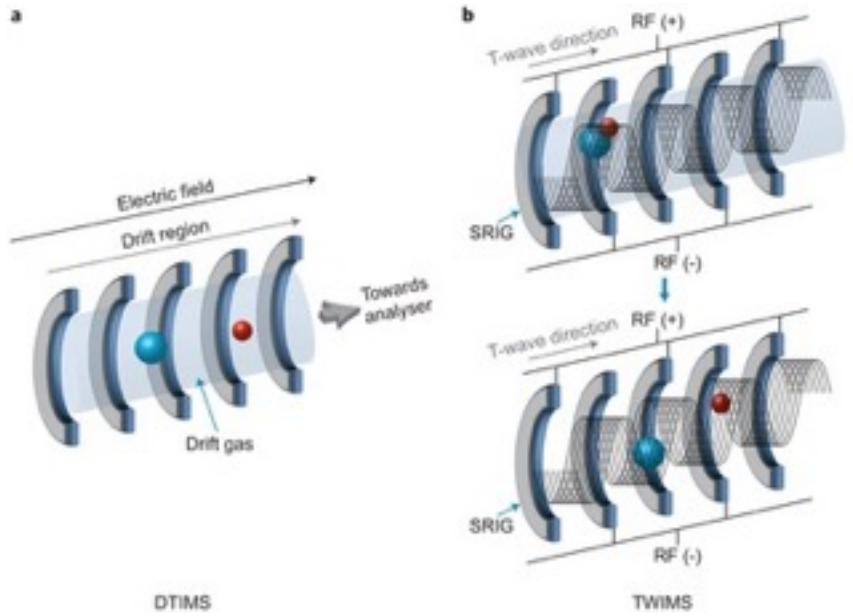



Figure 1A

Number of peer-reviewed papers published annually (to end of 2013) combining ion mobility and mass spectrometry




Lanucara, et al. The power of ion-mobility… Nature Chemistry. 2014

# Hydrogen-deuterium exchange coupled to MS (HDX-MS)

- Detect N-H, O-H, S-H exchange with deuterium in $D_2O$ by MS
- Provides information about structural flexibility due to protein folding/ unfolding, conformational changes, hydrogen bonding, or solvent exposure
- Recent improvements in instrumentation, sample preparation, and data analysis

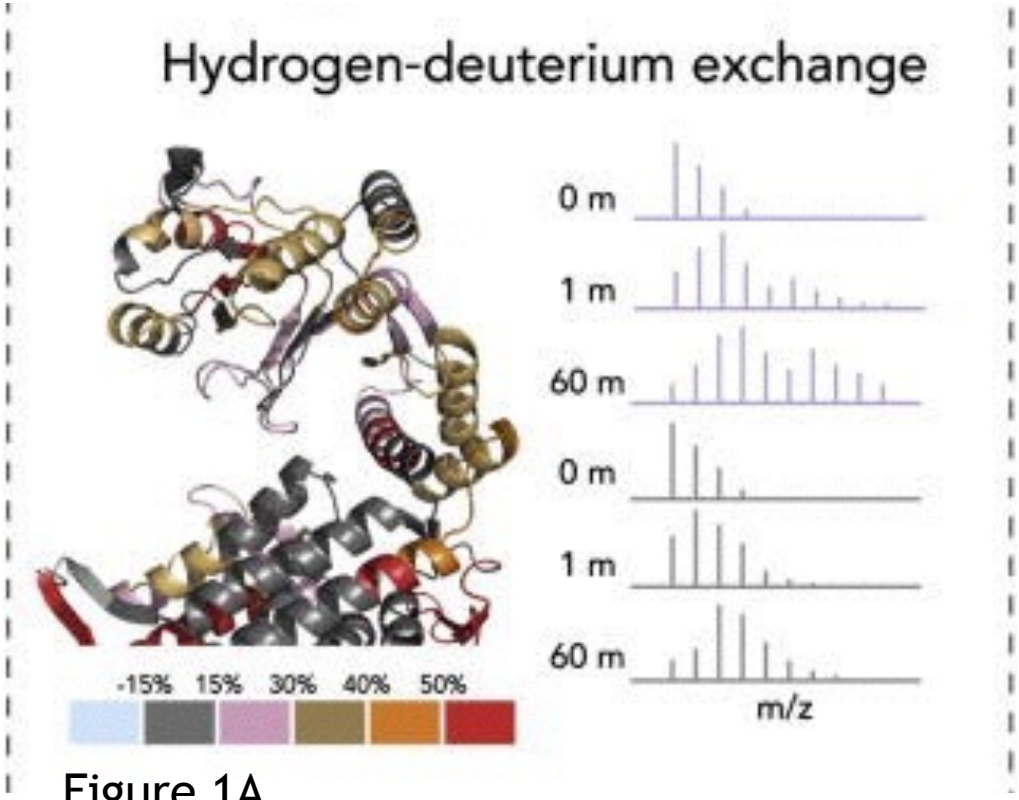
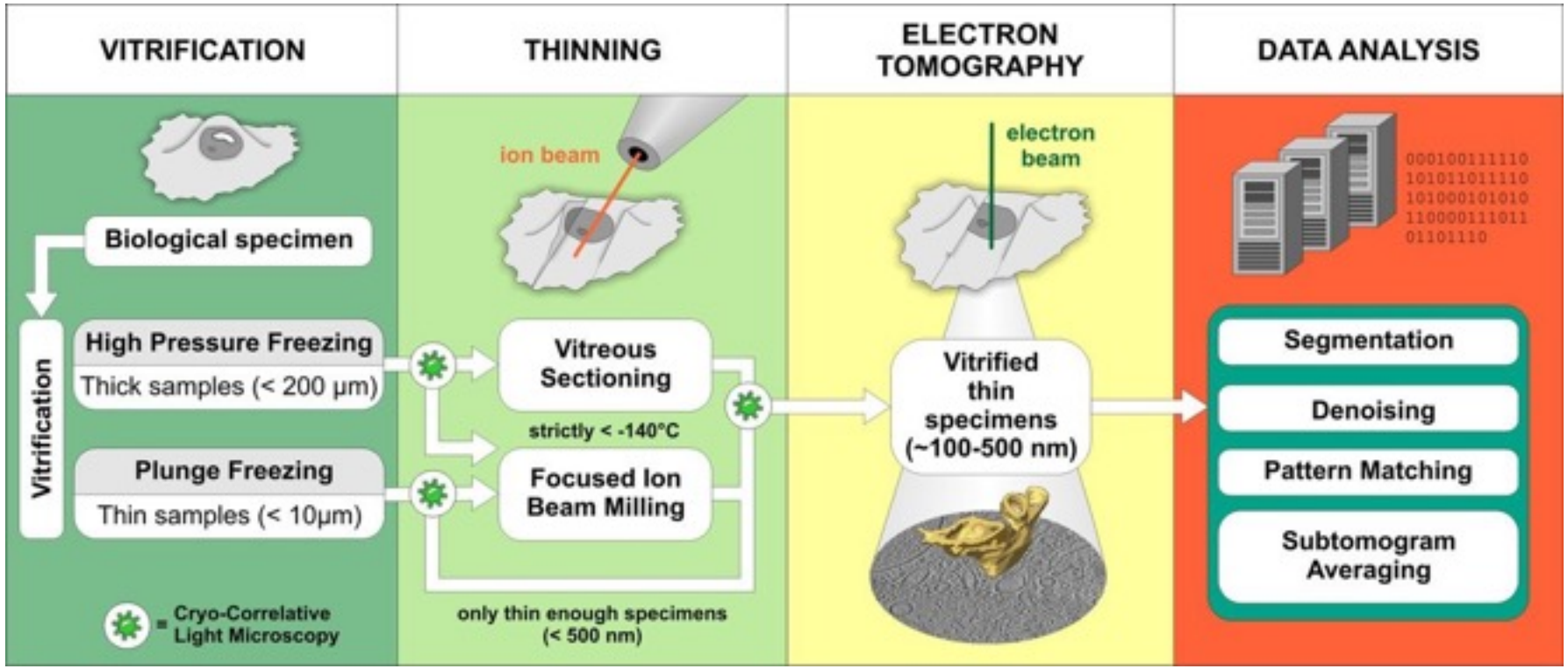



Figure 1A

# Cryo-electron tomography (Cryo-ET)

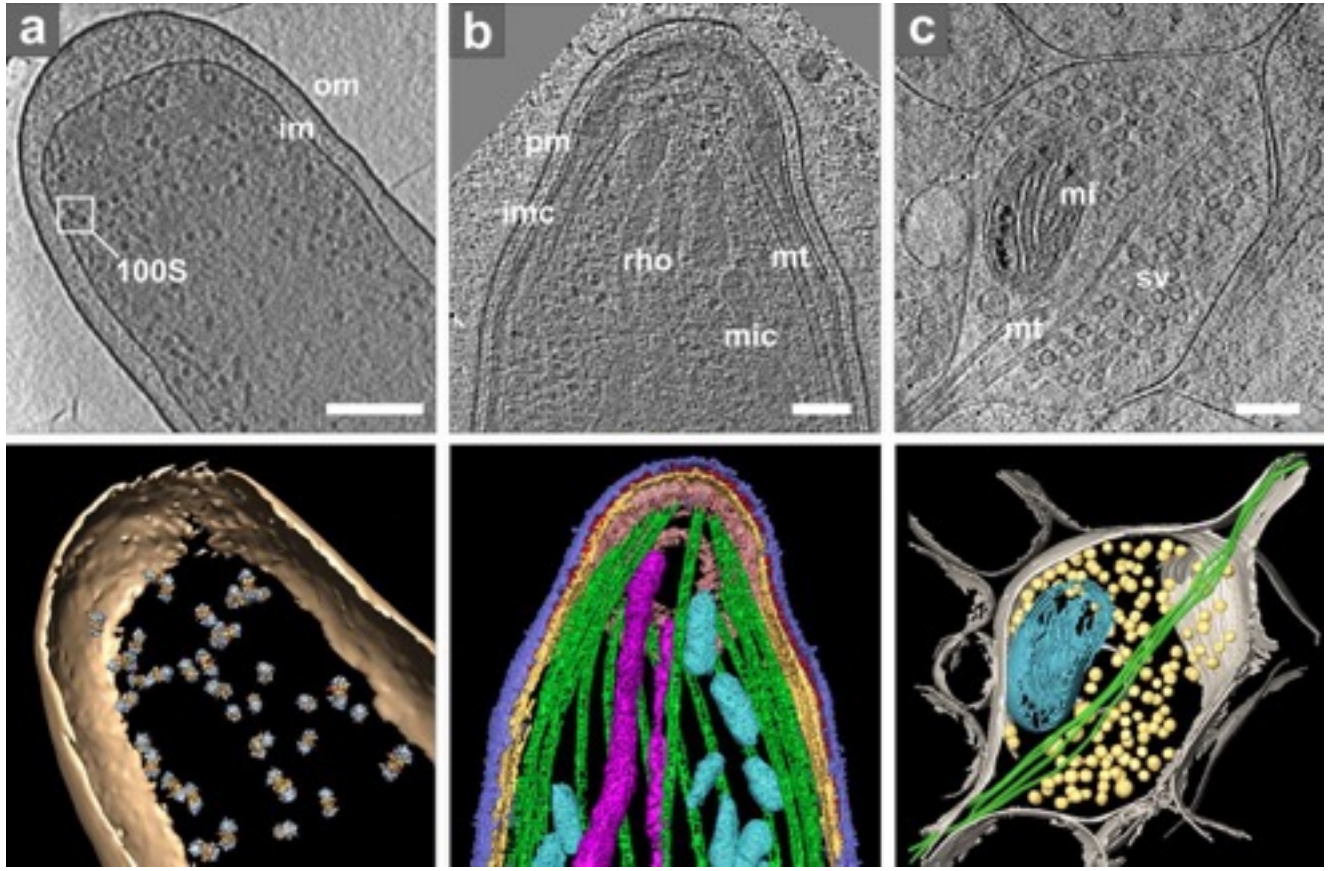



3-D images of the sample are from a reconstructed series of subtomograms collected at different tilt angles

Lucic, et al. Cryo-electron tomography: The challenge of doing structural biology in situ. Journal of Cell Biology 2013.

