

Visualizing Ribosome Assembly

*Time-Lapsed Single Particle EM of 30S Ribosome
Subunit Assembly*

November 9, 2009

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Automated Molecular Imaging Group

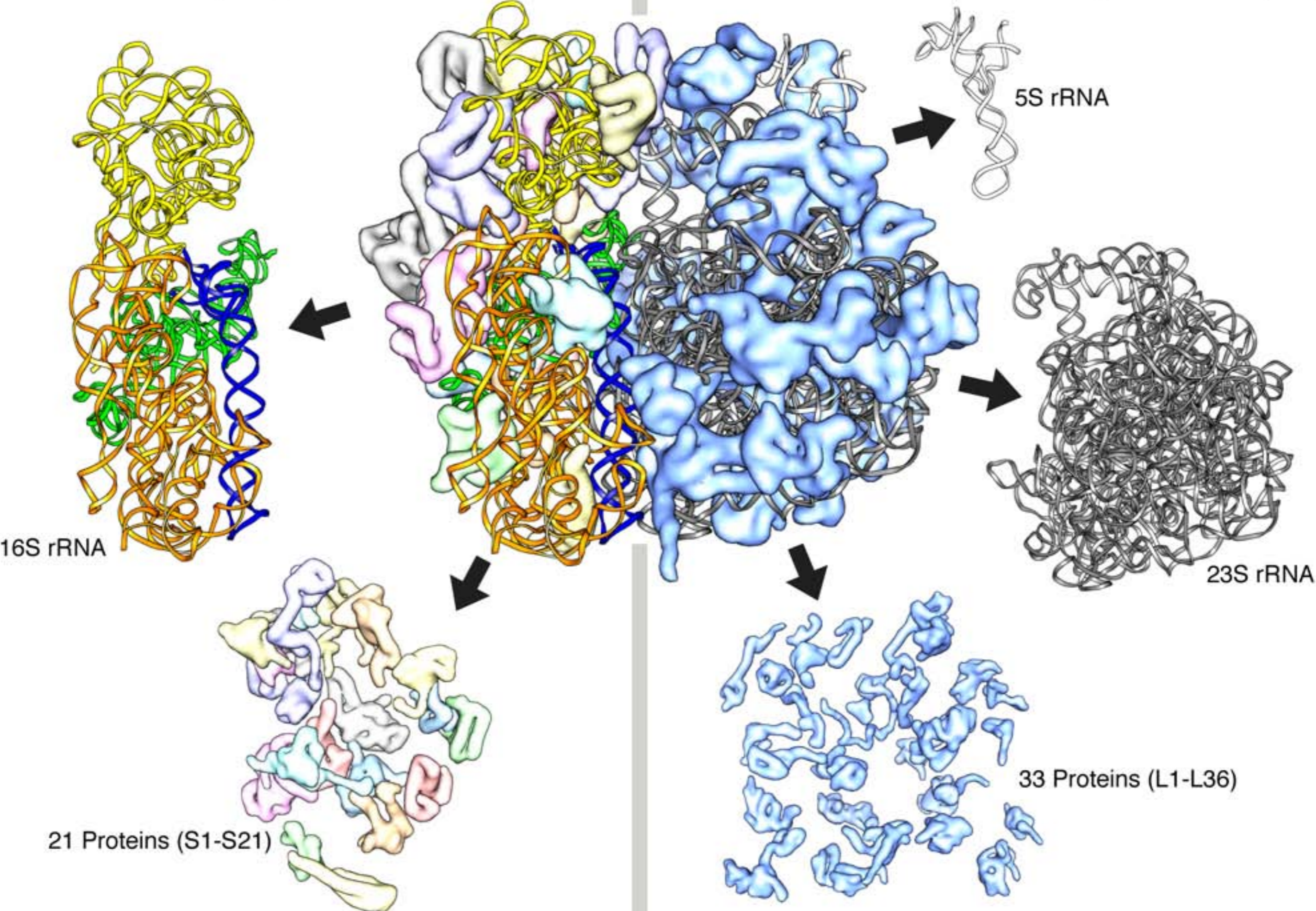
Milligan Laboratory

The Scripps Research Institute, La Jolla, CA

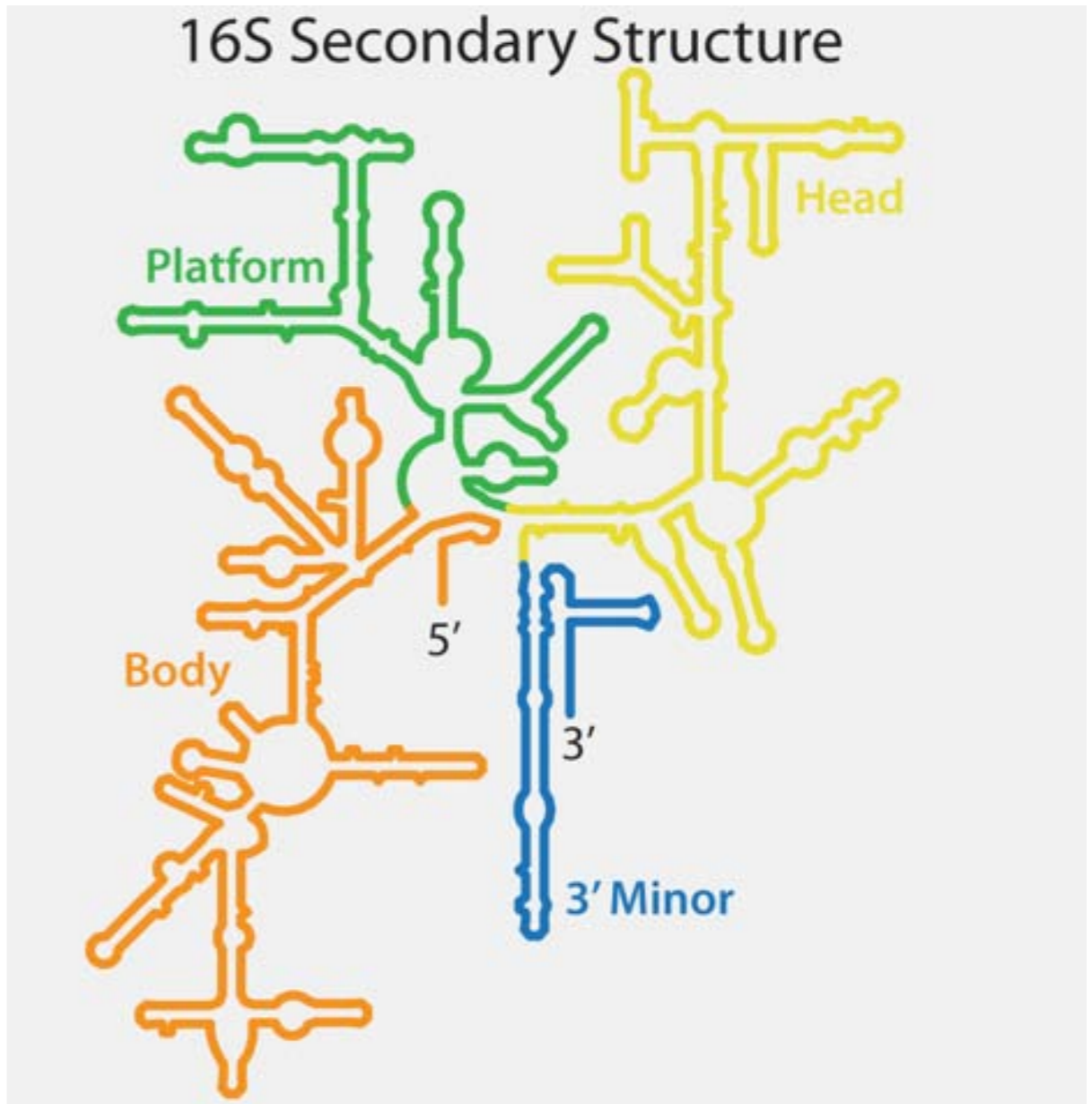
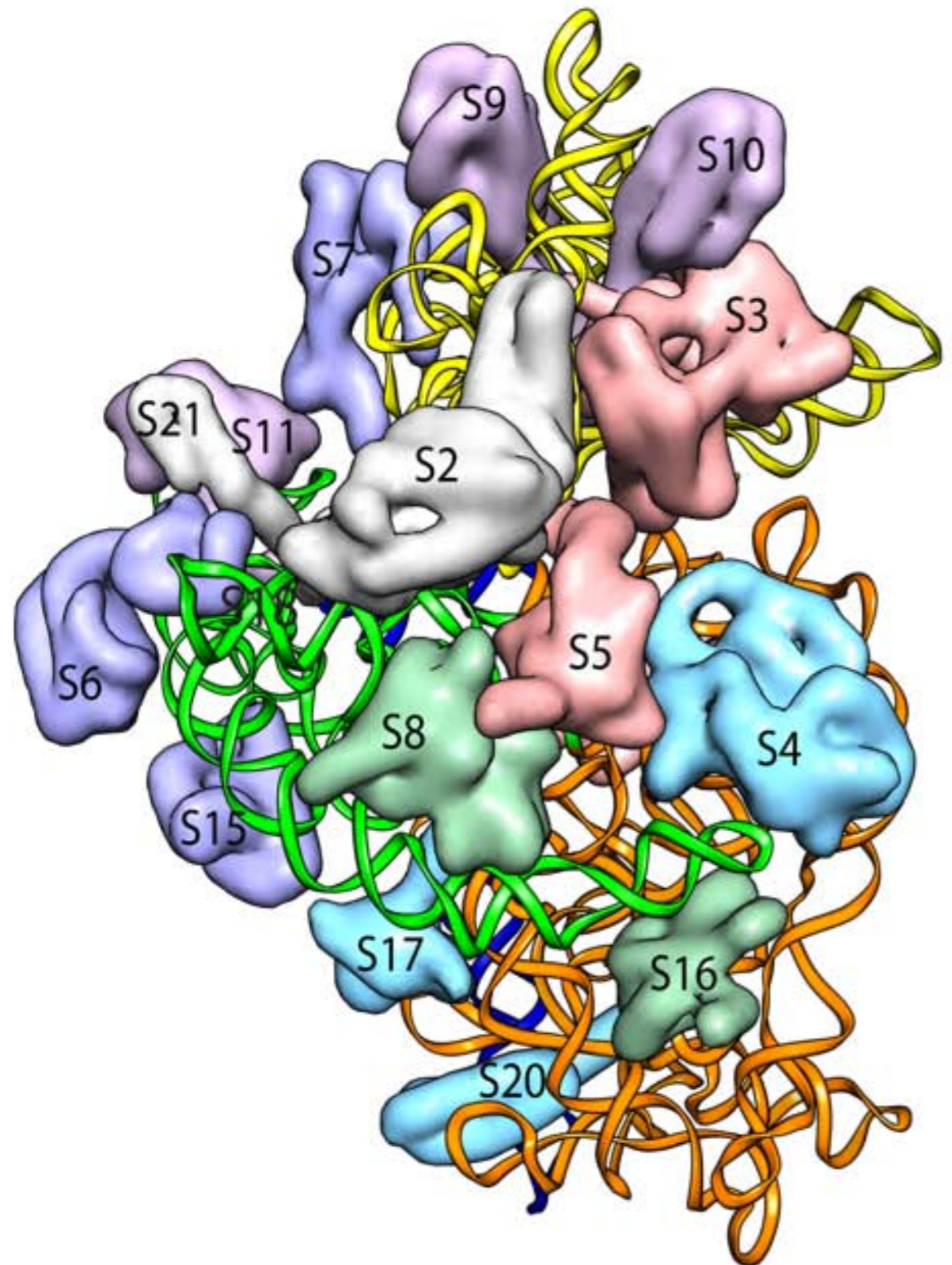
Ribosomes Review

