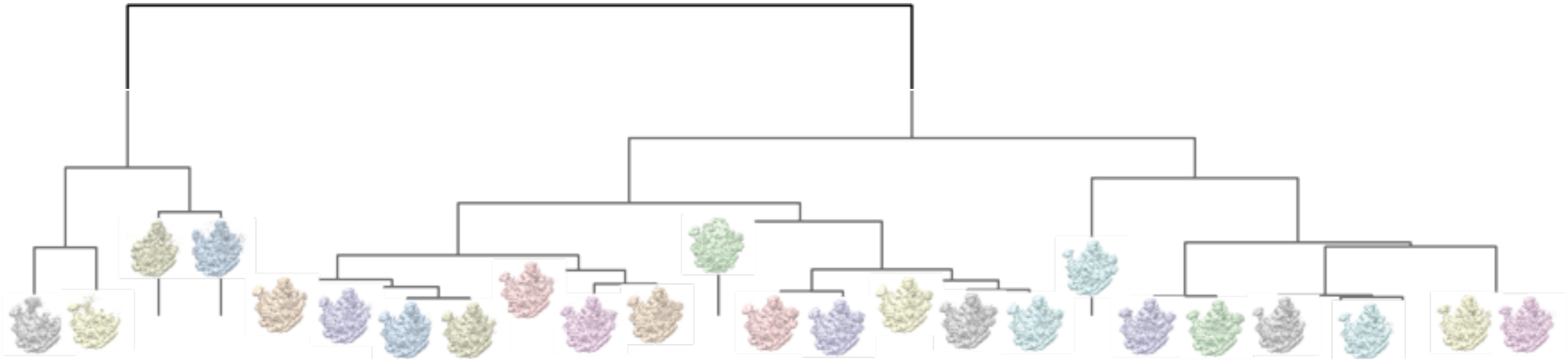
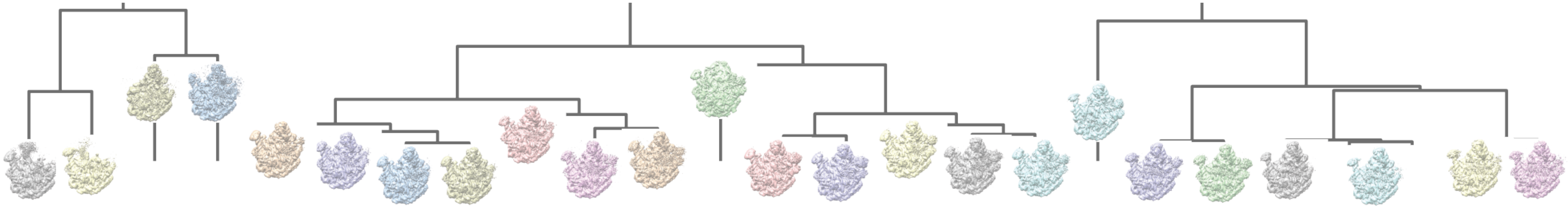


# Characterizing Late Roadblocks in Ribosome Assembly

**Jessica Rabuck-Gibbons**[1,2], Joseph Davis[1], Dmitry Lyumkis[2], James Williamson[1]

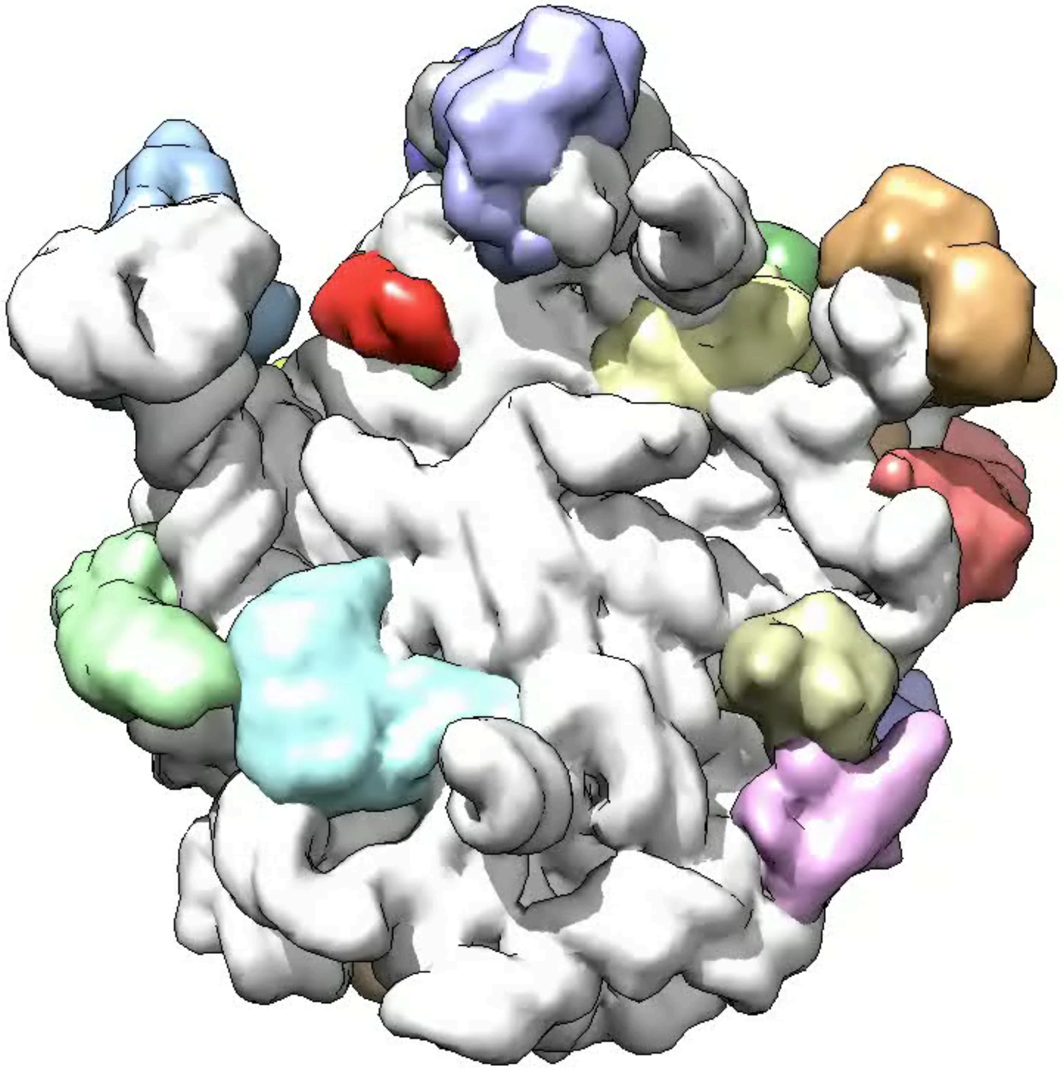
[1] The Scripps Research Institute, Department of Integrative Structural and Computational Biology, La Jolla, Ca 92037

[2]Laboratory of Genetics and Helmsley Center for Genomic Medicine, The Salk Institute for Biology Studies, La Jolla, Ca 92037



# How does the 50S subunit assemble into its mature form?

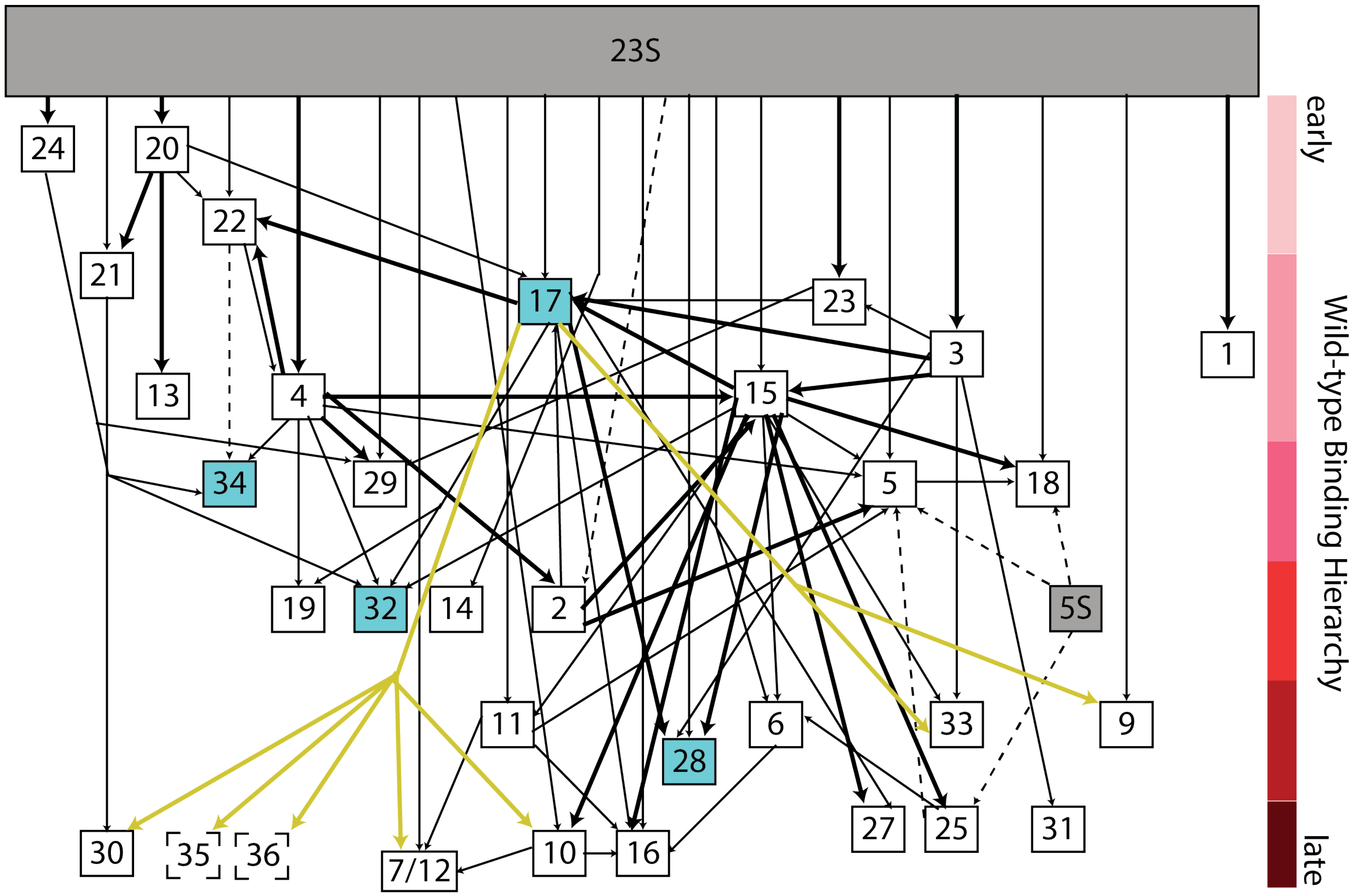
large 50S (bacterial)
ribosomal subunit



5S RNA
Proteins

- Ribosomes are responsible for protein synthesis in cells
- Highly complex — 2 subunits, multiple long stretches of folded RNA, ~50 proteins
- All these components must assemble into an ordered complex