# Cryo-electron tomography techniques

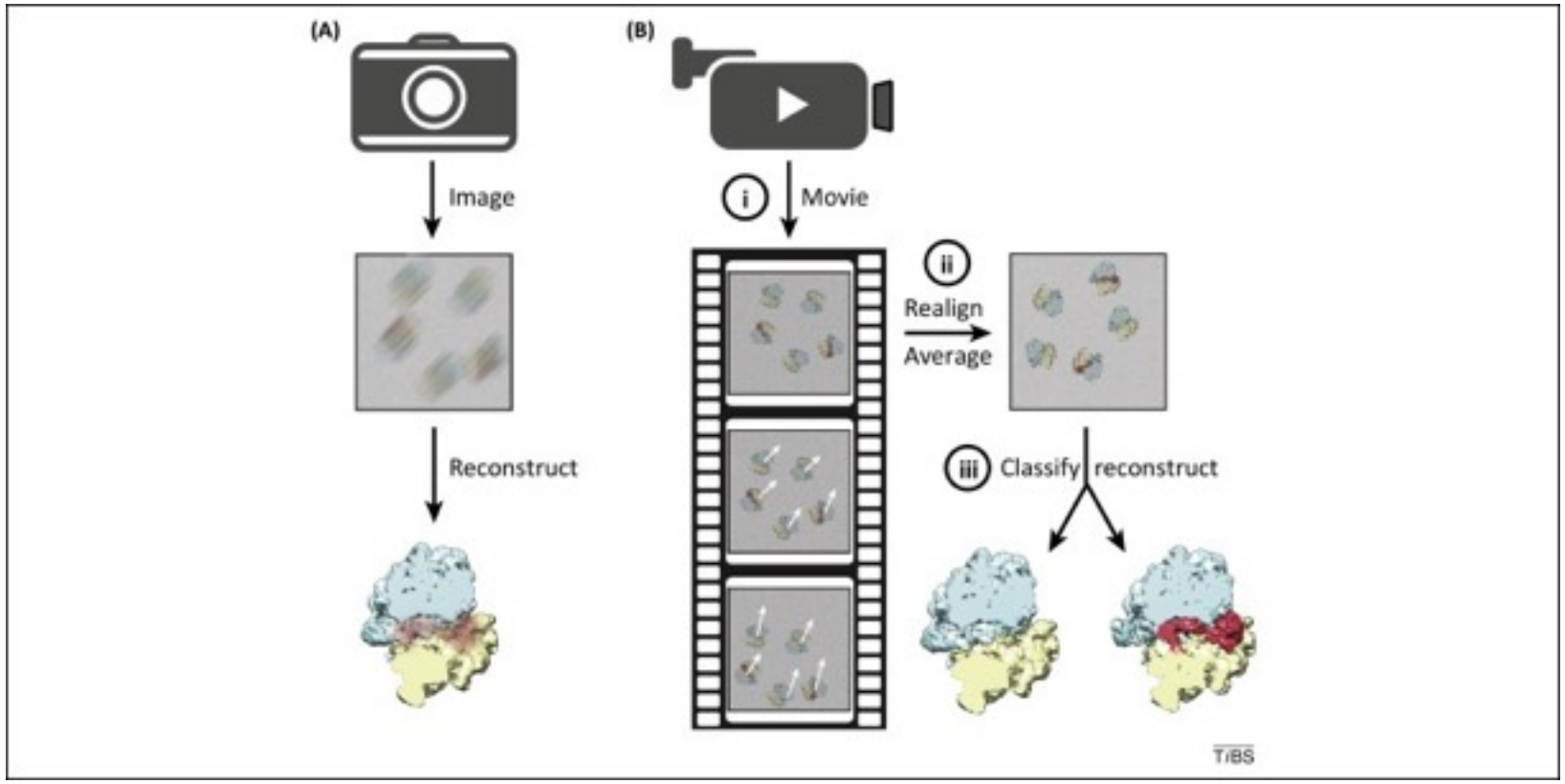
- *In situ* structure determination of complexes
- High noise level, crowded cellular compartments
- Subtomogram image processing is computationally intensive




Lucic, et al. Cryo-electron tomography: The challenge of doing structural biology in situ. Journal of Cell Biology 2013.

# Cryo-electron microscopy (Cryo-EM)

Reconstruct 3-D structures from a series of images of the protein of interest taken at different angles




Bai, Xiao-chen, et al. How Cryo-EM is revolutionizing structural biology. Trends in Biochemical Sciences. 2015

# Low resolution (5-25Å) EM density maps

- Fit atomic models from X-ray crystallography, NMR, structure prediction, etc into EM density maps
- Multiple factors: model accuracy, electron density resolution, scoring functions, number of components, etc
- Rigid fitting: 6 degrees of freedom to fit atomic model to density map
- Flexible fitting: Deform atomic model using molecular mechanics force field and forces that match electron density map

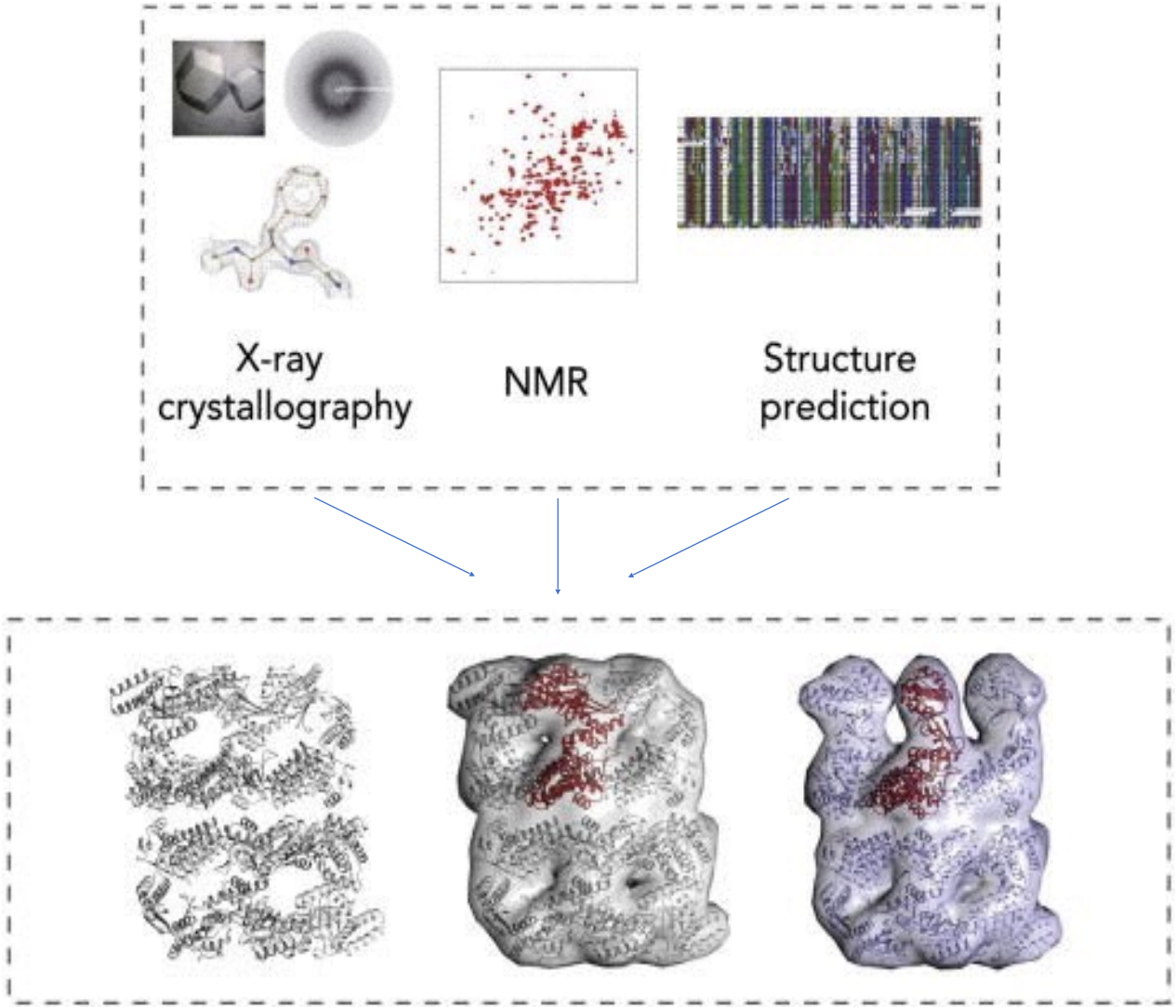
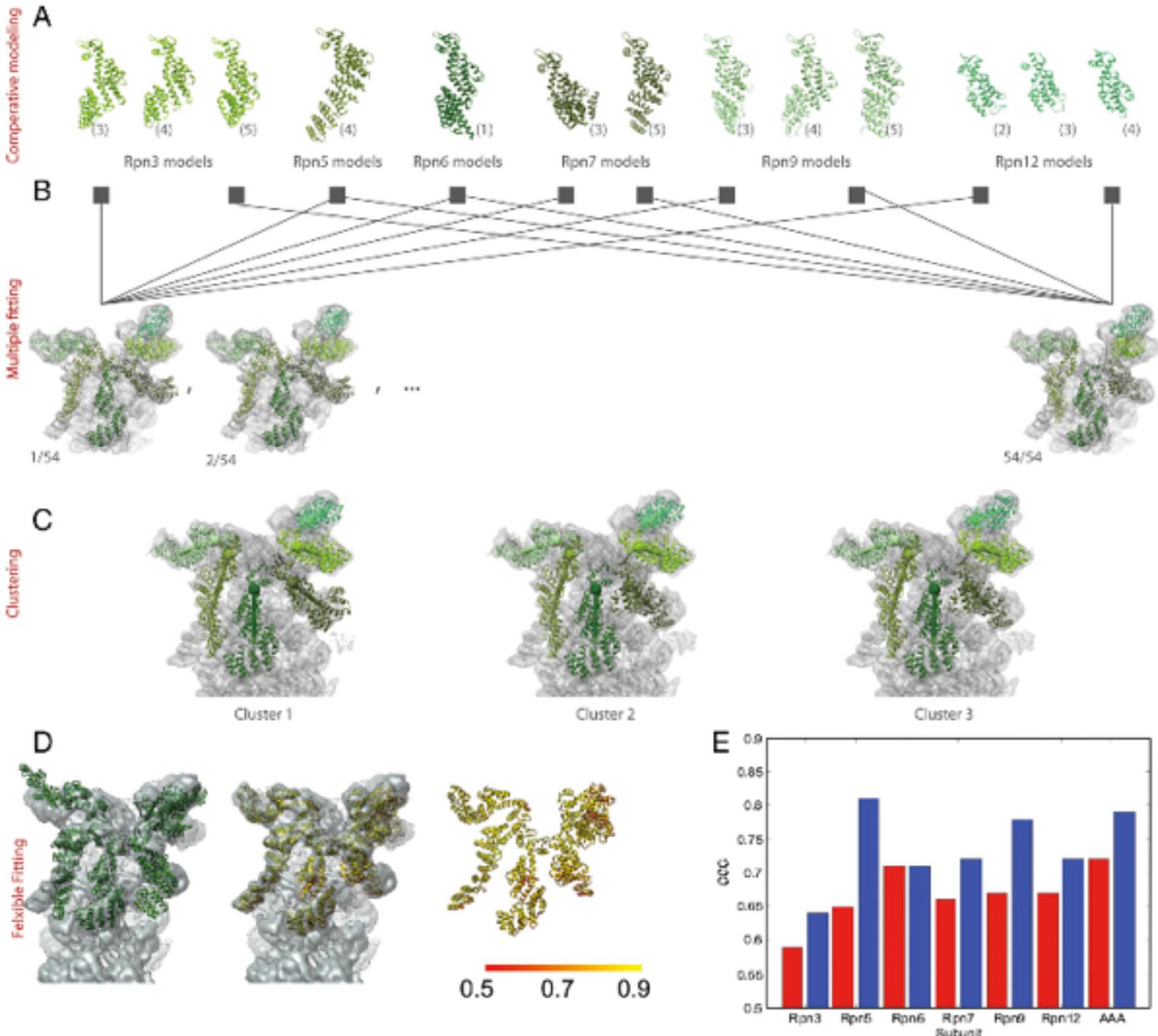



Figure 1C, D

# Example: Integrative modeling of the 26S proteasome holocomplex

- Data: cryo-EM density map, 12 residue-specific cross-links, interactions from proteomic studies, atomic structures of individual subunits
- Convert data into spatial restraints
- Localize subunits (spatial restraints; message passing algorithm) -> fit subunits (MultiFit) -> flexible fitting refinement (molecular dynamics flexible fitting, MDFF)
- Evaluate models based on restraints, completeness, similarity between models, non-integrated structural data

Lasker, et al. Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. PNAS 2012.




"Multiple comparative models were built for each subunit, fitted into the cryo-EM map, and the best-scoring configurations were subjected to flexible fitting."

Figure S3
Lasker, et al. Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. PNAS 2012.