Small Subunit (30S)

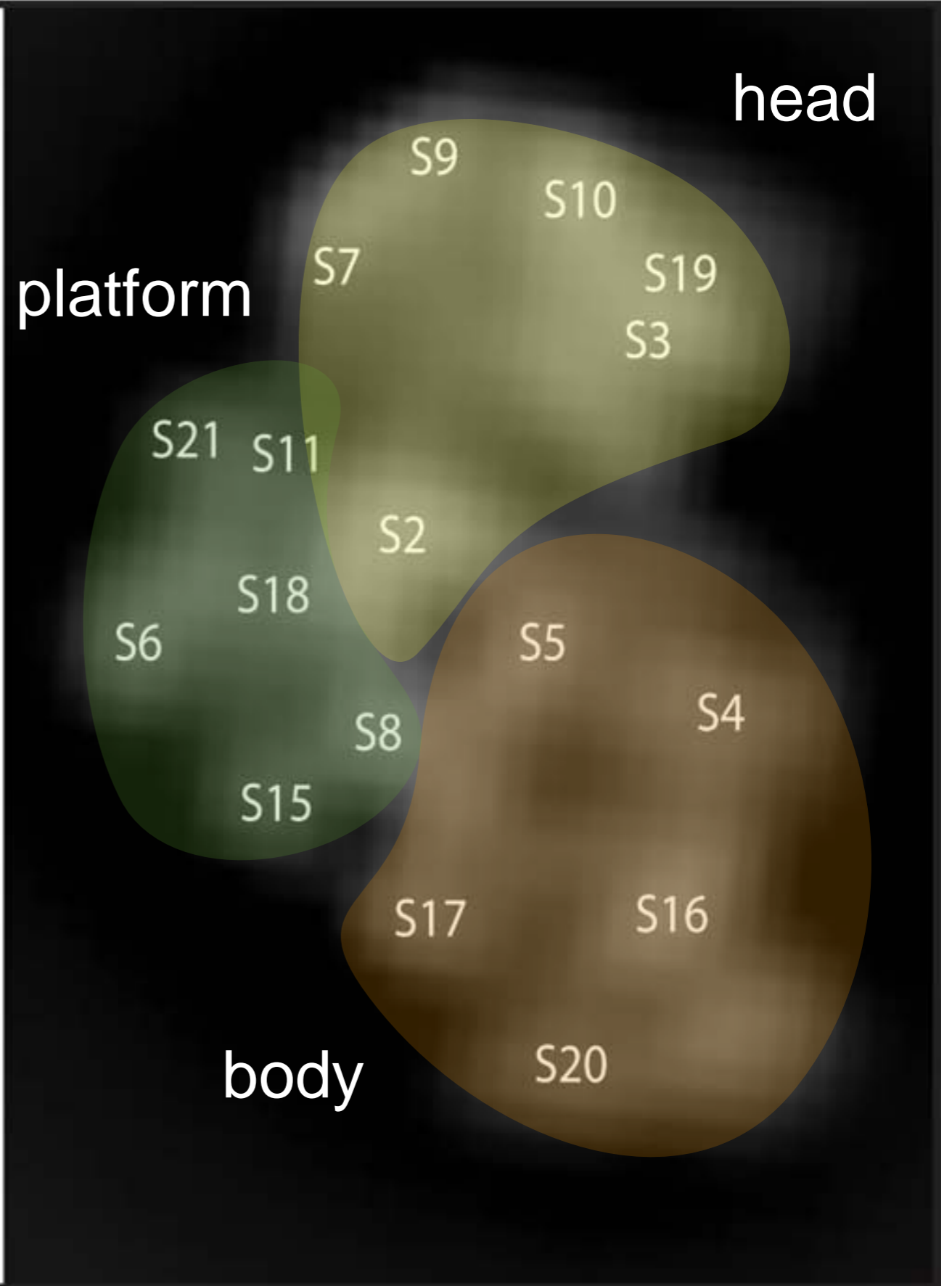
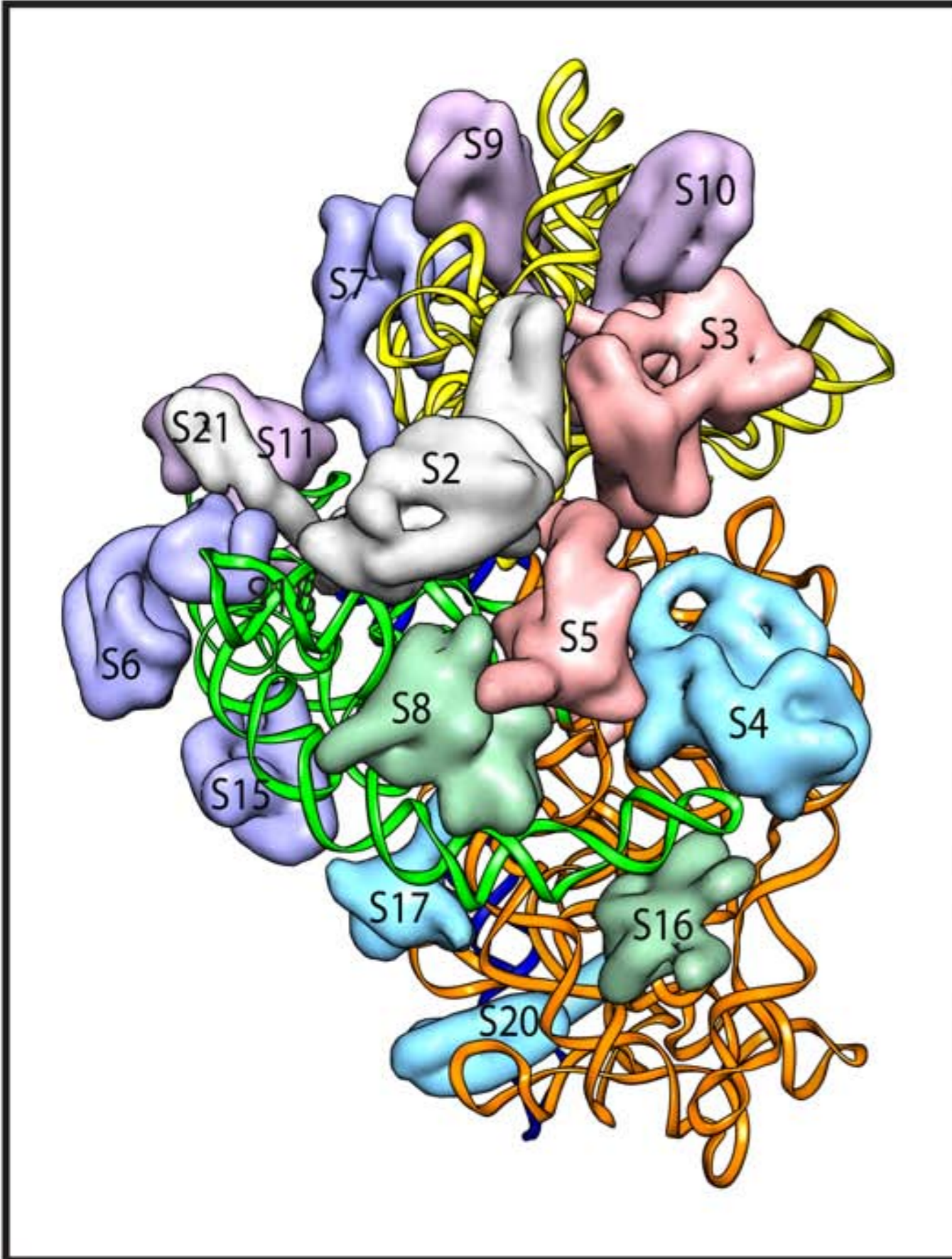
Large Subunit (50S)