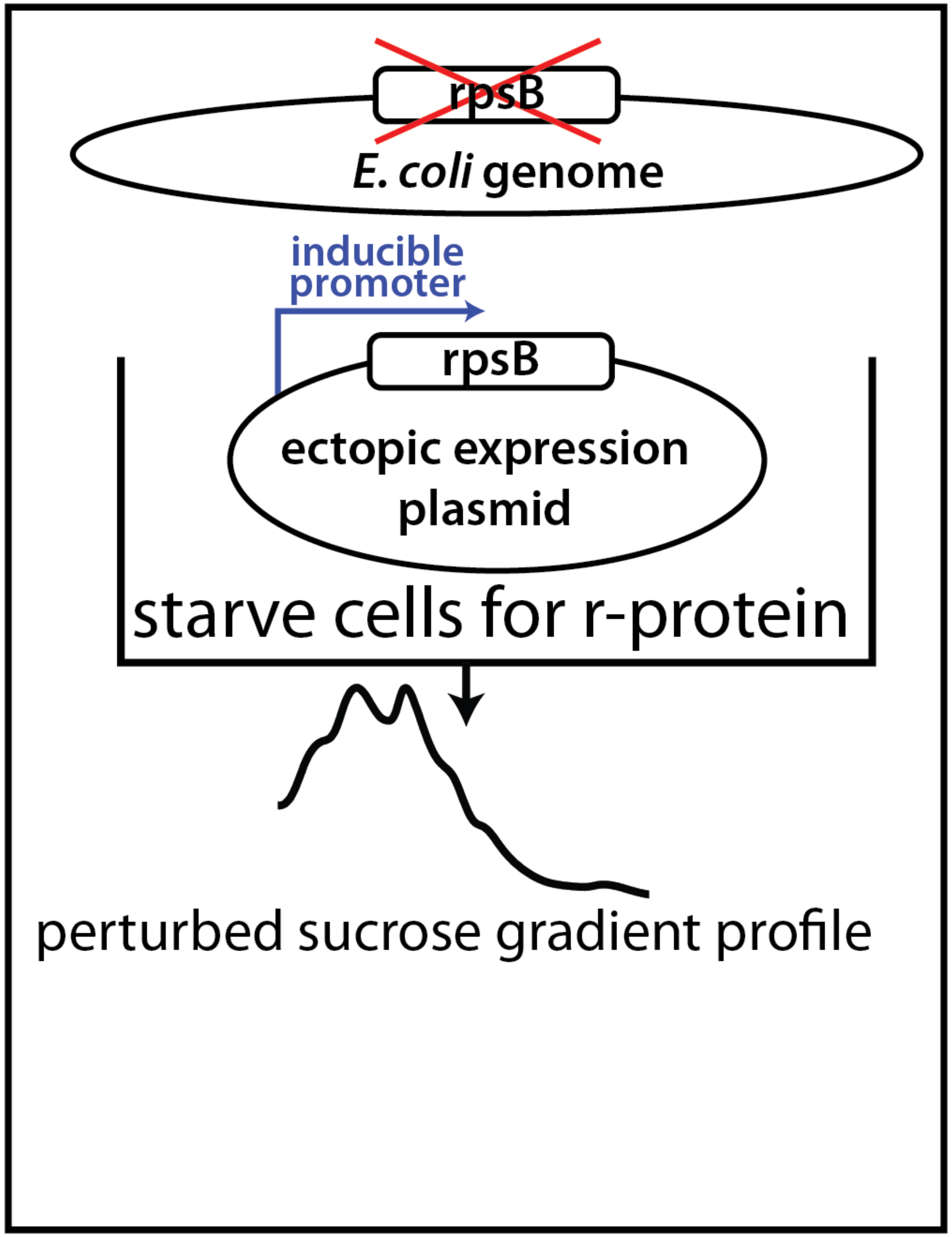
# Nierhaus Assembly Map



🟦 Data presented

— new interaction based on previous bL17 data

# A genetic system to perturb large subunit biogenesis — in vivo



~~rpsB~~

*E. coli* genome

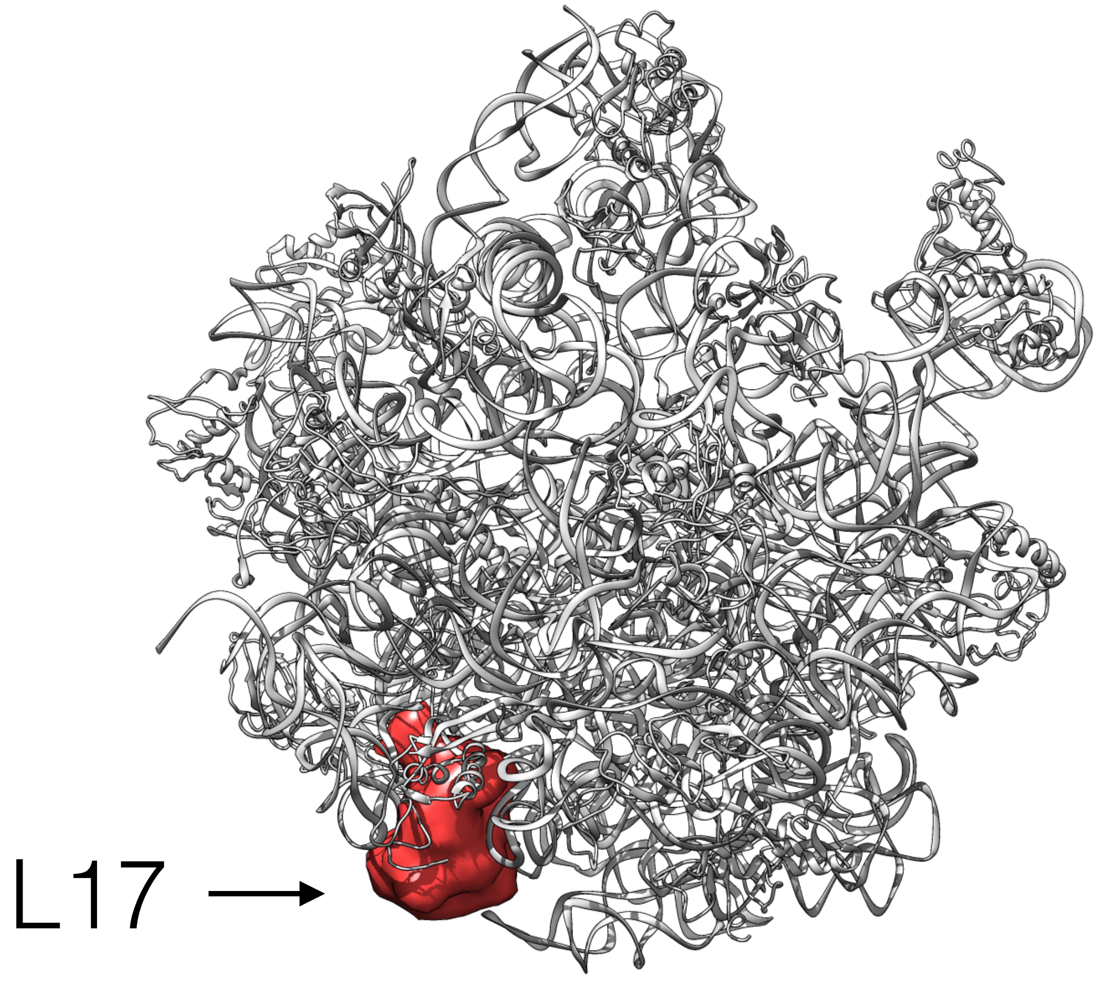
inducible promoter

rpsB

ectopic expression plasmid

starve cells for r-protein

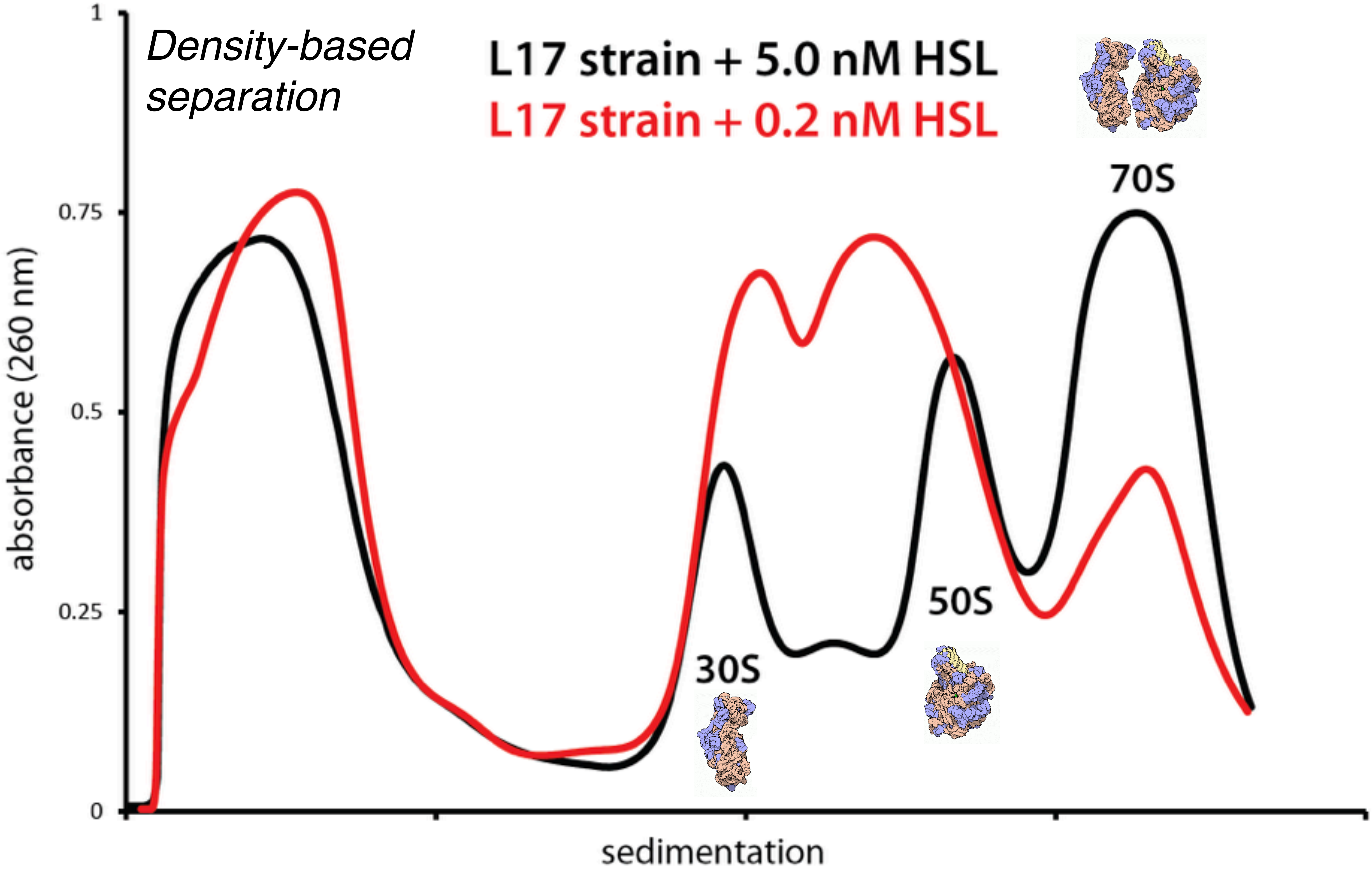