# Putting it all together

- Use a scoring function to integrate MS data and distance constraints
- XL-MS spatial constraints have been combined with 3D density fitting and 2D class-average images to improve structure determination
- Data can also be used to validate/support model structures (IM-MS)
- Resolution of data determines how well it can be integrated

# QUESTIONS?

Vol 450|29 November 2007|doi:10.1038/nature06404

nature

ARTICLES

# Determining the architectures of macromolecular assemblies

Frank Alber[1]*, Svetlana Dokudovskaya[2]*†, Liesbeth M. Veenhoff[2]*†, Wenzhu Zhang[3], Julia Kipper[2]†, Damien Devos[1]†, Adisetyantari Suprapto[2]†, Orit Karni-Schmidt[2]†, Rosemary Williams[2], Brian T. Chait[3], Michael P. Rout[2] & Andrej Sali[1]

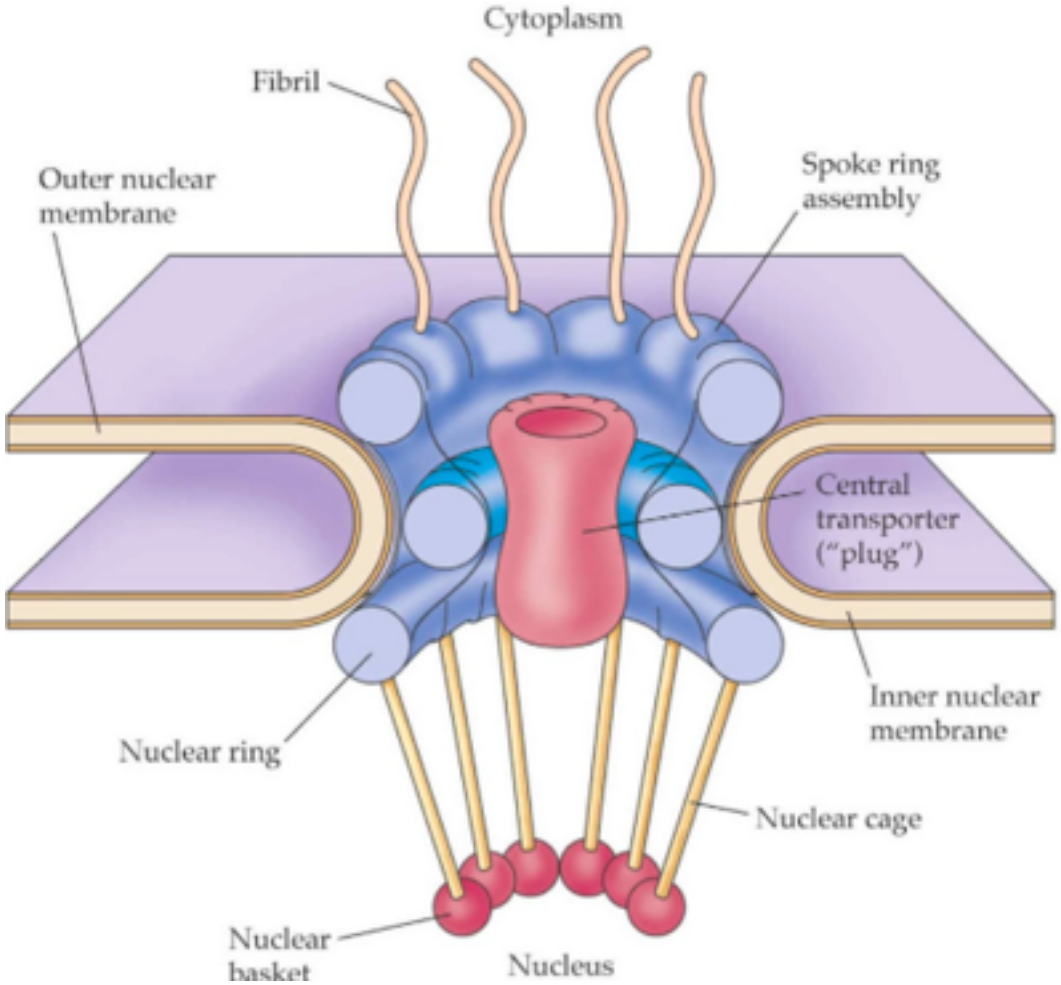
# Nuclear Pore Complex

**Why do we care?**

- Allows the transport of molecules in and out of the nucleus of a cell
- **2000** Nuclear Pore Complexes in a single vertebrate cell
- Structure? → **Function!**

**Why is solving the structure such a challenge?**

- **30** distinct proteins, total of **456** proteins
- **X-ray Crystallography** and **NMR** could not handle such a large complex
- **Lower-resolution** methods do not give us atomic information

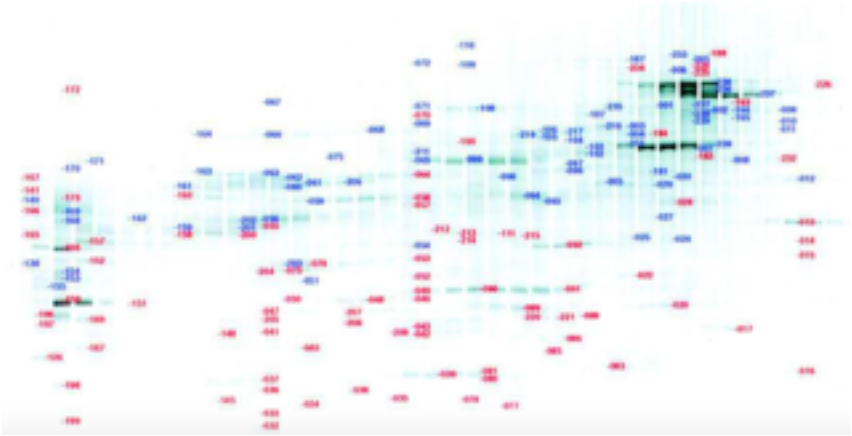


# Presentation Outline:

1. Data Generation
2. Integrating the Data
    - Optimization
    - “Ensemble Analysis”
3. Validation
4. Strengths and Weaknesses of Study

# Data Generation

## 1. **Components List:** Previously determined



???
Structure of the Nuclear Pore Complex

Rout, M. P. *et al*. **The yeast nuclear pore complex: composition, architecture, and transport mechanism.** *J. Cell Biol*. **148,**

# Data Generation

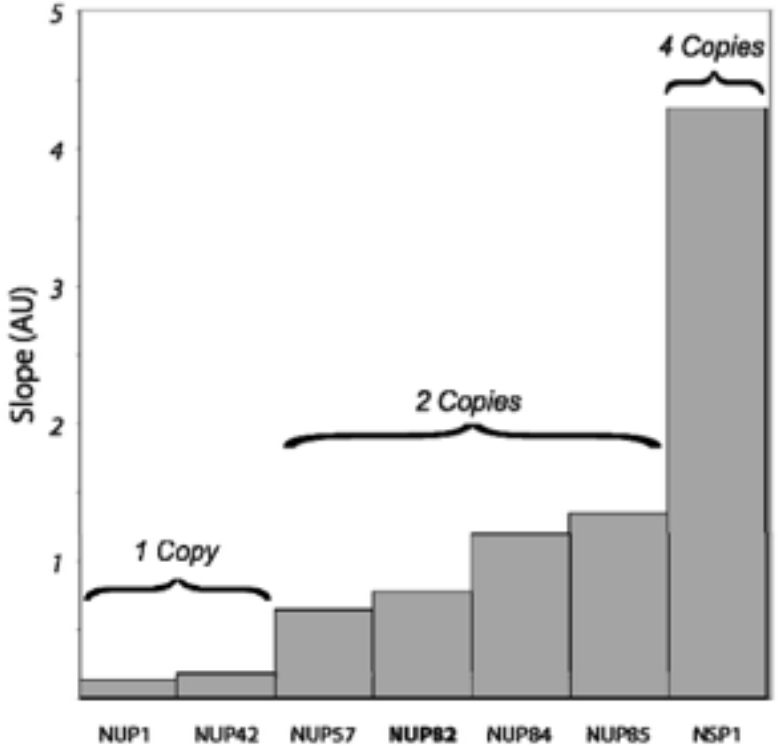
## **2. Shape and Size:** Ultracentrifugation

High speed spinning:

# Data Generation

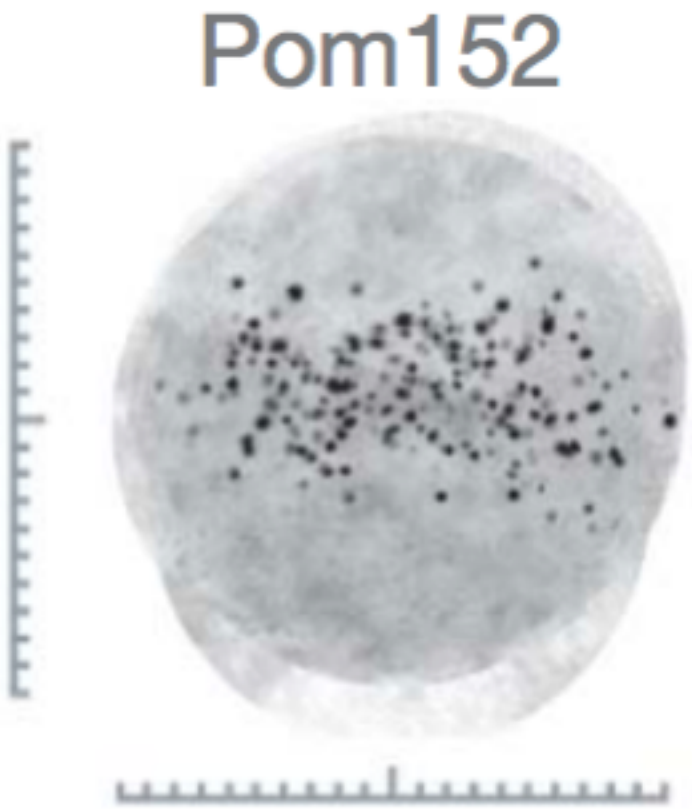
## **3. Amount of each protein (stoichiometry):** Immunoblotting

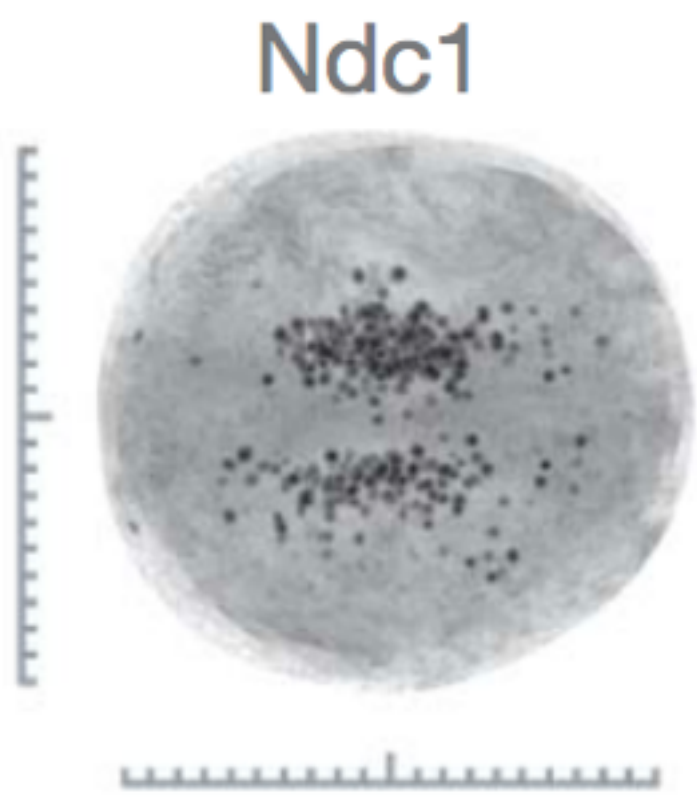
NUP1 NUP42 NUP57 NUP82 NSP1



# Data Generation

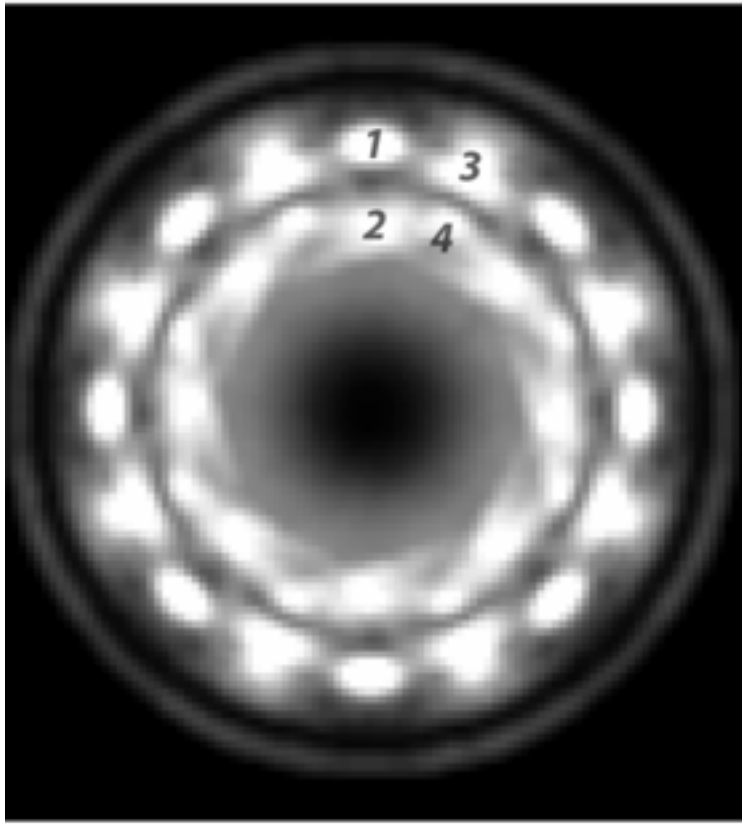
**4. Low resolution position of proteins:** Immuno-Electron Microsco