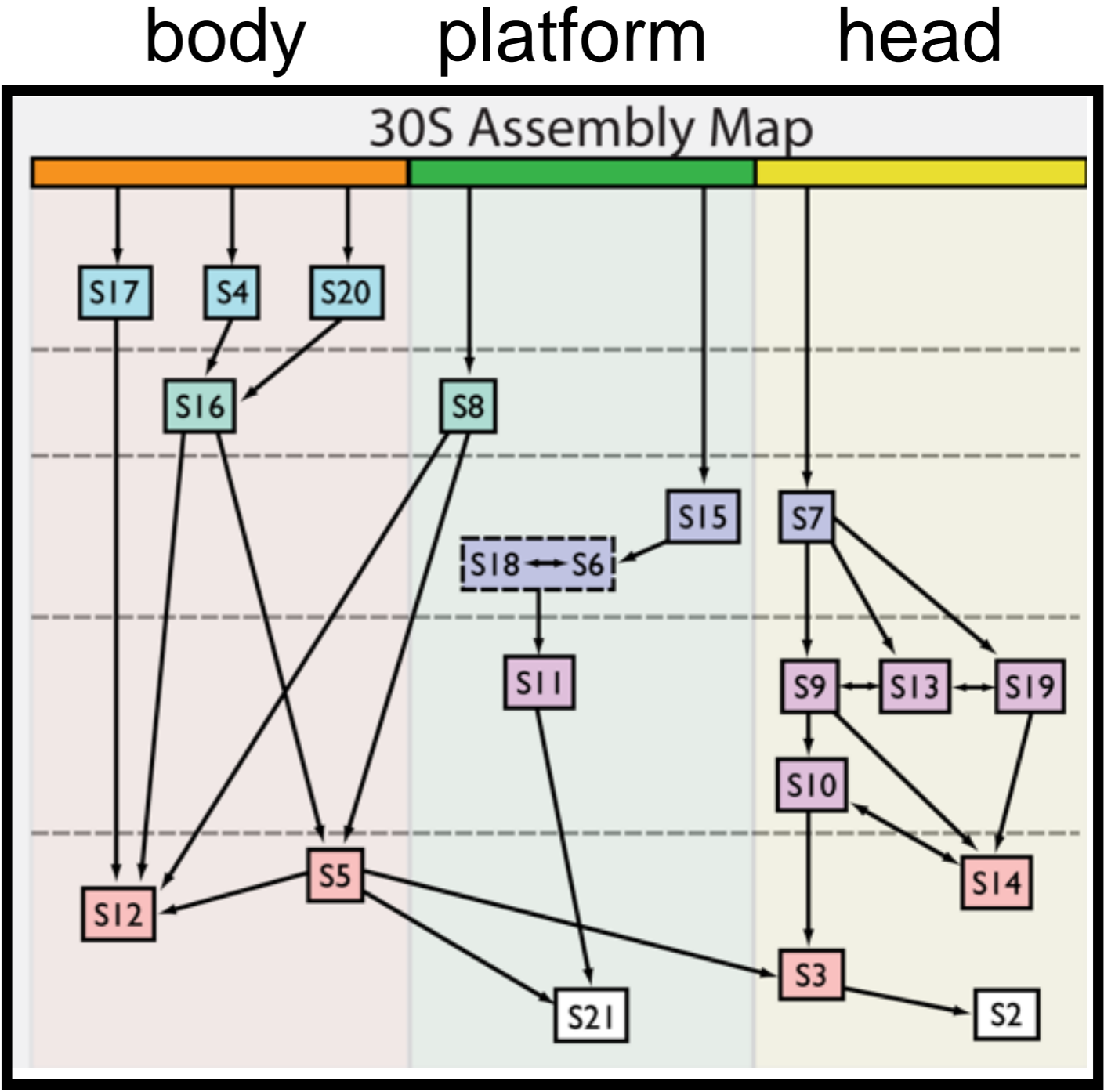
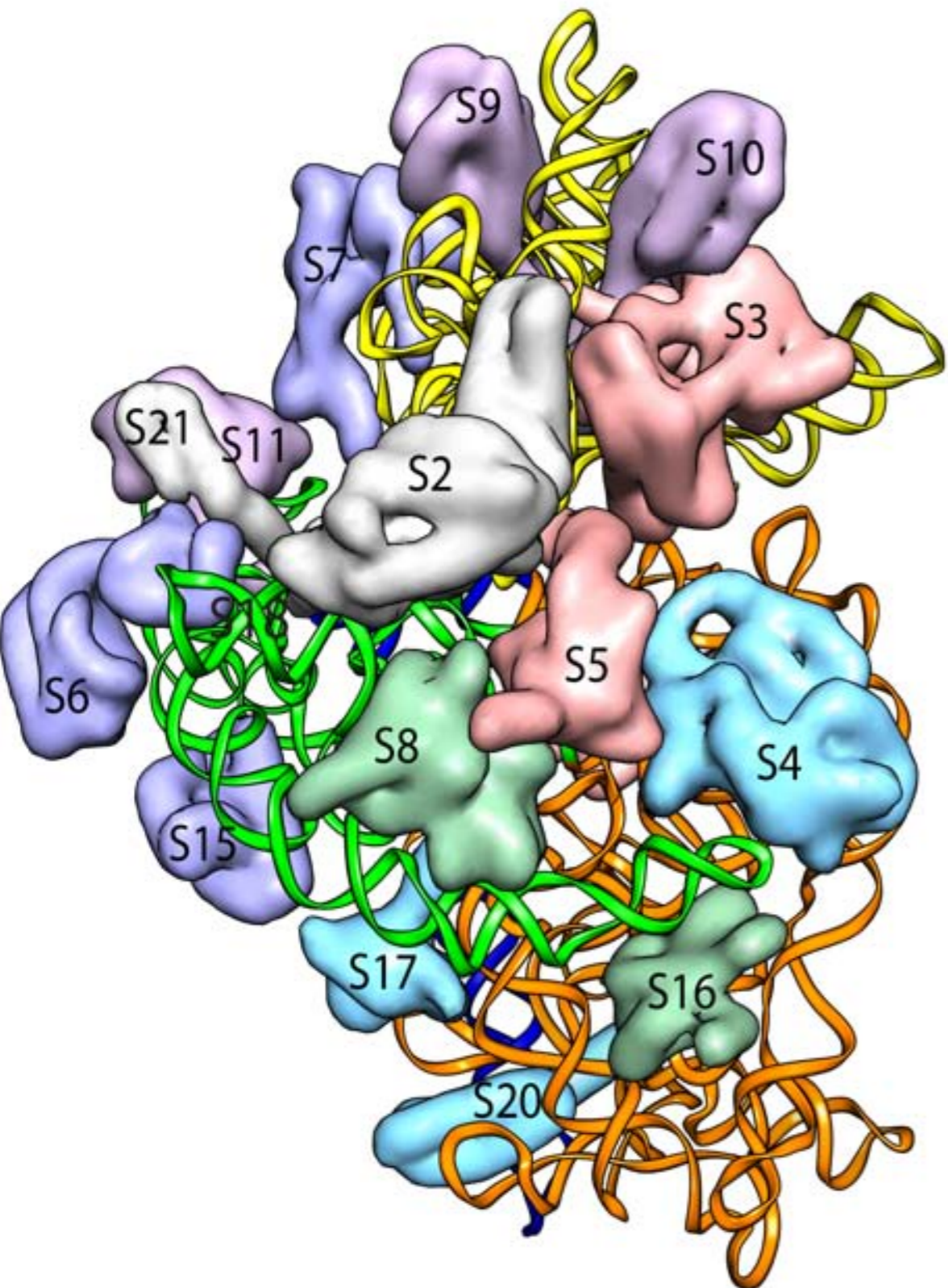
The small, or 30S, subunit of E.coli Ribosome