perturbed sucrose gradient profile



Defined quantities of ribosomal protein L17 provides titratable population of assembling ribosomes

Slide by Dmitry Lyumkis

# Ribosomal protein (rpL17) depletion perturbs sucrose density gradient profiles



Slide by Dmitry Lyumkis

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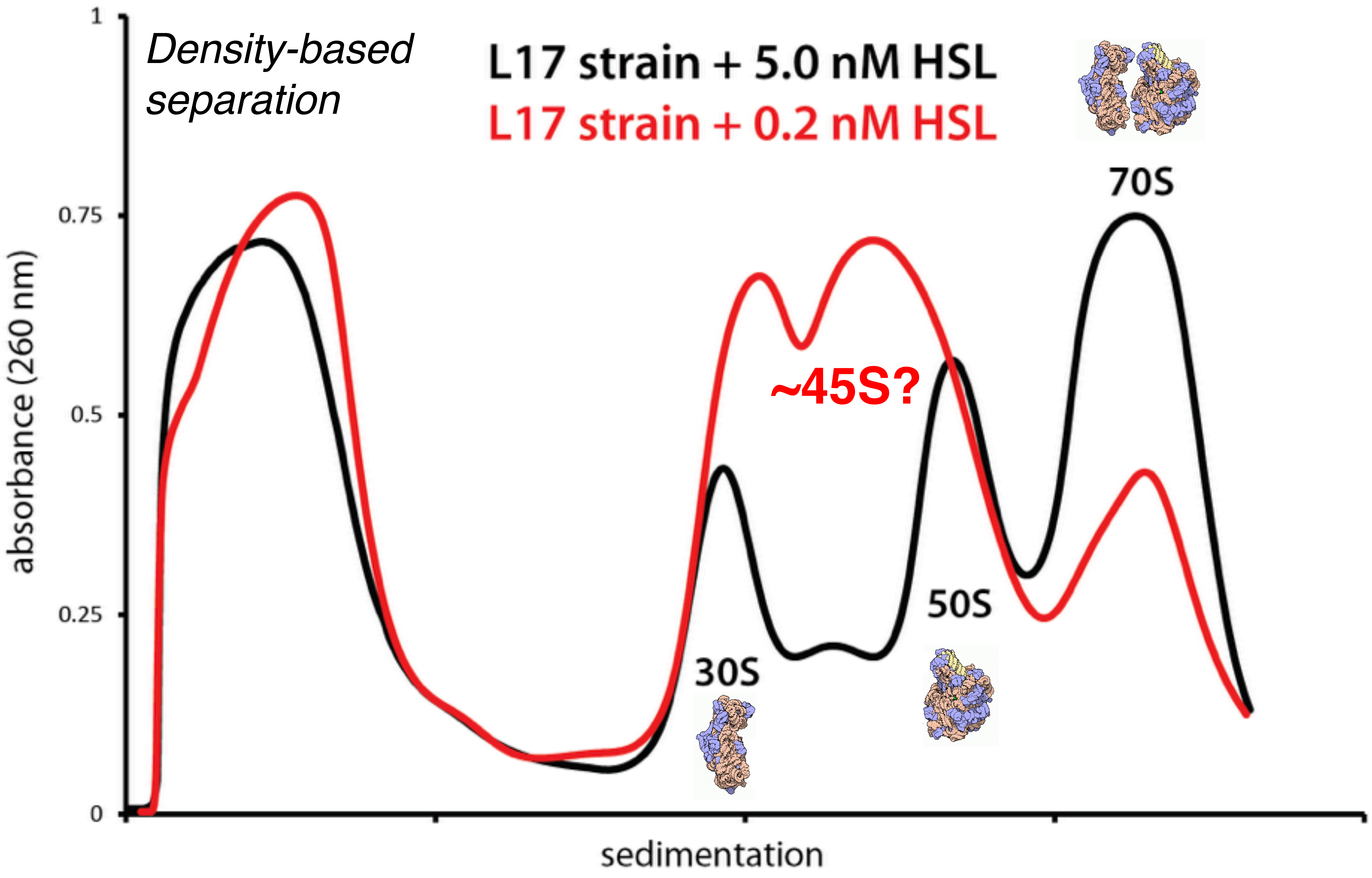