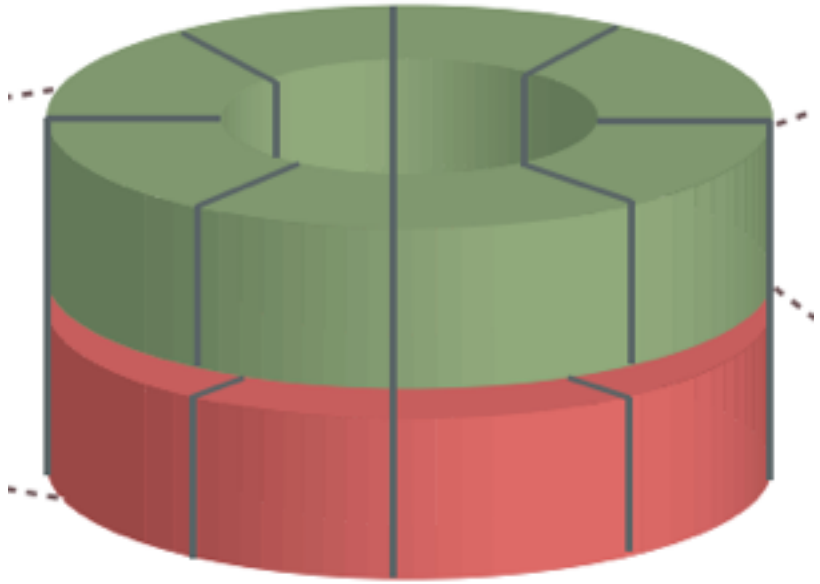



# Data Generation

## **5. Overall Shape:** Cryo-Electron Microscopy



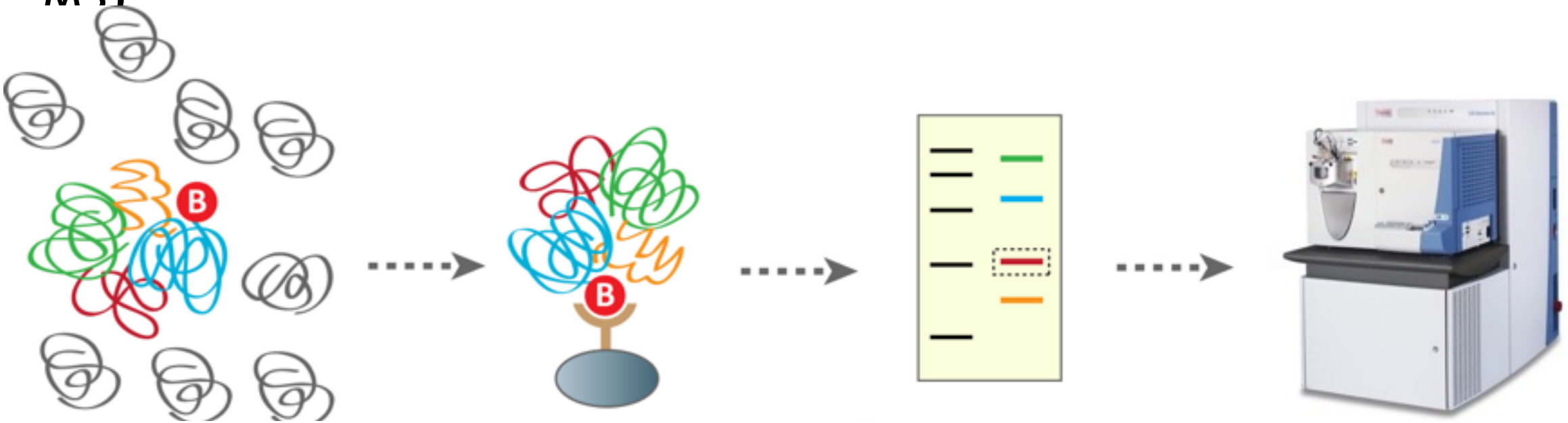

Cryo-EM bird's eye view



Cartoon Reconstructed Side View

# Data Generation

**6. Protein Connectivity:** Affinity Purification-Mass Spectrometry (A
MS)




Tagged Protein in Solution

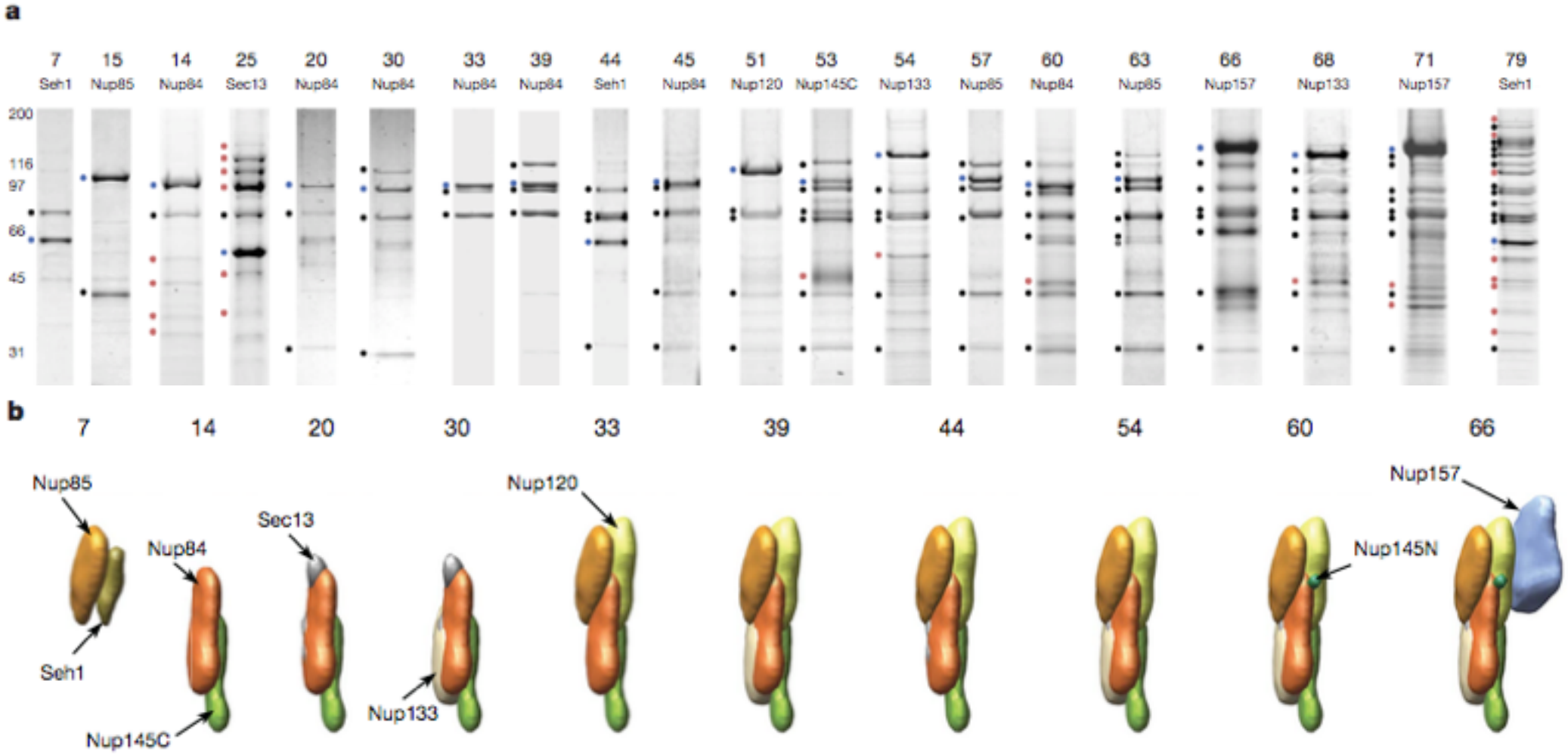
Immunoprecipitation

Gel Visualization

Identification by Mass-Spectrometry

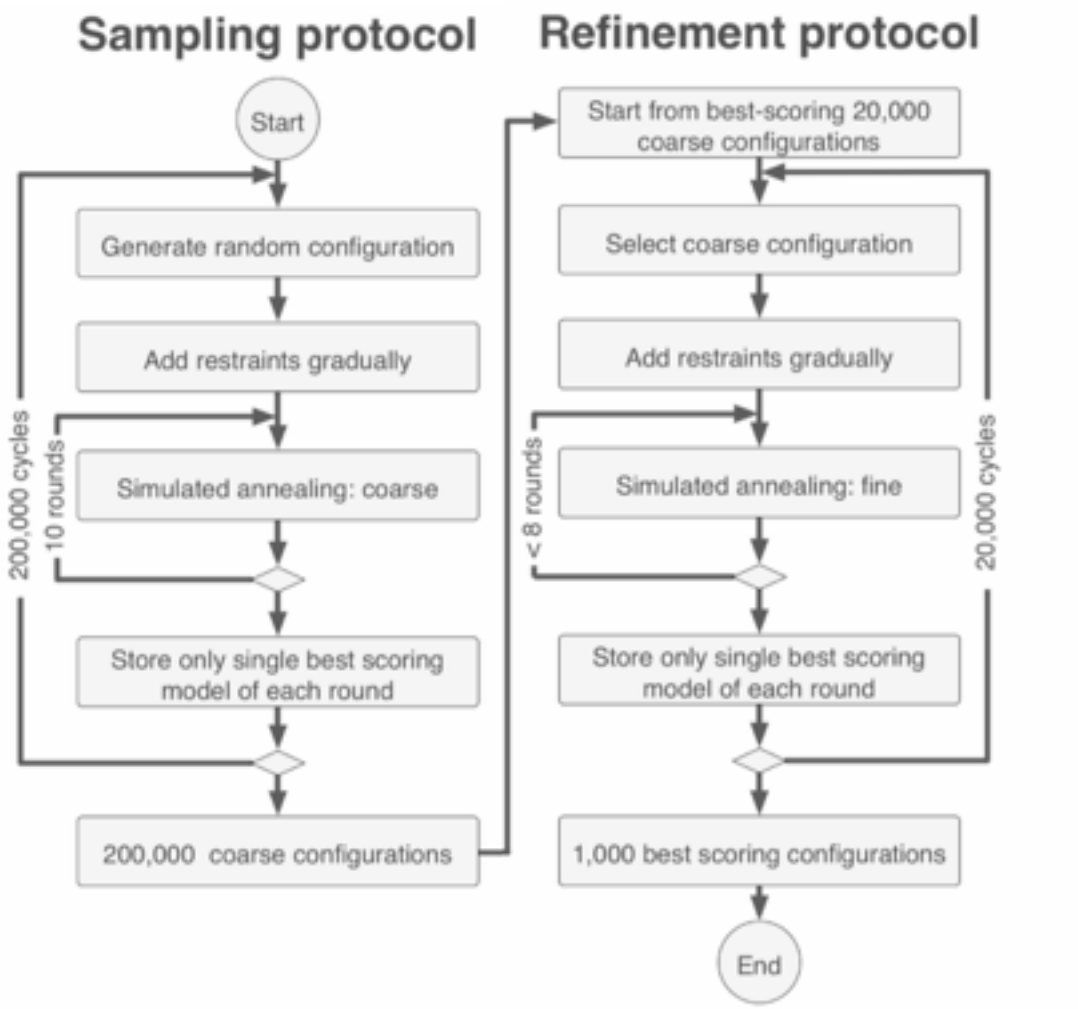
# Data Generation

## 6. **Protein Connectivity:** Affinity Purification-Mass Spectrometry (A



# Integrating the Data: Optimization

1. Components List
2. Shape and Size of Proteins
3. Amount of each protein
4. Localization of each type of Protein
5. Overall Shape
6. Protein Connectivity



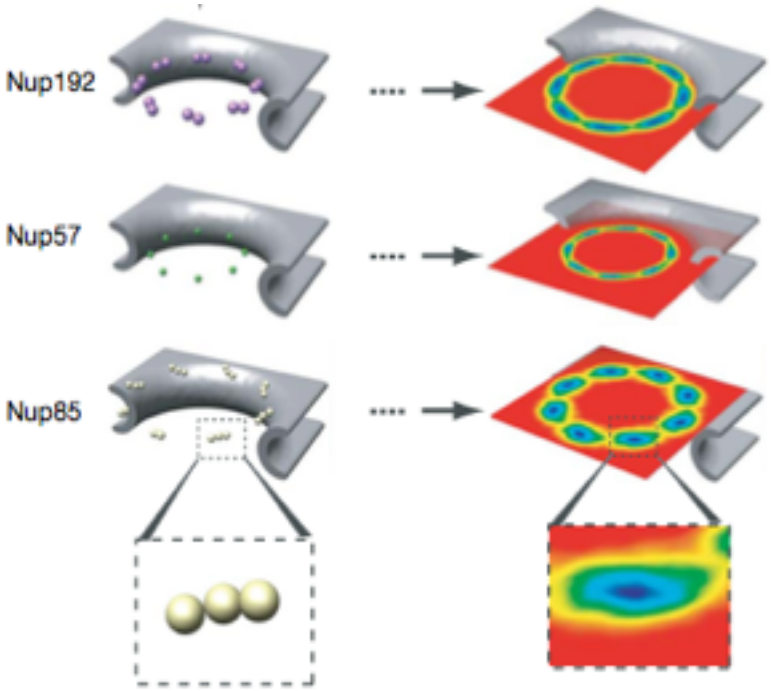

$$p(C/I) = \prod_f p_f(C/I_f)$$

Structural information from the Experimental Data

Cartesian Coordinates of components

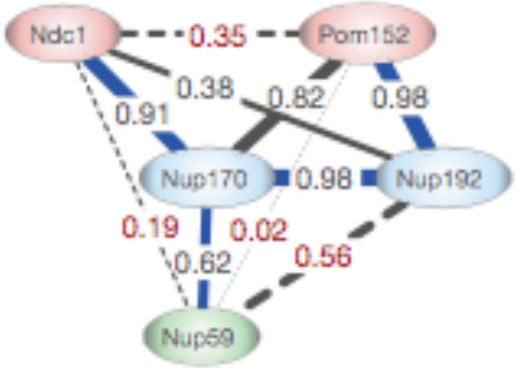
# Integrating the Data: Ensemble Analysis