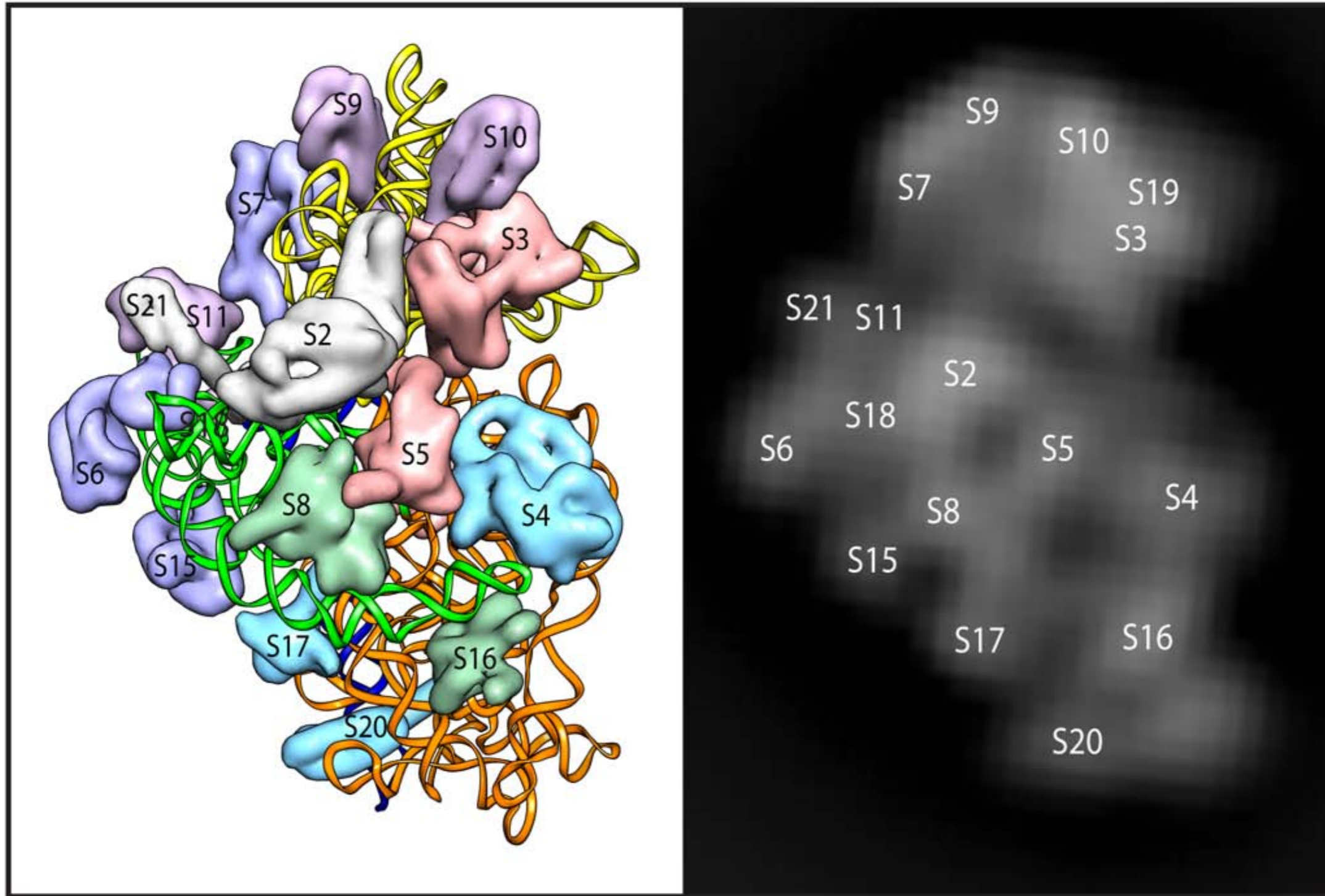
The small, or 30S, subunit of E.coli Ribosome