Slide by Dmitry Lyumkis

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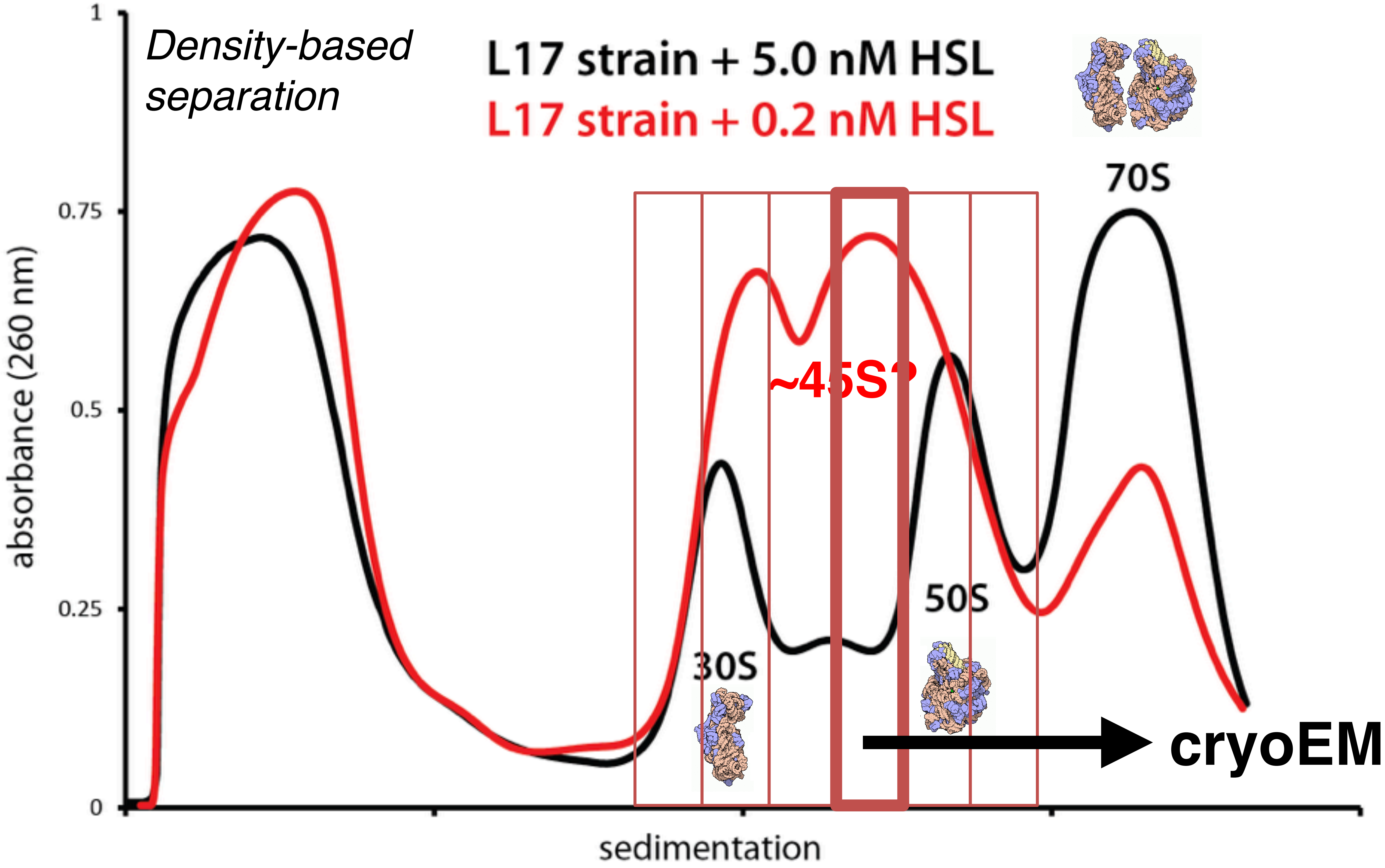
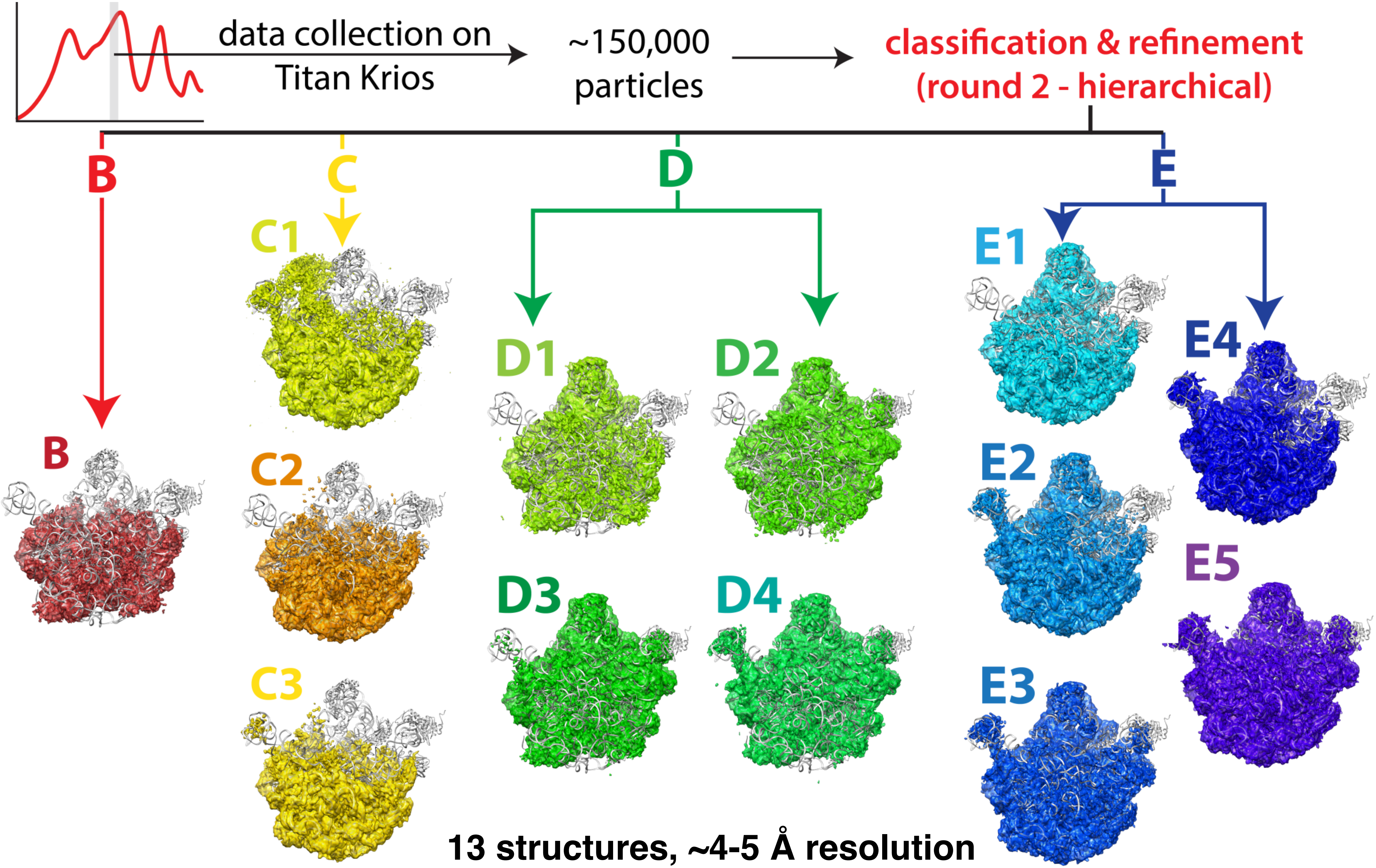


Slide by Dmitry Lyumkis

# Disparate structures revealed through single-particle analysis



data collection on Titan Krios

~150,000 particles

classification & refinement (round 2 - hierarchical)

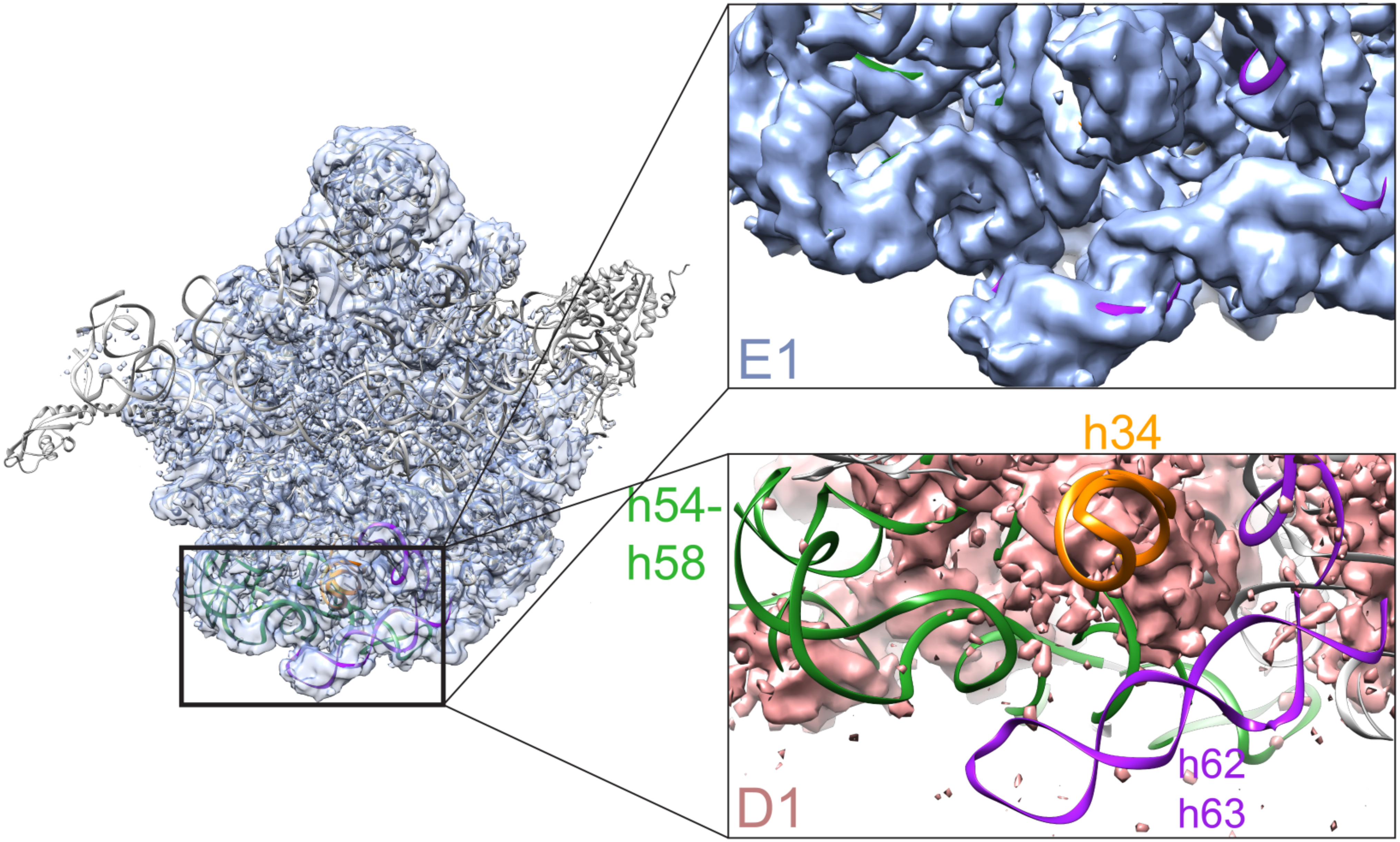
**13 structures, ~4-5 Å resolution**

***** NOT dead-end or degradation products *****

Joey Davis, Yong Zi Tan, and Jamie Williamson

# helix (RNA) and protein occupancy differs between maps

1. Theoretical density generated for each helix/protein from docked PDB
2. For each map, calculated fraction of mature density occupied