- Goal: 1000 possible structures ---------> 1 highly probably native structure



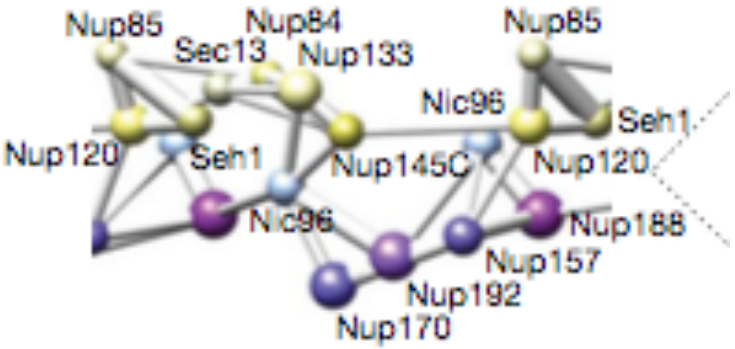

**Protein Positioning**

+




**Protein Contacts**

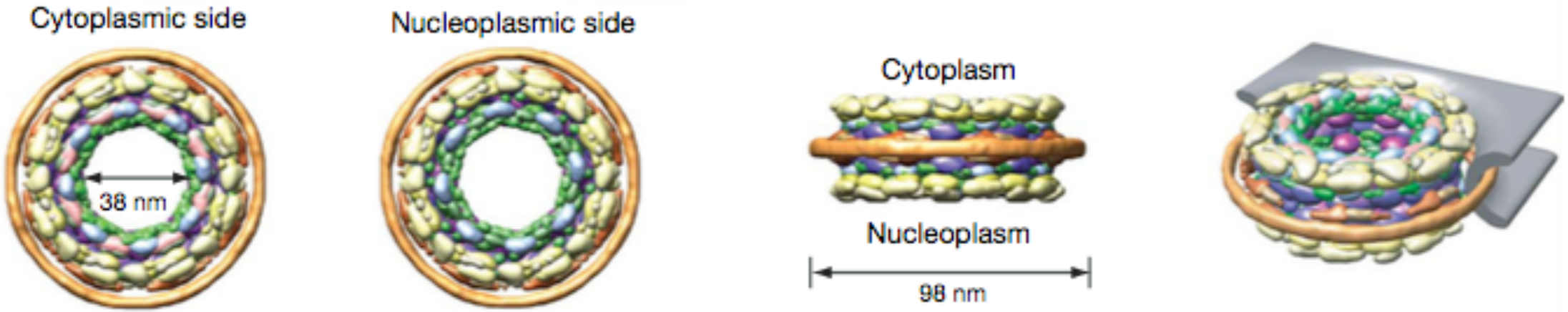
=




**Protein Configuration**

# Validation

- **Cross-validated:** leaving 10% of the data out still converged on the same global maximum
- **Consistent**…with unused data including, but not limited to:
  - electron microscopy images



# Weaknesses

- Experimental data difficult to generate
- Questionable transferability of methods

*“Indeed, it is hard to conceive of any combination of errors that could have biased our structure towards a single solution…”*

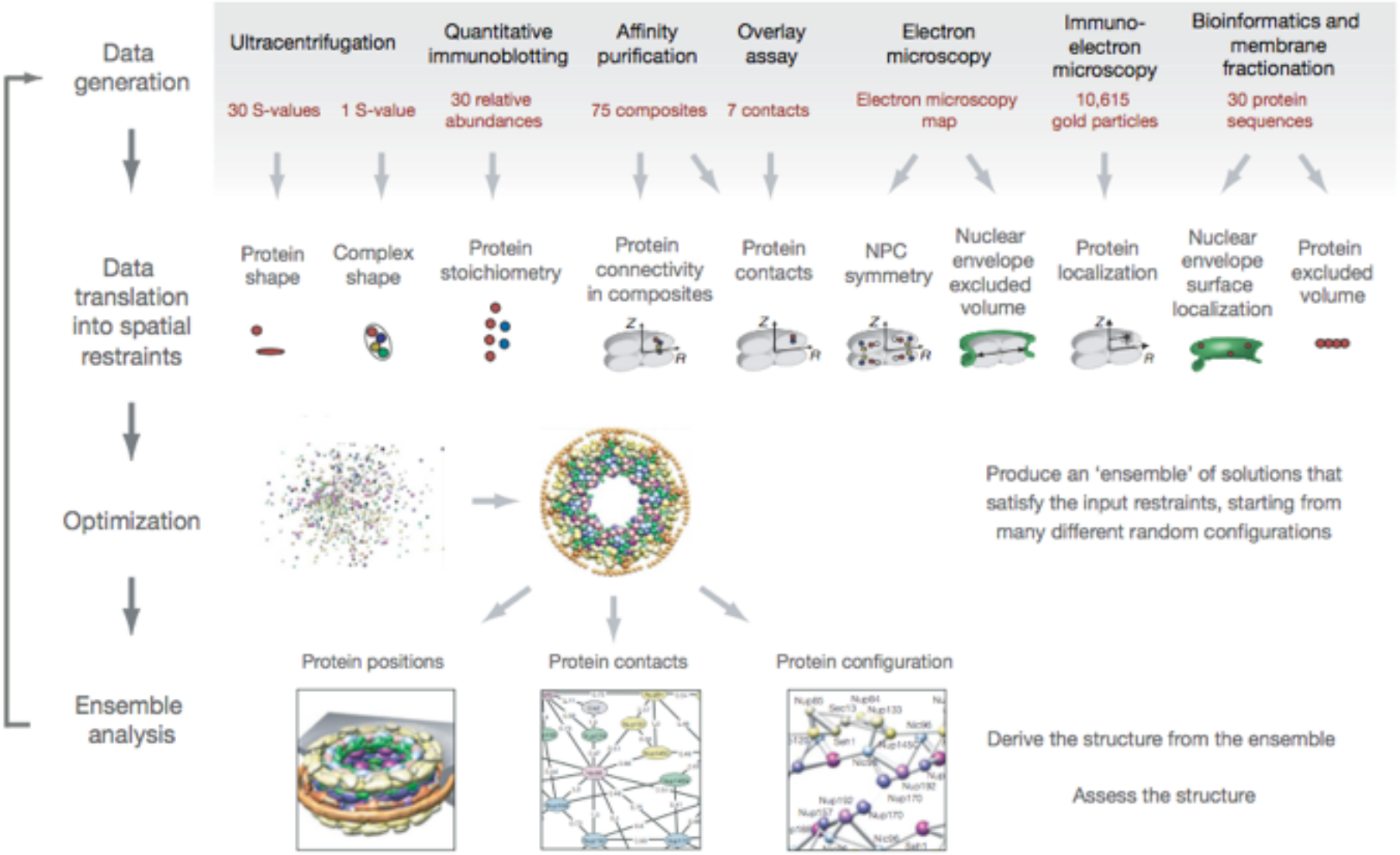
# Strengths

- Plethora of experimental data
- Novel use of data types and integration methods
- Really well written

# Beyond this paper…

- Evaluation biological implication of the solved structure
- Application on a different macromolecular complex
- Make it high-throughput? ☺️

# Questions?


Data generation
Ultracentrifugation
Quantitative immunoblotting
Affinity purification
Overlay assay
Electron microscopy
Immuno-electron microscopy
Bioinformatics and membrane fractionation
30 S-values
1 S-value
30 relative abundances
75 composites
7 contacts
Electron microscopy map
10,615 gold particles
30 protein sequences
Data translation into spatial restraints
Protein shape
Complex shape
Protein stoichiometry
Protein connectivity in composites
Protein contacts
NPC symmetry
Nuclear envelope excluded volume
Protein localization
Nuclear envelope surface localization
Protein excluded volume
Optimization
Produce an 'ensemble' of solutions that satisfy the input restraints, starting from many different random configurations
Protein positions
Protein contacts
Protein configuration
Ensemble analysis
Derive the structure from the ensemble
Assess the structure

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RESEARCH ARTICLE

# Molecular architecture of the yeast Mediator complex

**Philip J Robinson[1†], Michael J Trnka[2†], Riccardo Pellarin[3,4†], Charles H Greenberg[3], David A Bushnell[1], Ralph Davis[1], Alma L Burlingame[2], Andrej Sali[3], Roger D Kornberg[1*]**

[1]Department of Structural Biology, Stanford University School of Medicine, Stanford, United States; [2]Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, United States; [3]Department of Bioengineering and Therapeutic Sciences, Department of Pharmaceutical Chemistry, California Institute for Quantitative Biosciences, University of California, San Francisco, San Francisco, United States; [4]Structural Bioinformatics Unit, Institut Pasteur, CNRS UMR 3528, Paris, France

# Mediator

- Massive protein complex conserved in eukaryotes
- Essential for RNA pol II transcription and regulation of transcription

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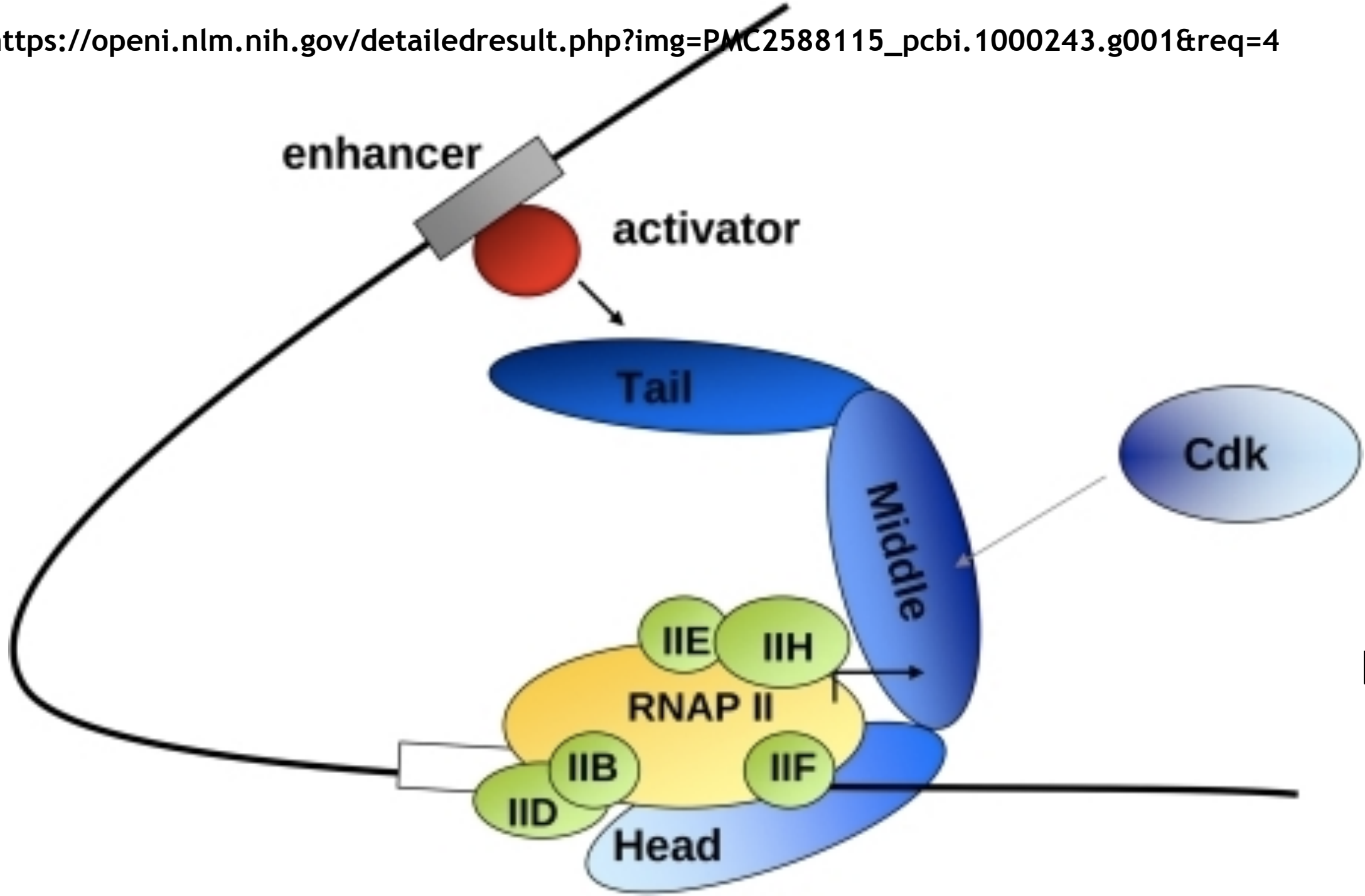
- Massive protein complex conserved in eukaryotes
- Essential for RNA pol II transcription and regulation of transcription

# What was previously known about it?

- Three modules: **Head**, **Middle**, and **Tail**
- Structural rearrangement occurs upon interaction with RNA pol II
  - In the RNA polymerase holoenzyme, RNA pol II is surrounded by the Head and Middle modules

https://openi.nlm.nih.gov/detailedresult.php?img=PMC2588115_pcbi.1000243.g001&req=4



# Goal of paper:
# Determine mediator's structure

# Integrative Structure Determination

Four stages of Integrative Structure Determination:

1. Gathering data
2. Representing and translating the data into spatial restraints
3. Sampling the conformational space and identifying good scoring solutions
4. Analyzing and assessing the ensemble of solutions

Integrative modeling platform (IMP) software used for integrative structure determination

# Integrative Structure Determination

**Data:**

XS-MS dataset, X-ray crystal structures, homology models, cryo-EM density maps

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XS-MS dataset, X-ray crystal structures, homology models, cryo-EM density maps

**Spatial restraints:**
Constructed a set of 21 Mediator subunit model representations
Selected model that best “docked” into cryo-EM maps

# Integrative Structure Determination

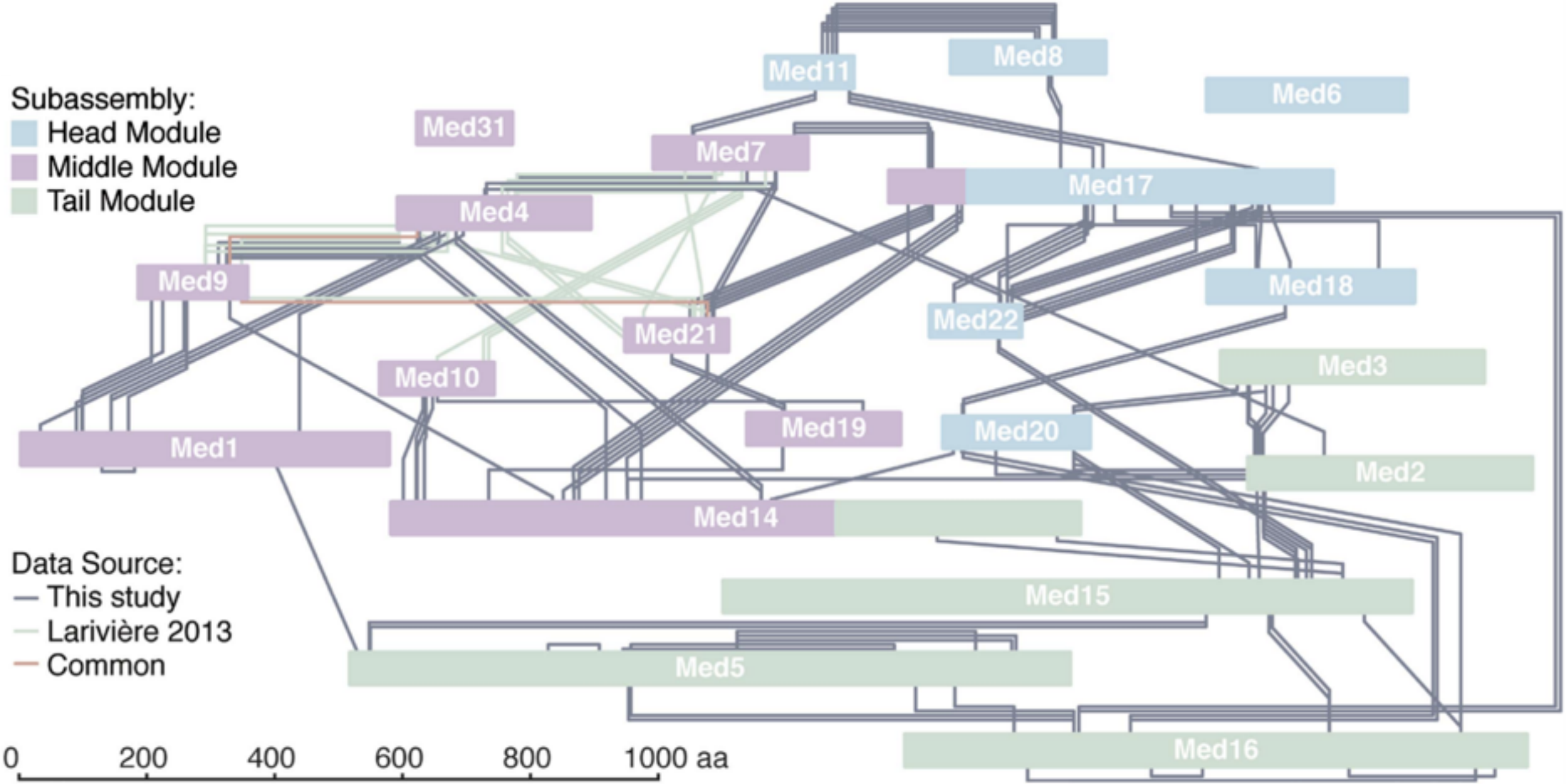
**Data:**
XS-MS dataset, X-ray crystal structures, homology models, cryo-EM density maps

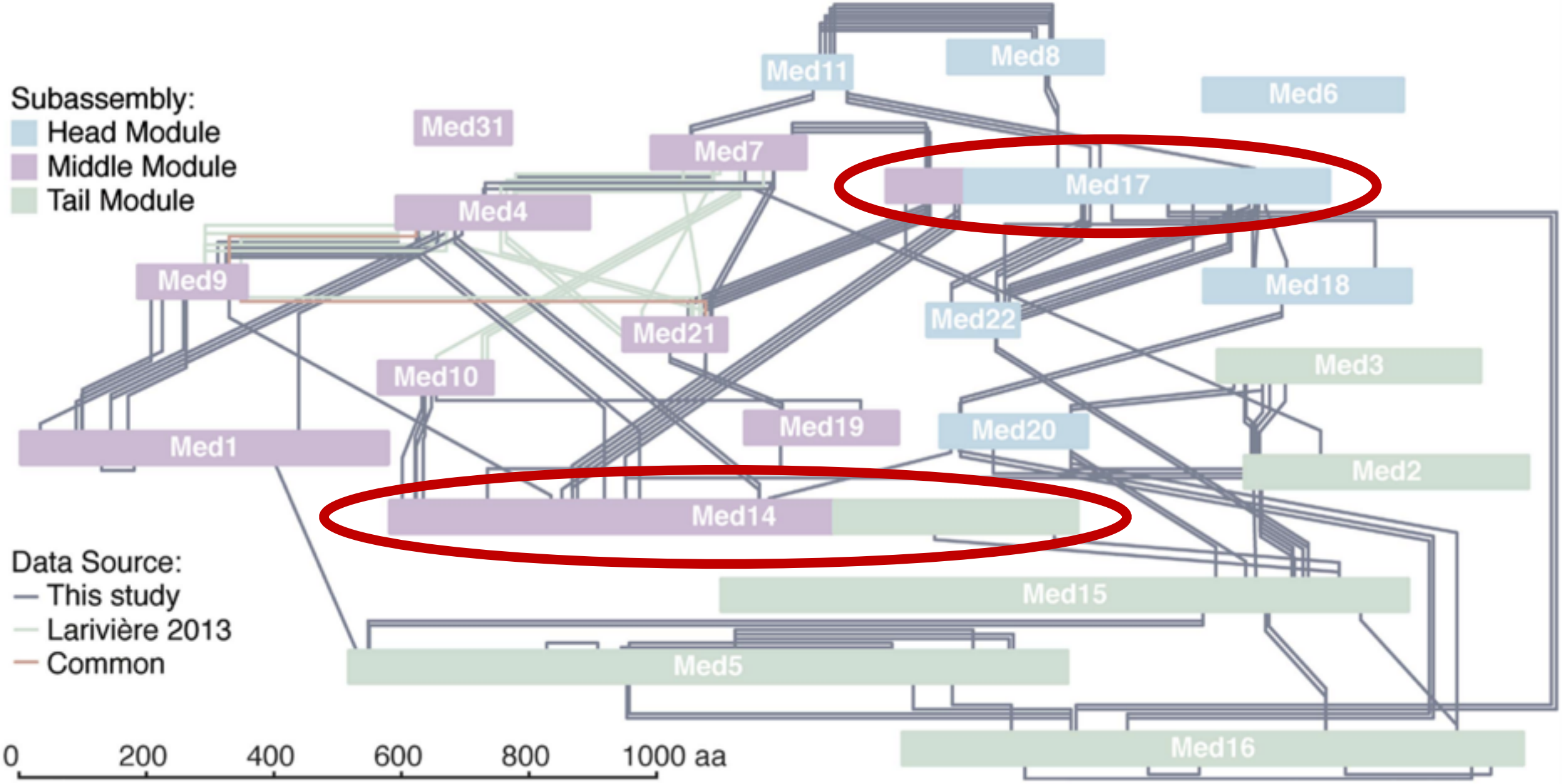
**Spatial restraints:**
Constructed a set of 21 Mediator subunit model representations
Selected model that best “docked” into cryo-EM maps

**Sampling the conformational space:**
165,523 Mediator model configurations produced
Selected the 500 best and grouped them into four clusters (based on RMSD)

# XL-MS Results

Note: XL-MS was performed on Mediator when Mediator was in the form of a complex with pol II (holoenzyme)


Subassembly:
Head Module
Middle Module
Tail Module
Med11
Med8
Med6
Med31
Med7
Med17
Med4
Med9
Med18
Med22
Med21
Med10
Med3
Med19
Med20
Med1
Med2
Med14
Data Source:
This study
Larivière 2013
Common
Med15
Med5
Med16
0
200
400
600
800
1000 aa


Subassembly:
Head Module
Middle Module
Tail Module
Med11
Med8
Med6
Med31
Med7
Med17
Med4
Med9
Med18
Med22
Med21
Med3
Med10
Med19
Med20
Med1
Med2
Med14
Med15
Med5
Med16
Data Source:
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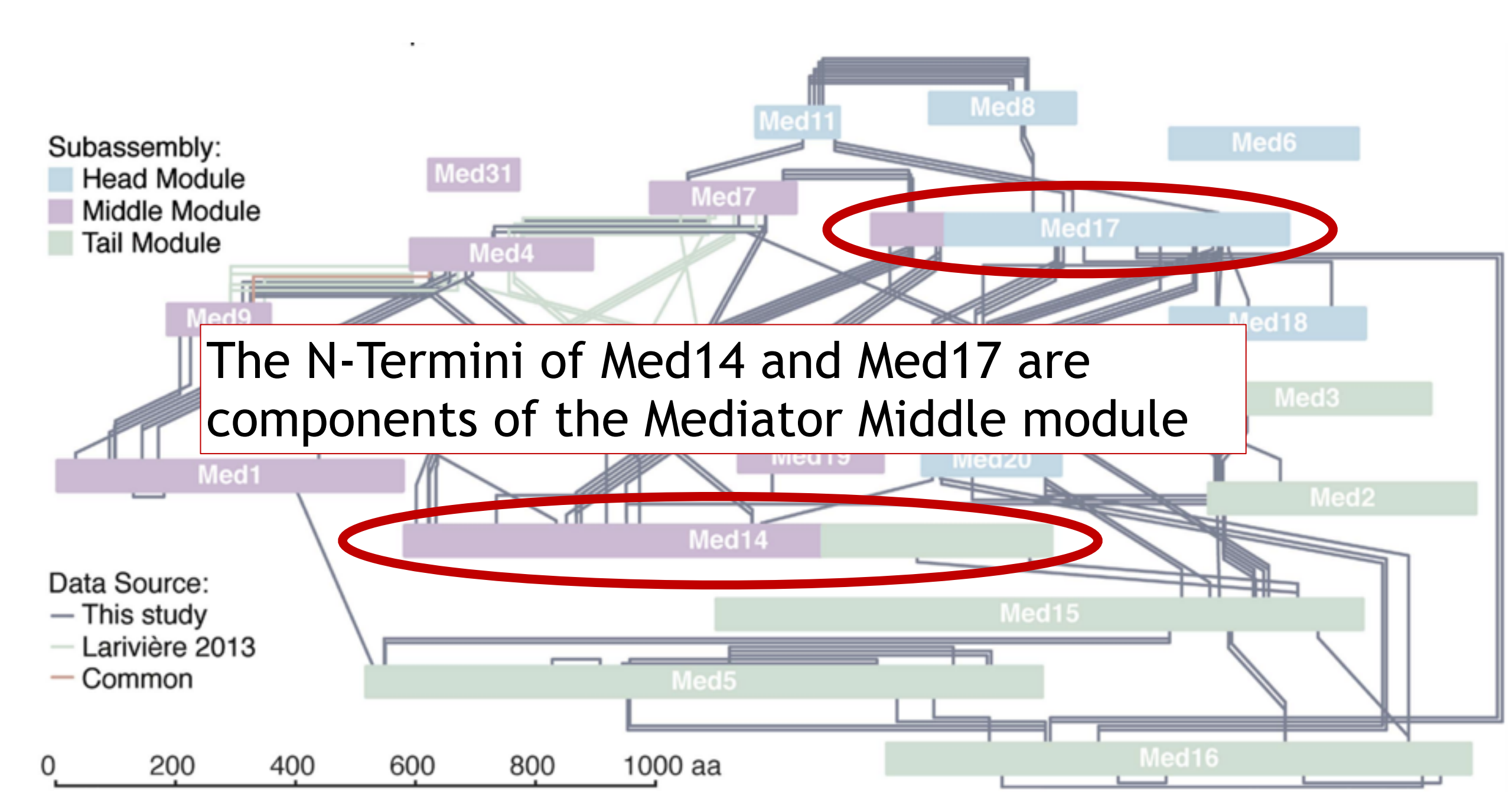
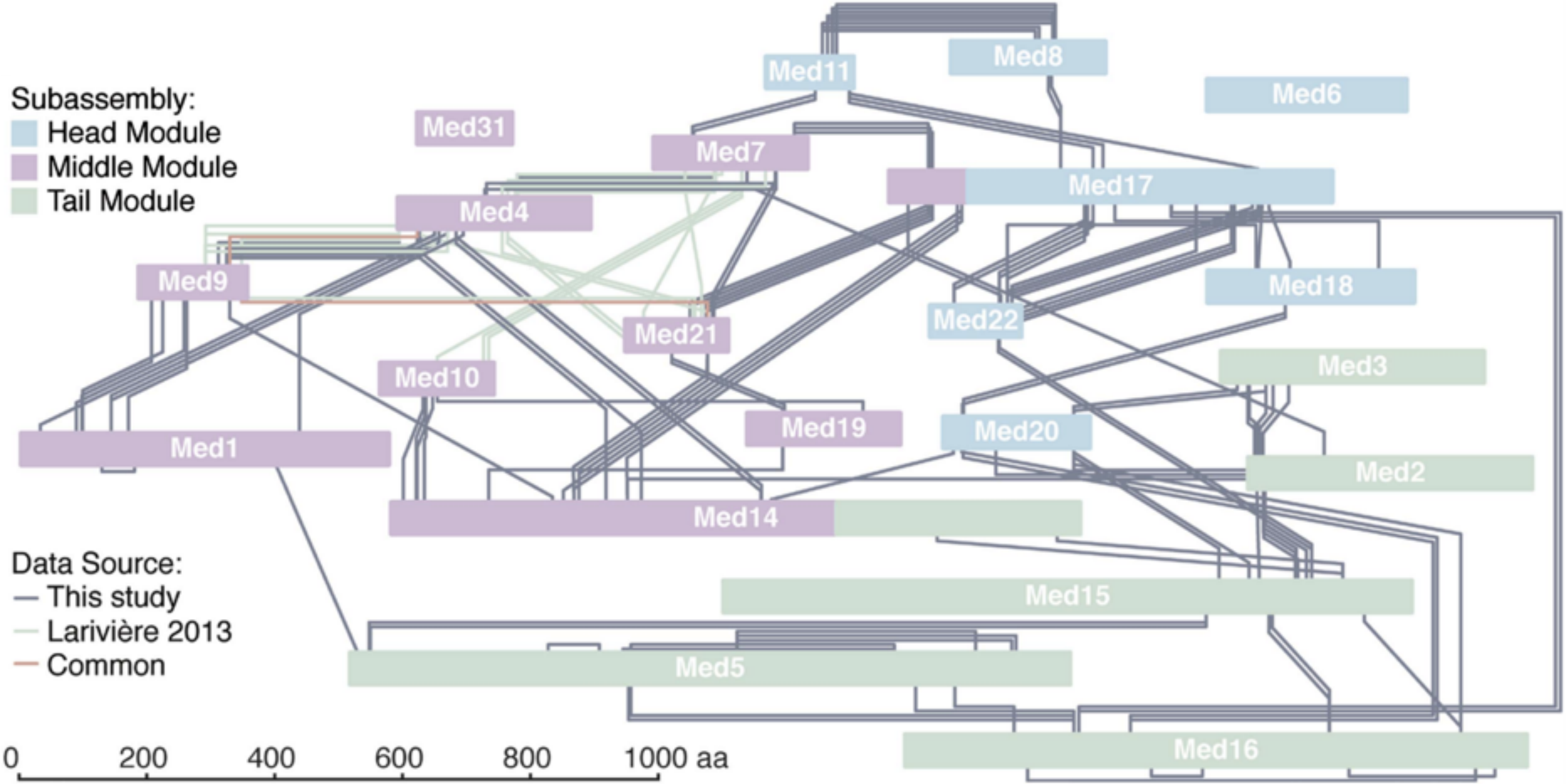