Slide by Joey Davis

**Davis, *et al.* Cell 2016**

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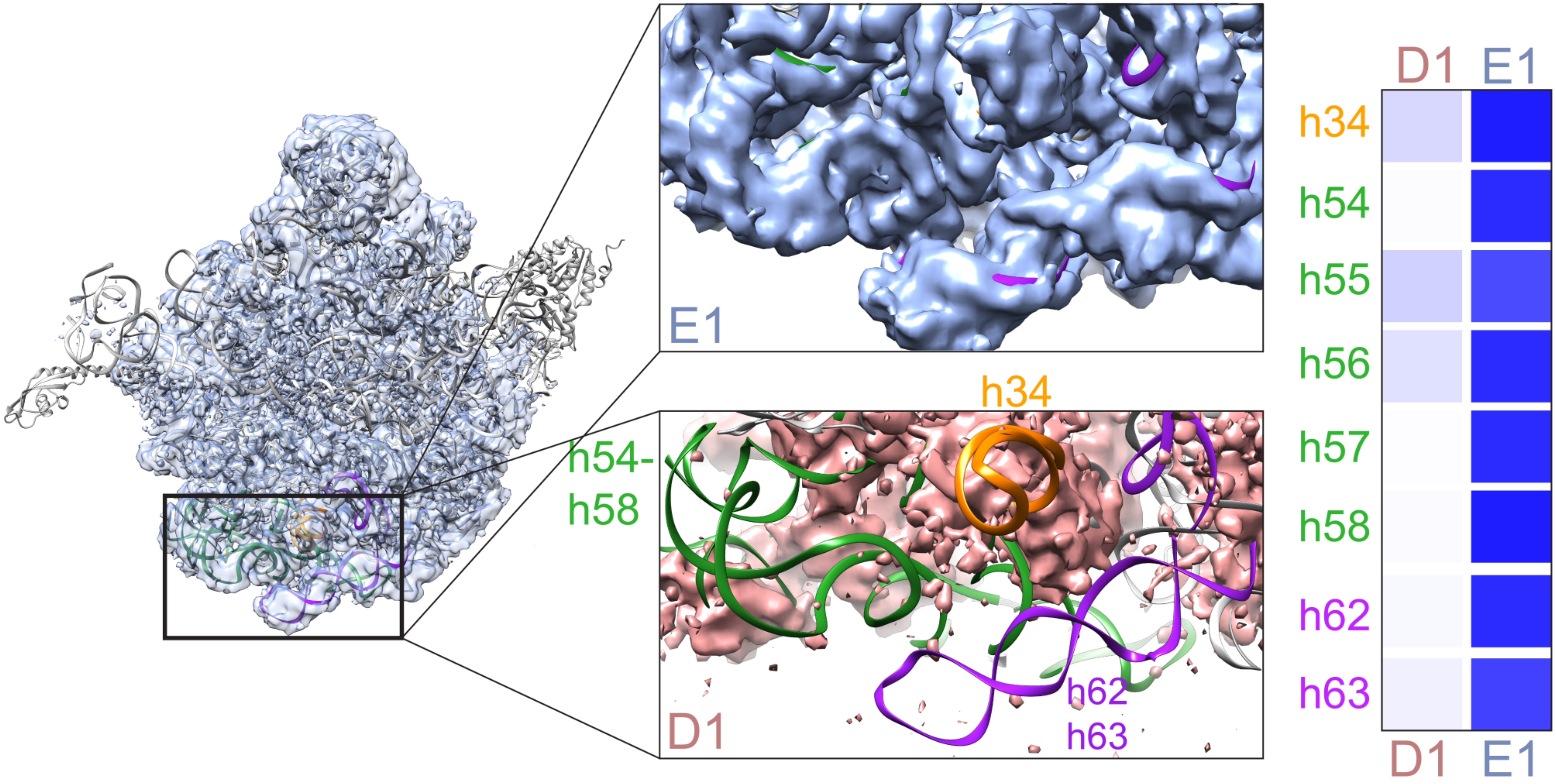
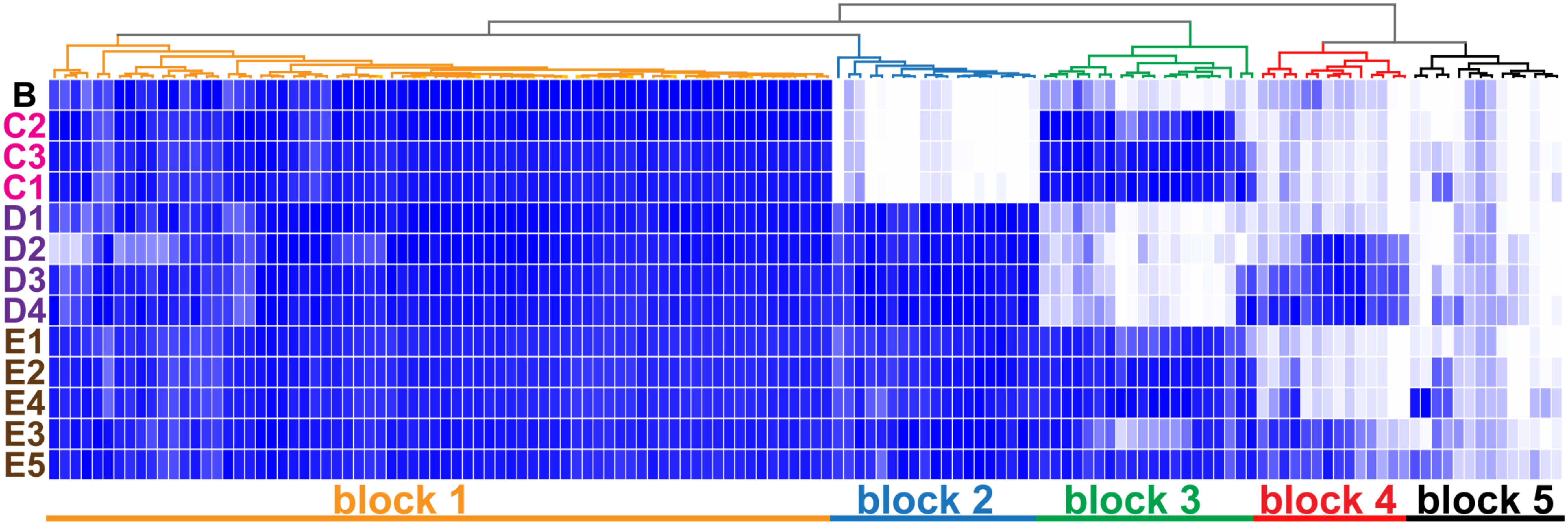


- **How does occupancy of each helix and each protein vary across intermediates?**

Slide by Joey Davis

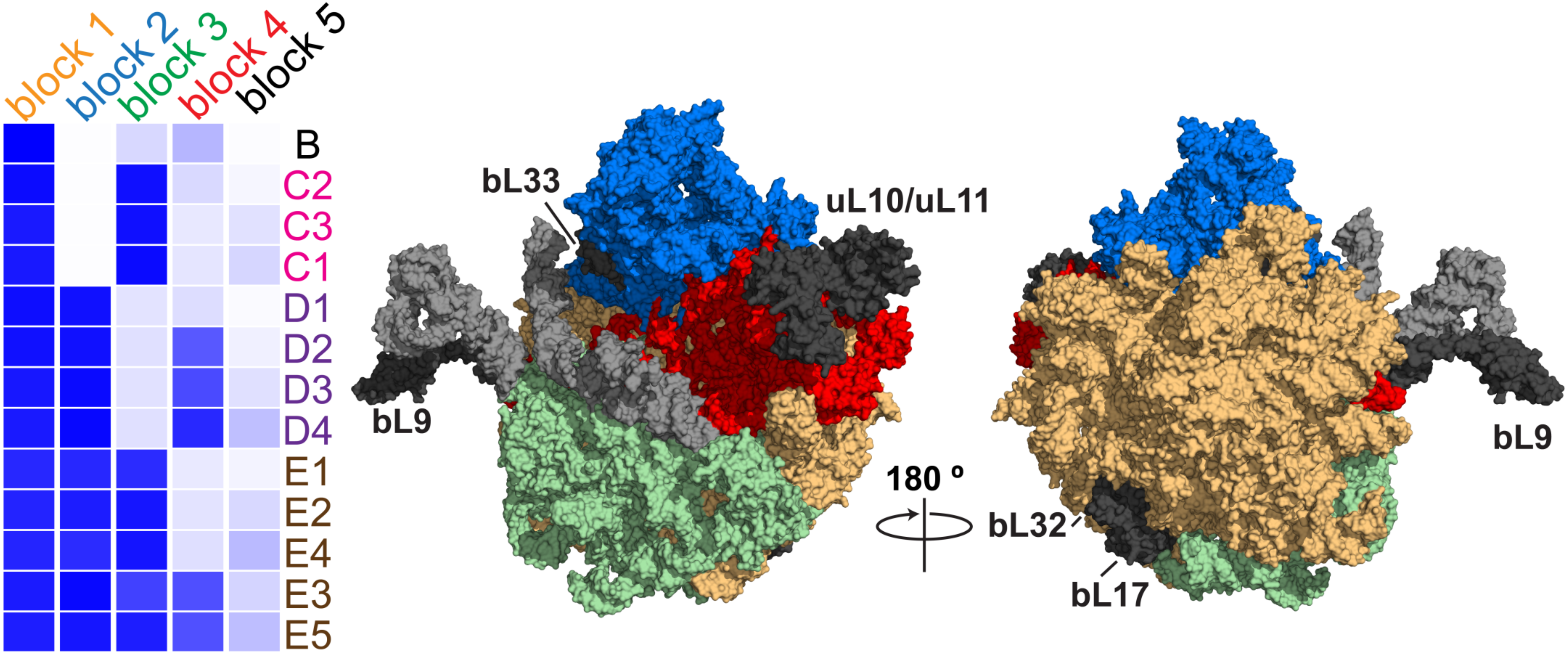
**Davis, *et al.* Cell 2016**

# helix (RNA) and protein occupancy differs between maps



- Occupancy calculated across all proteins/helices and intermediates.
- Occupancy map can be simplified to 'blocks' using the median value.