The N-Termini of Med14 and Med17 are components of the Mediator Middle module


Subassembly:
Head Module
Middle Module
Tail Module
Med11
Med8
Med6
Med31
Med7
Med17
Med4
Med9
Med18
Med22
Med21
Med10
Med3
Med19
Med20
Med1
Med2
Med14
Data Source:
This study
Larivière 2013
Common
Med15
Med5
Med16
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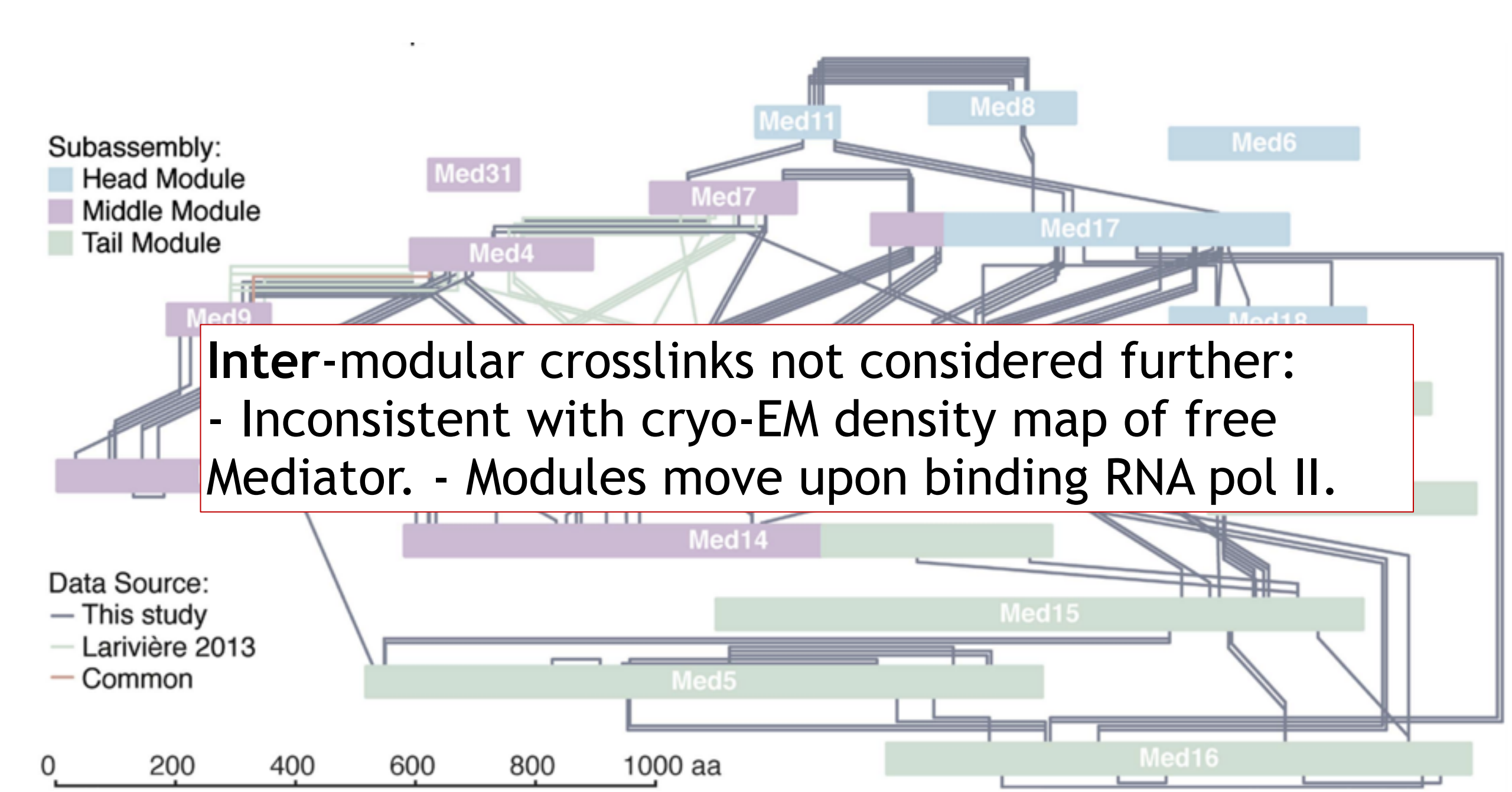
800
1000 aa



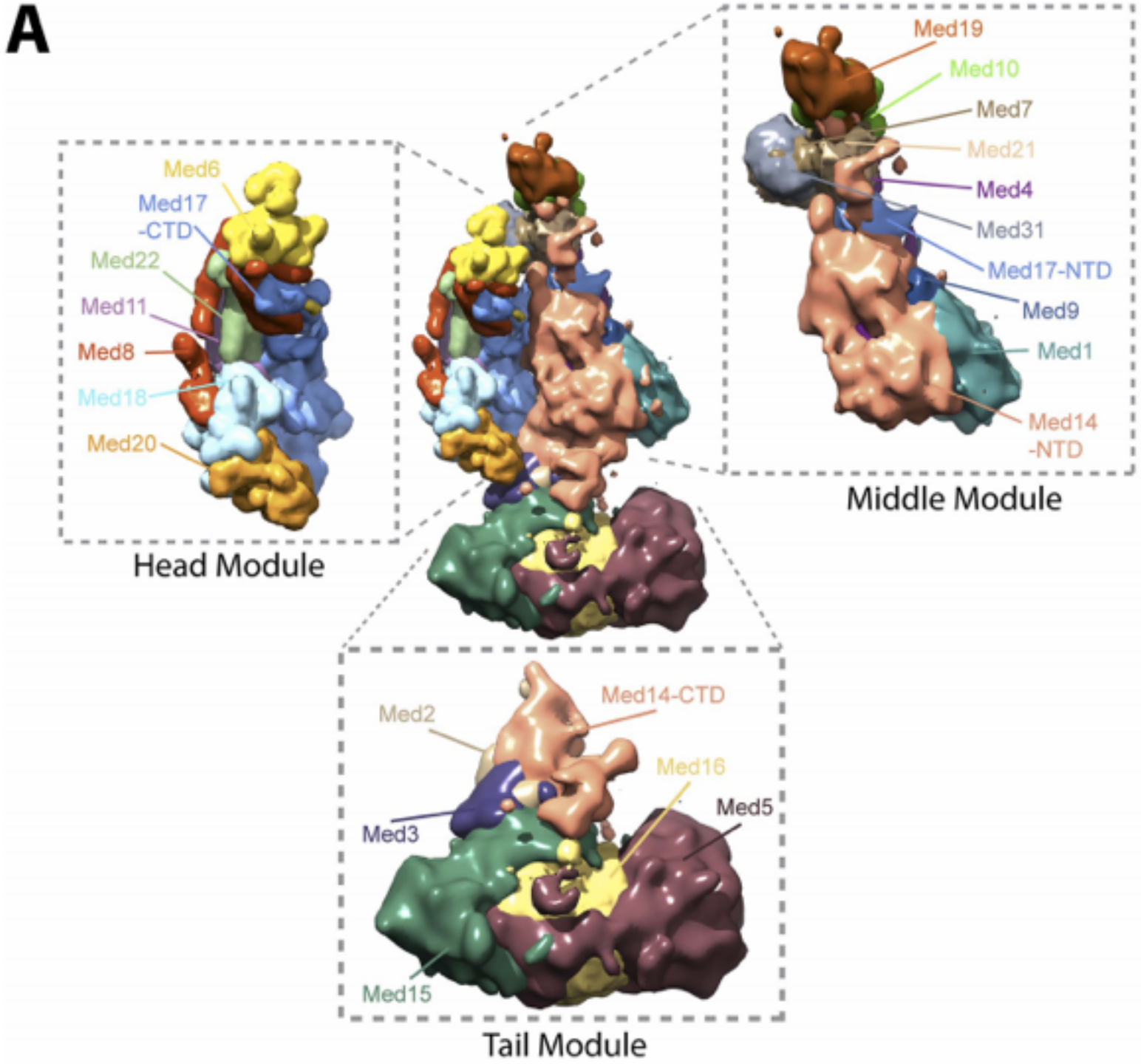

**Inter**-modular crosslinks not considered further:
- Inconsistent with cryo-EM density map of free Mediator. - Modules move upon binding RNA pol II.

# Integrative Structure Determination: Cluster 1

Eventually narrowed down to one cluster:

- Cluster 3 had Mediator with orientation inverted (which is inconsistent with prior experiments).
- **Cluster 1** also showed the best cross-link satisfaction statistics and had the best average score.

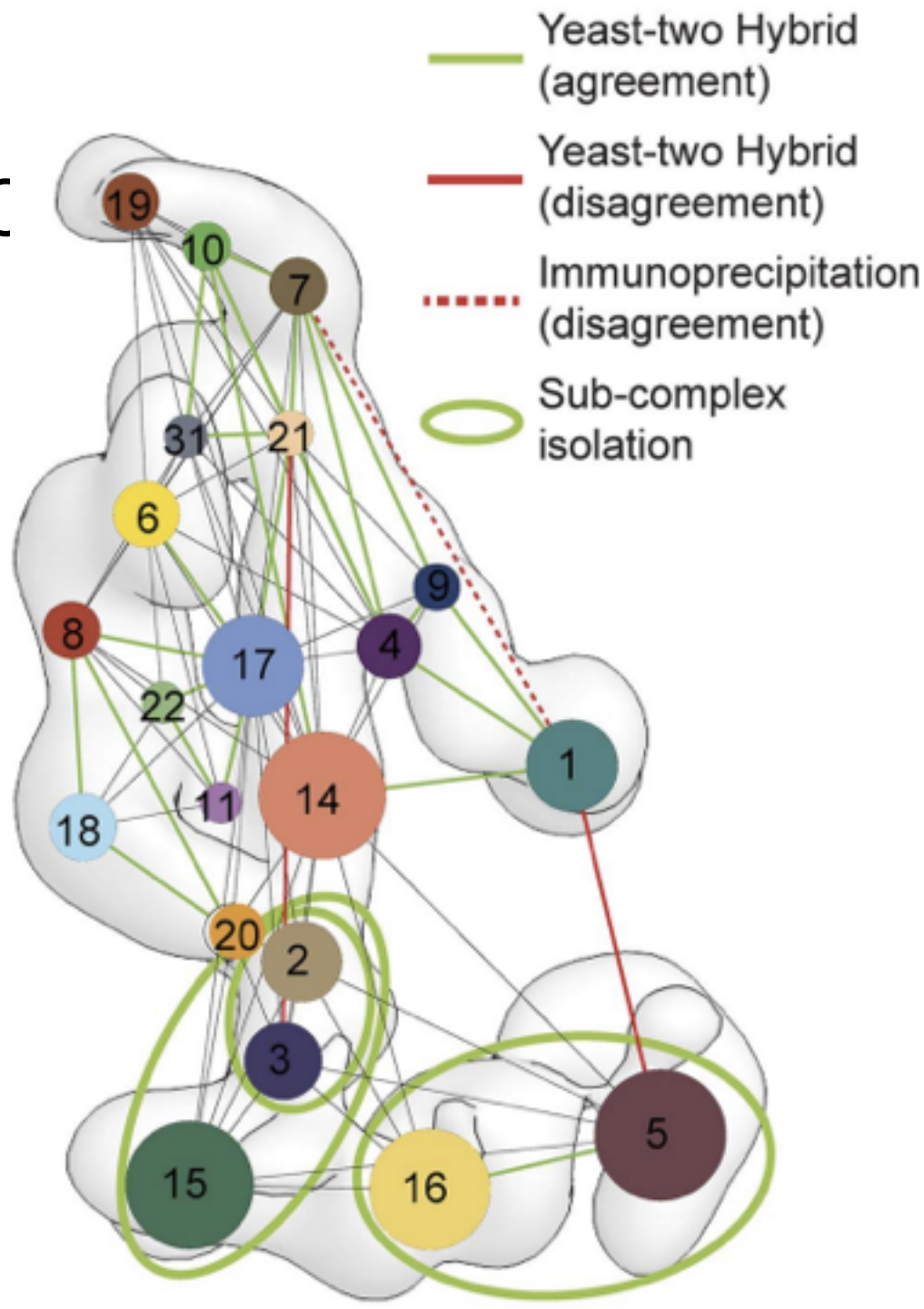
# Mediator Model



# Mediator Model: Verificatic

- Verification based on previous data from yeast two-hybrid assays and immunoprecipitation assays.