# Folding blocks co-localize on tertiary structure

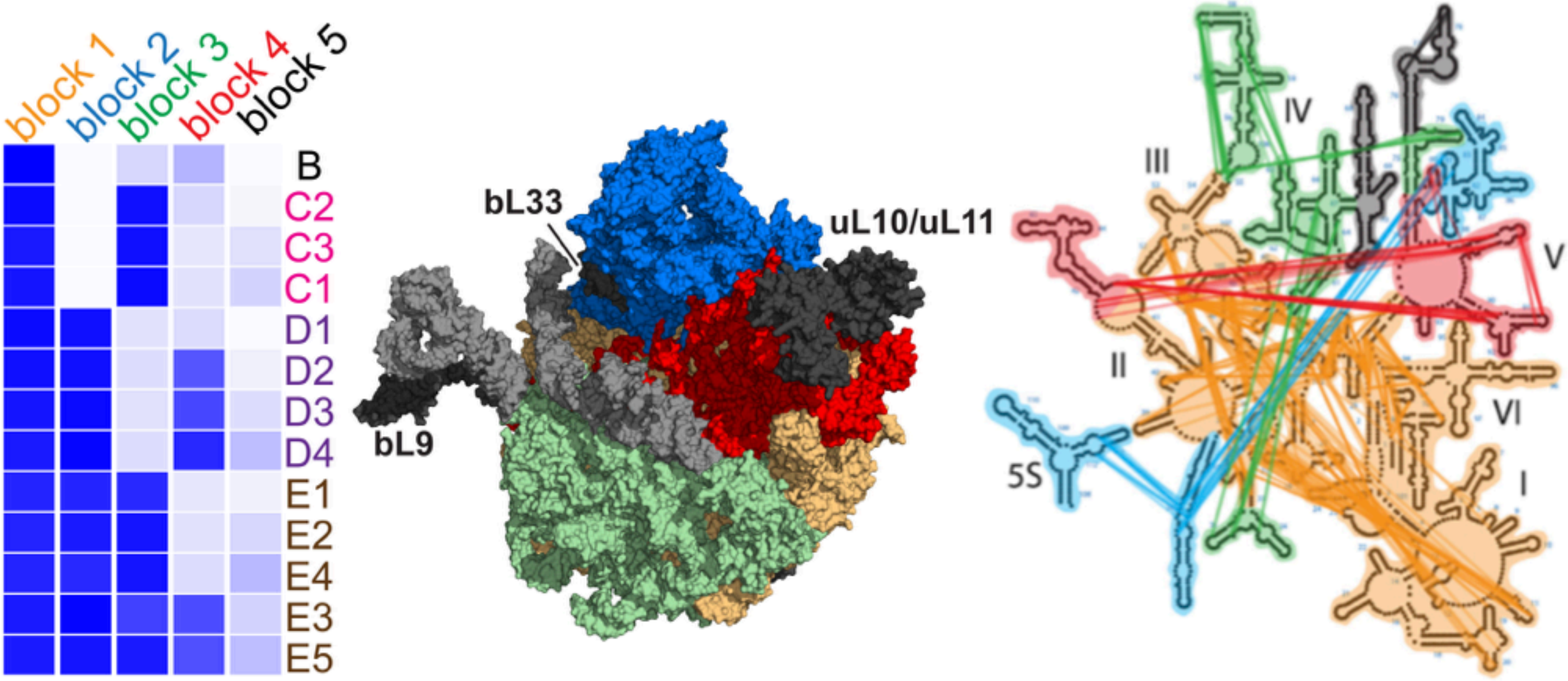


- **Blocks co-localize on tertiary structure and identify folding domain boundaries.**

Slide by Joey Davis

**Davis, *et al.* Cell 2016**

# Folding blocks co-localize on tertiary structure … but not in sequence space



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**Davis, *et al.* Cell 2016**

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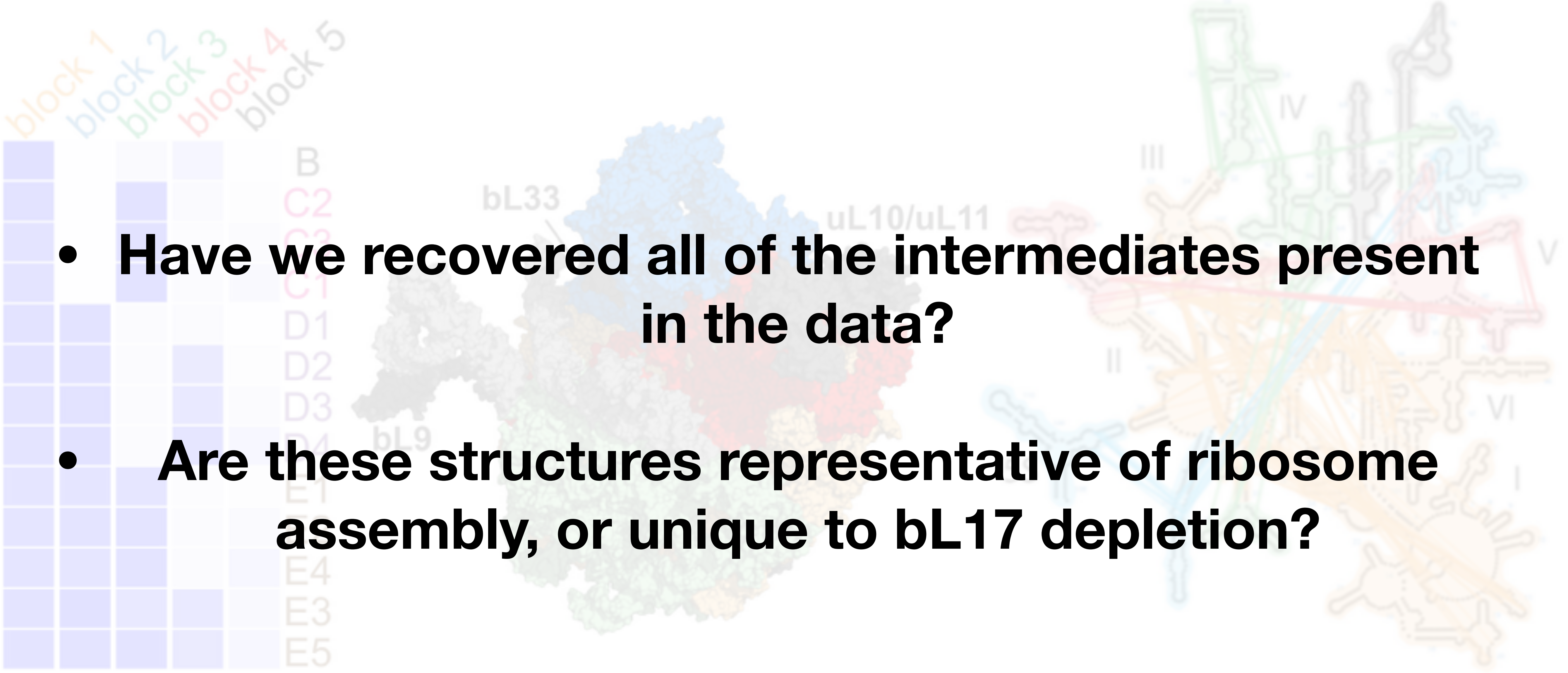


- **Have we recovered all of the intermediates present in the data?**

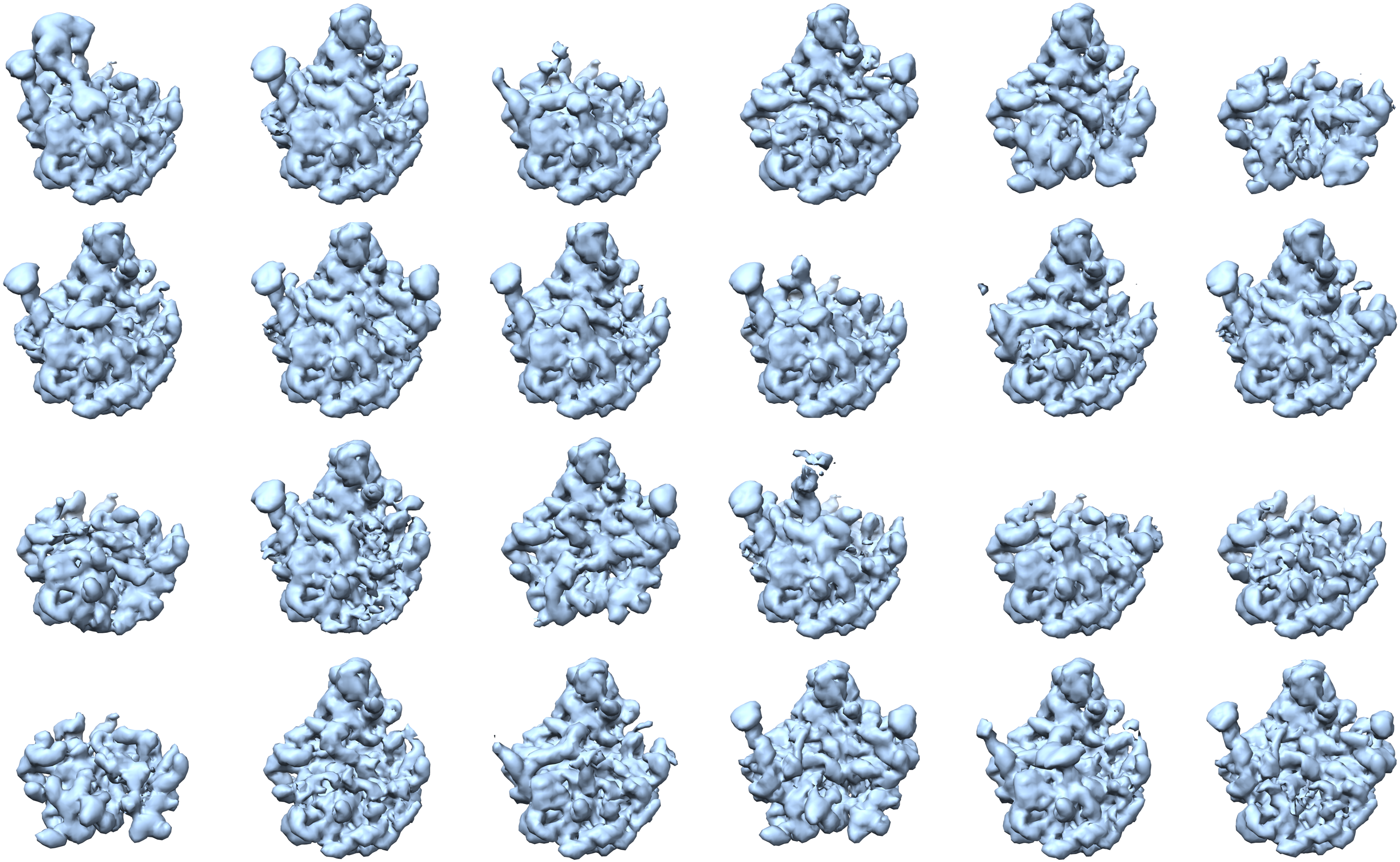
- **Are these structures representative of ribosome assembly, or unique to bL17 depletion?**

- Blocks co-localize on tertiary structure and identify folding domain boundaries.

Slide by Joey Davis

Davis, *et al.* Cell 2016

# Have we recovered all of the intermediates present in the data? No.

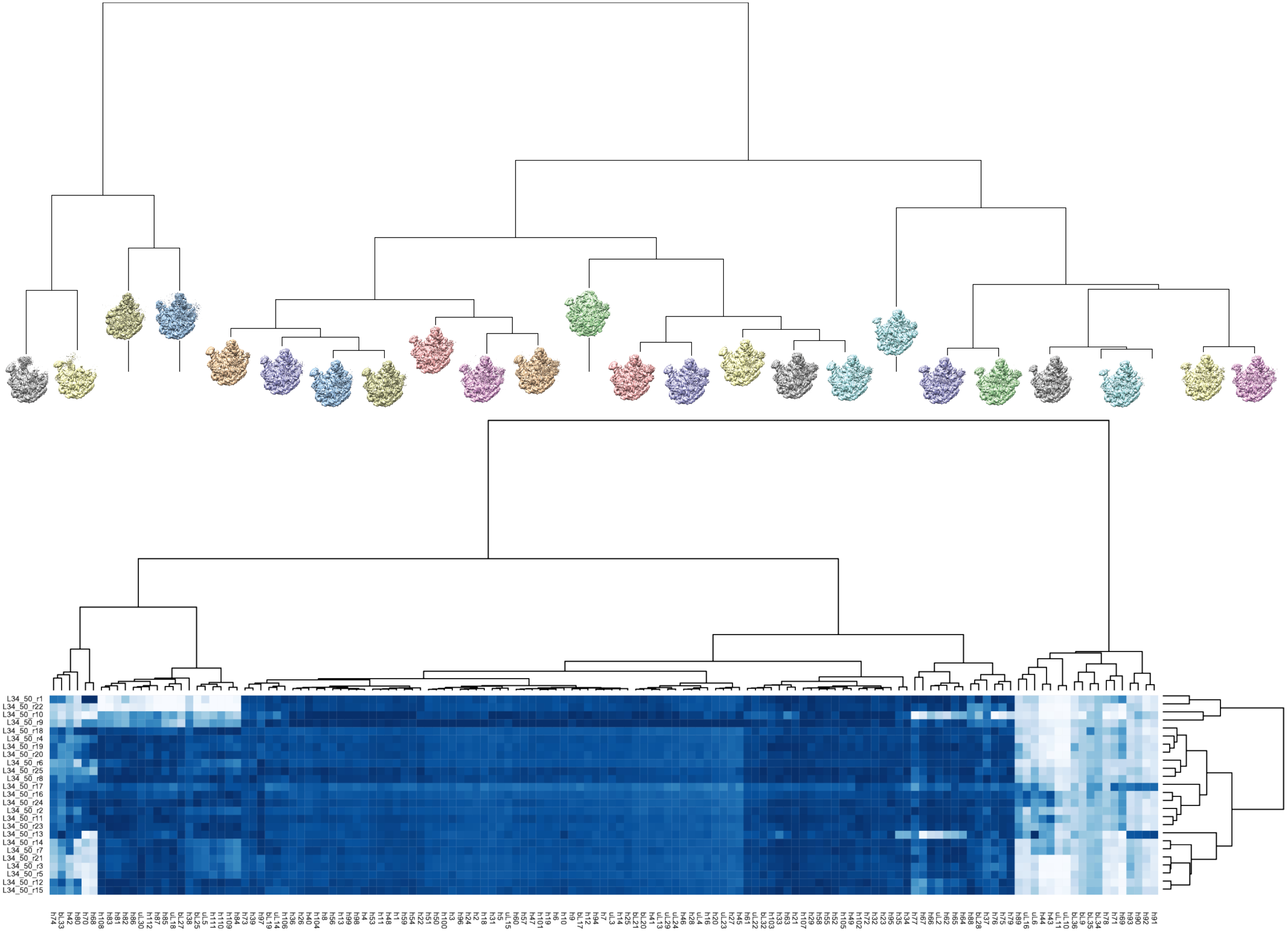


Slide by Dmitry Lyumkis

# State of current library of protein depletion strains

| Dataset | MotionCorr/ CTF/etc | Initial 2D classification | Making a stack | gCTF | Relion 2D Classification | Relion 3D Classification | Frealign /Occ. Analysis | Hi-Res Model |
|---|---|---|---|---|---|---|---|---|
| L17 | + | + | + | + | + | + | + | IP |
| L28 | + | + | + | + | + | + | + | IP |
| L32 | + | + | + | + | + | + | + | IP |
| L34 | + | + | + | + | + | + | + | IP |
| L19 | + | | | | | | | |
| L36 | + | | | | | | | |
| L35 | | | | | | | | |
| L33 | | | | | | | | |

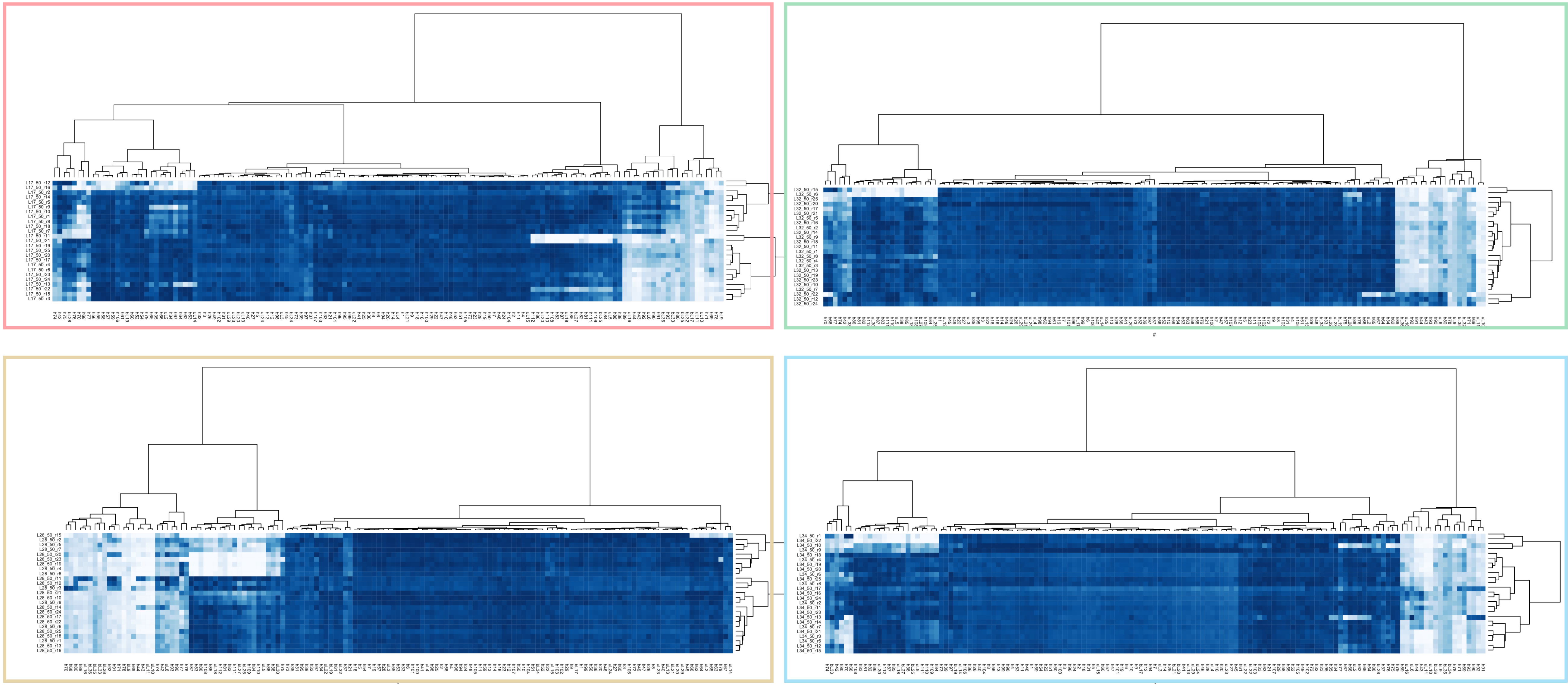
# L34 depletion, FrealignX 25-model single-particle classification