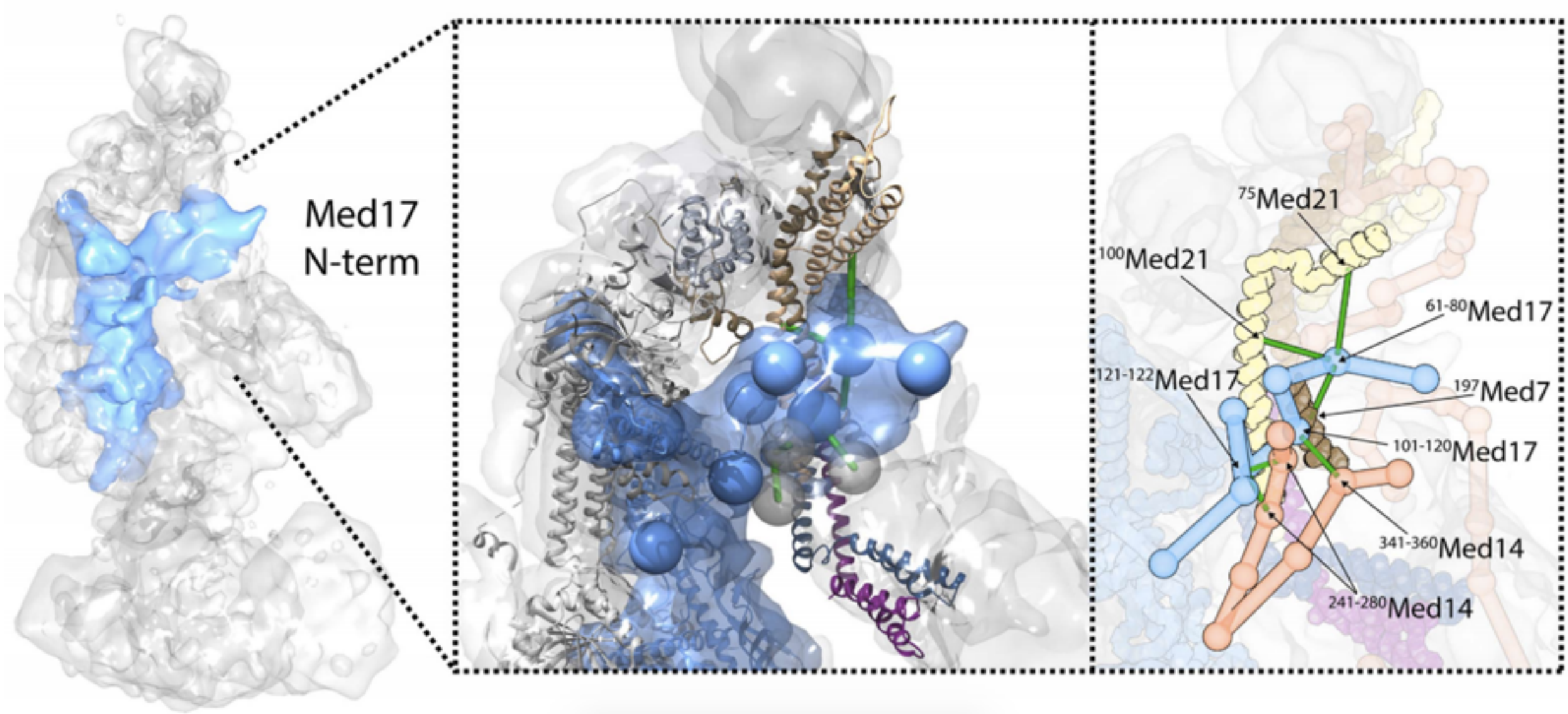
- Three disagreements:
  - Med3-Med21
  - Med1-Med7
  - Med1-Med5



# Mediator Model: Med17 and Med14

- Med17: Temperature-sensitive mutations of Med17 have been discovered that abolish all RNA pol II transcription at the restrictive temperature.
- The N-terminus of the Head subunit Med17 acts as an intermolecular bridge by forming an extensive cross-linking network within the Middle module

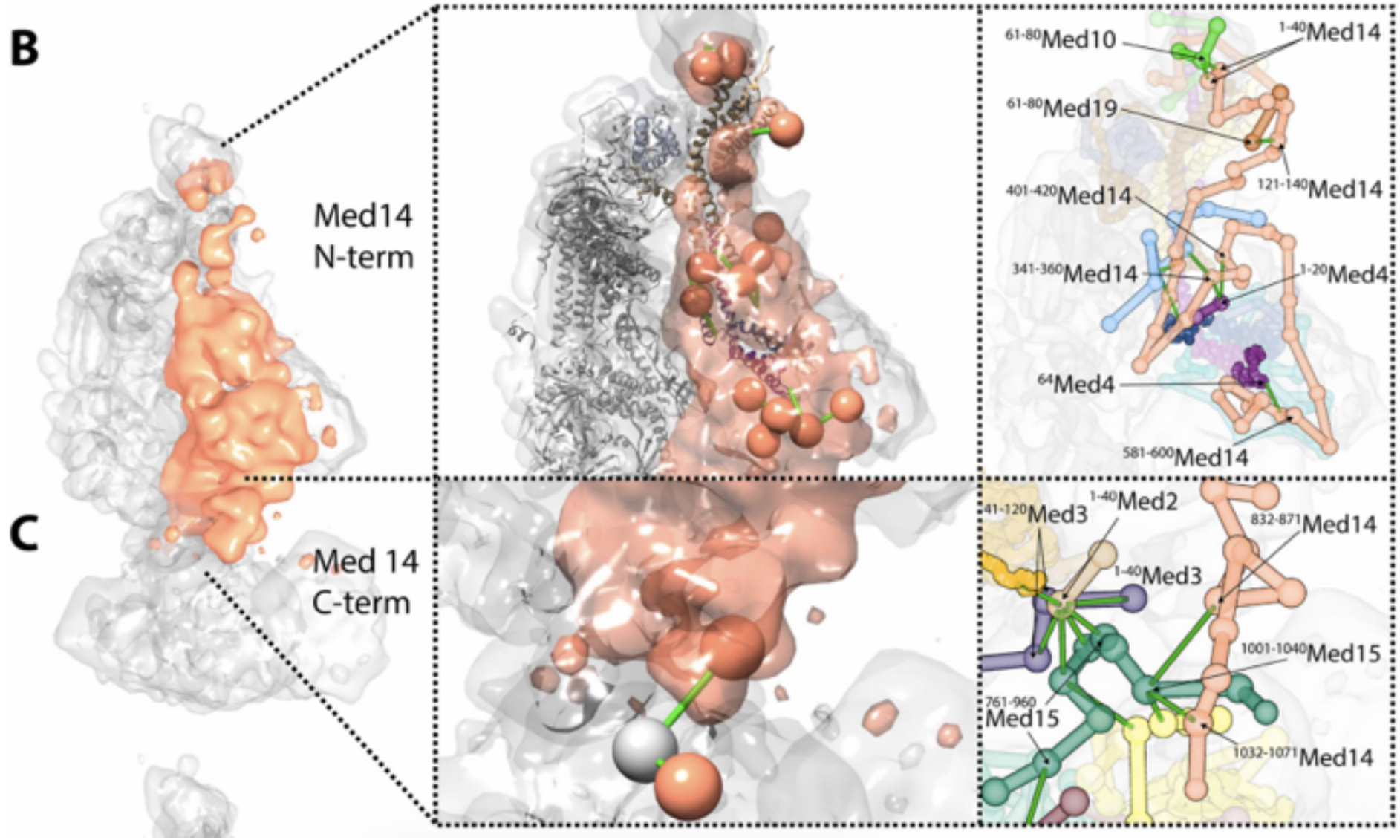
# Mediator Model: Med17 and Med14



# Mediator Model: Med17 and Med14

- Med14 subunit originally identified as a repressor protein in yeast
- Med14 makes extensive contacts with proteins from all three modules, and is the only Mediator subunit that does so

# Mediator Model: Med17 and Med14



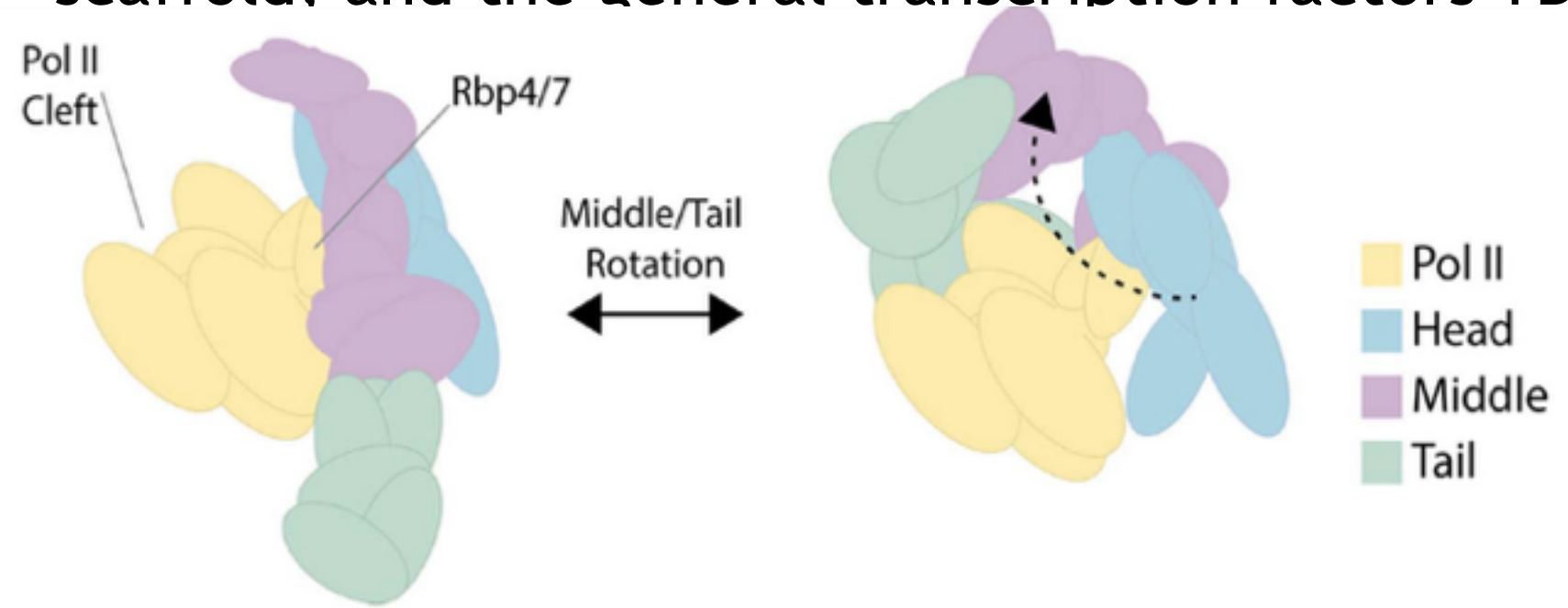
# Mediator & RNA Pol II: Core Initiation Complex

- Core initiation complex:  comprising the Mediator Head module, a minimal Middle module, pol II, a nucleic acid scaffold, and the general transcription factors TBP, TFIIB and TFIIF

- Cross-links between Head module and RNA pol II consistent with holoenzyme cryo-EM data.
- Cross-links between Tail and Middle modules to RNA pol II not consistent with holoenzyme cryo-EM data.
- This suggests that with the tail present and the absence of the nucleic acid scaffold and general factors, the Mediator-polymerase holoenzyme has a different configuration.

# Mediator & RNA Pol II: Core Initiation Complex

- Core initiation complex:  comprising the Mediator Head module, a minimal Middle module, pol II, a nucleic acid scaffold, and the general transcription factors TBP, TFIIB and



# Final Thoughts and Things to Work On

- What has been done:
  - A complete picture of the mediator complex and its modules has been constructed.
  - It has been shown that, in going from the core initiation complex to the holoenzyme, the mediator complex changes conformation to bring the Tail module in contact with RNA pol II.
- What needs to be worked on:
  - Resolve discrepancies with past immunoprecipitation and yeast two-hybrid results
  - A better description on how this model fits in with our understanding of transcription e.g. showing how the conformational change might turn transcription “on” or ”off”
  - Better characterizing the conformational change: What exactly changes?
  - A major weakness was reconciling data from mediator as part of the holoenzyme to d from mediator by itself.  Perhaps more consistency in further analysis would be nice.