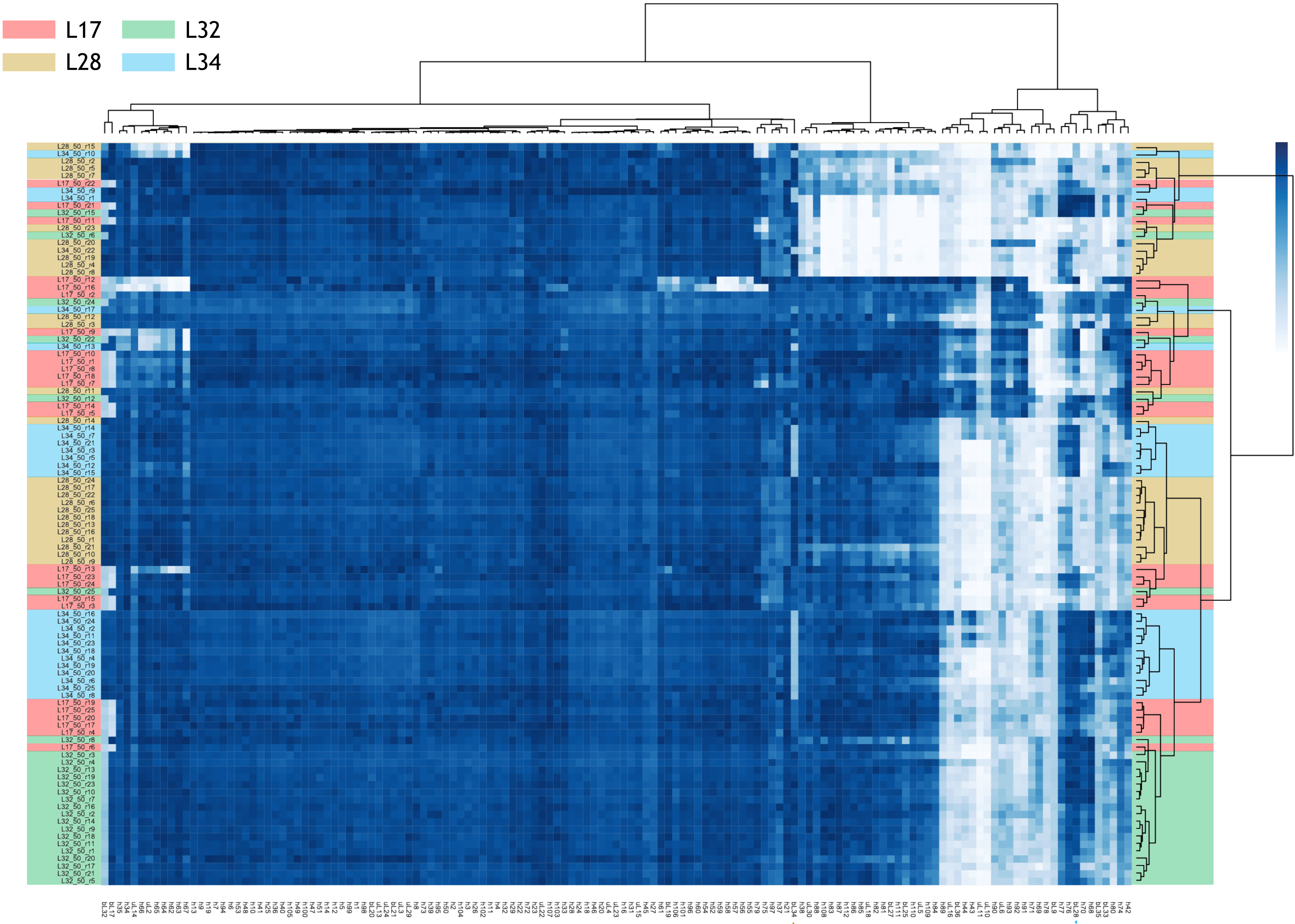
# Occupancy analysis across strains

🟥 L17 🟩 L32

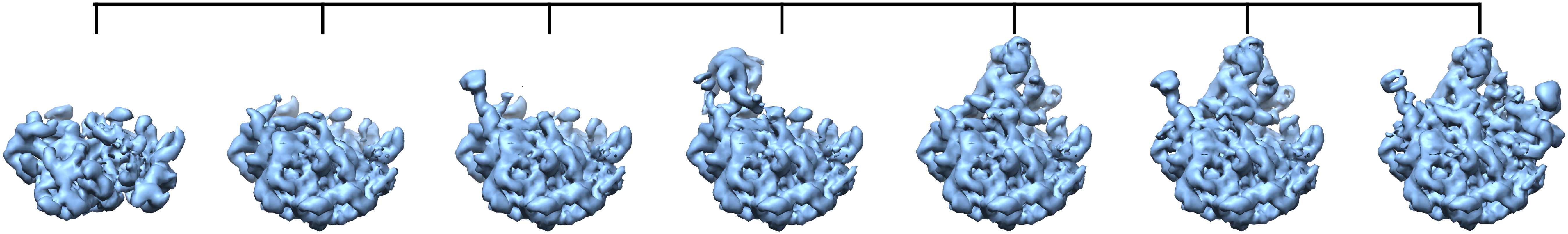
🟨 L28 🟦 L34



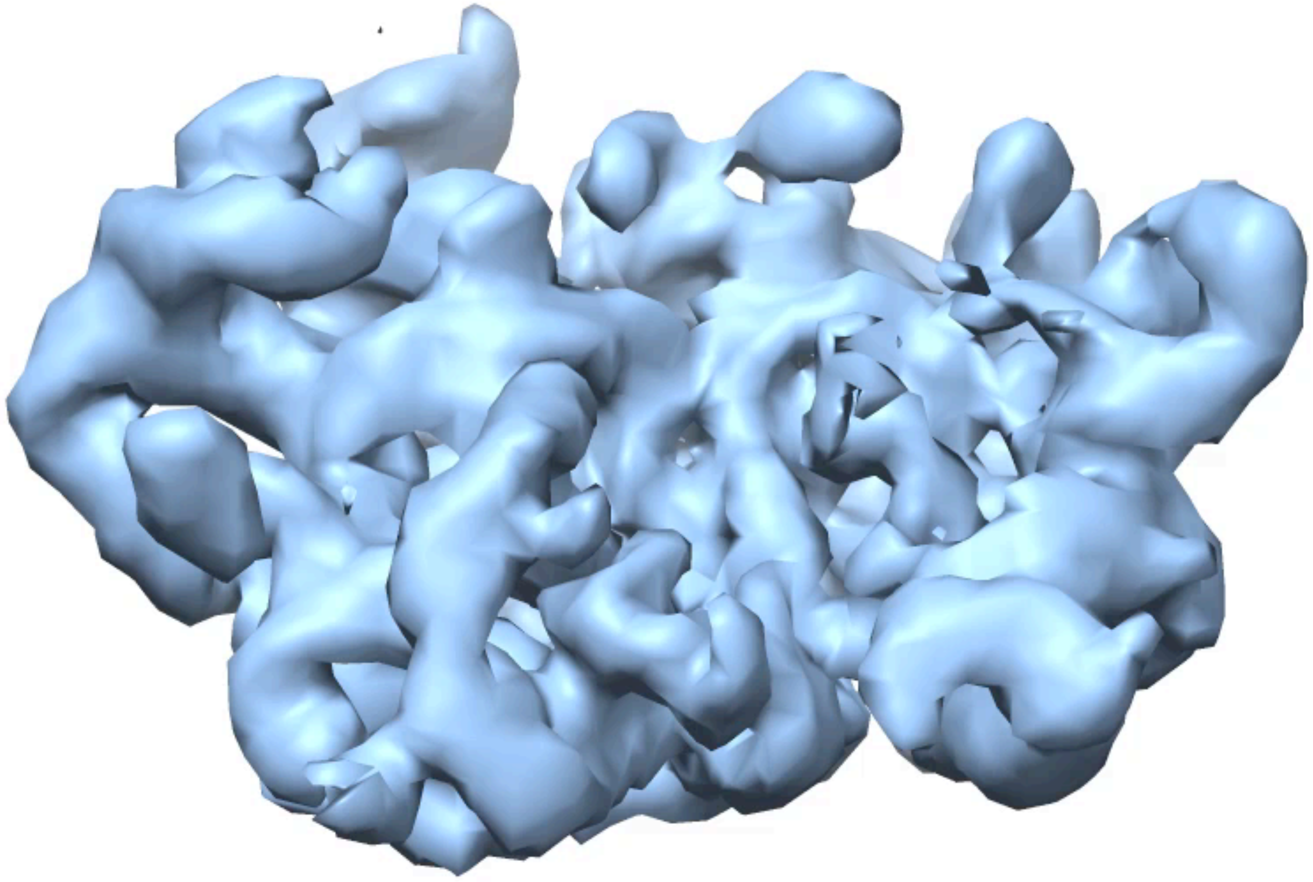
# L17, L28, L32, L34 depletions, combined!



# Harnessing cryo-EM to study macromolecular assembly



active assembly: a different way of thinking about macromolecular structure!



Slide by Dmitry Lyumkis

# Harnessing cryo-EM to study macromolecular assembly

- Challenges for cryoEM analysis
  - Careful classification strategies are needed
  - When are you done classifying?
  - How to determine statistically significant differences between intermediates?

# Acknowledgements

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**Helmsley Foundation**

# Harnessing cryo-EM to study macromolecular assembly

- Challenges for cryoEM analysis

  - Careful classification strategies are needed

  - When are you done classifying?

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