Assembly Occurs 5' to 3' and is Hierarchical

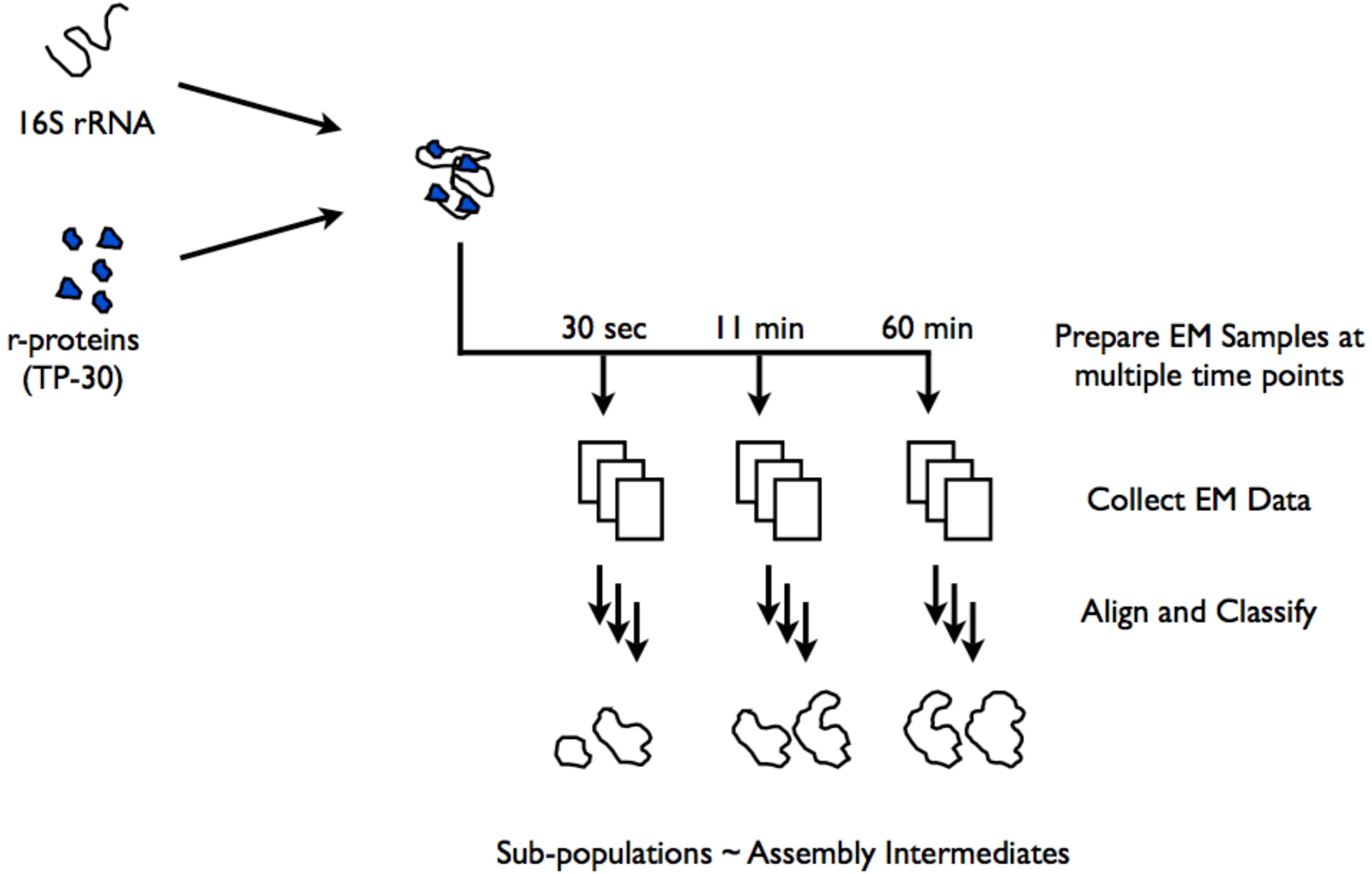


Single Particle EM of 30S Ribosomal Subunits:



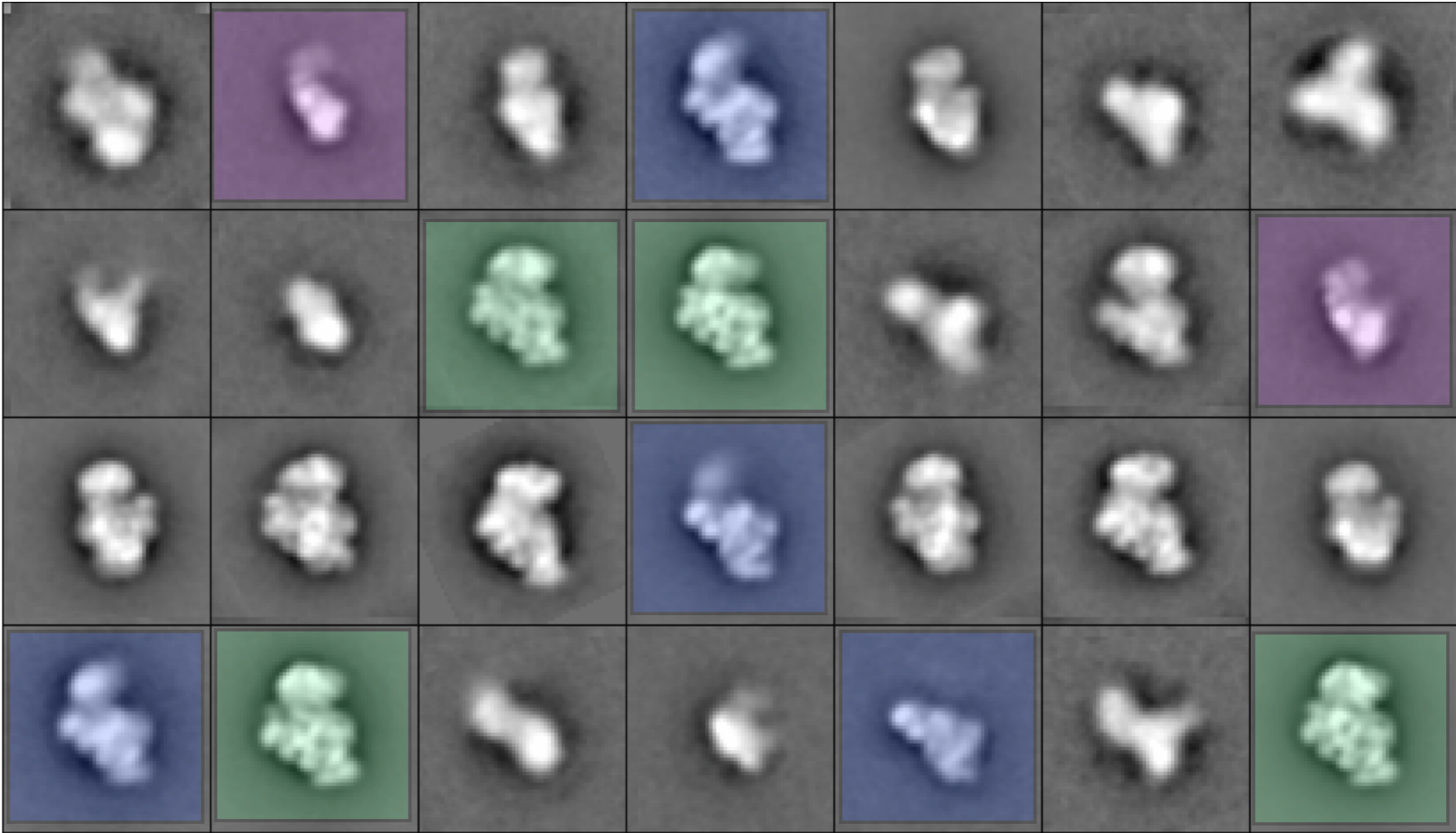
Can we monitor assembly with EM?

Time Lapsed Single Particle EM of 30S Assembly:



Classes at $t = 11$ min Time Point

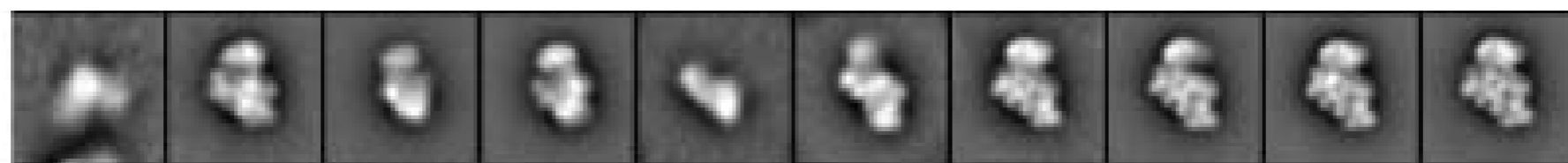
Reference Free Alignment, 30 Classes



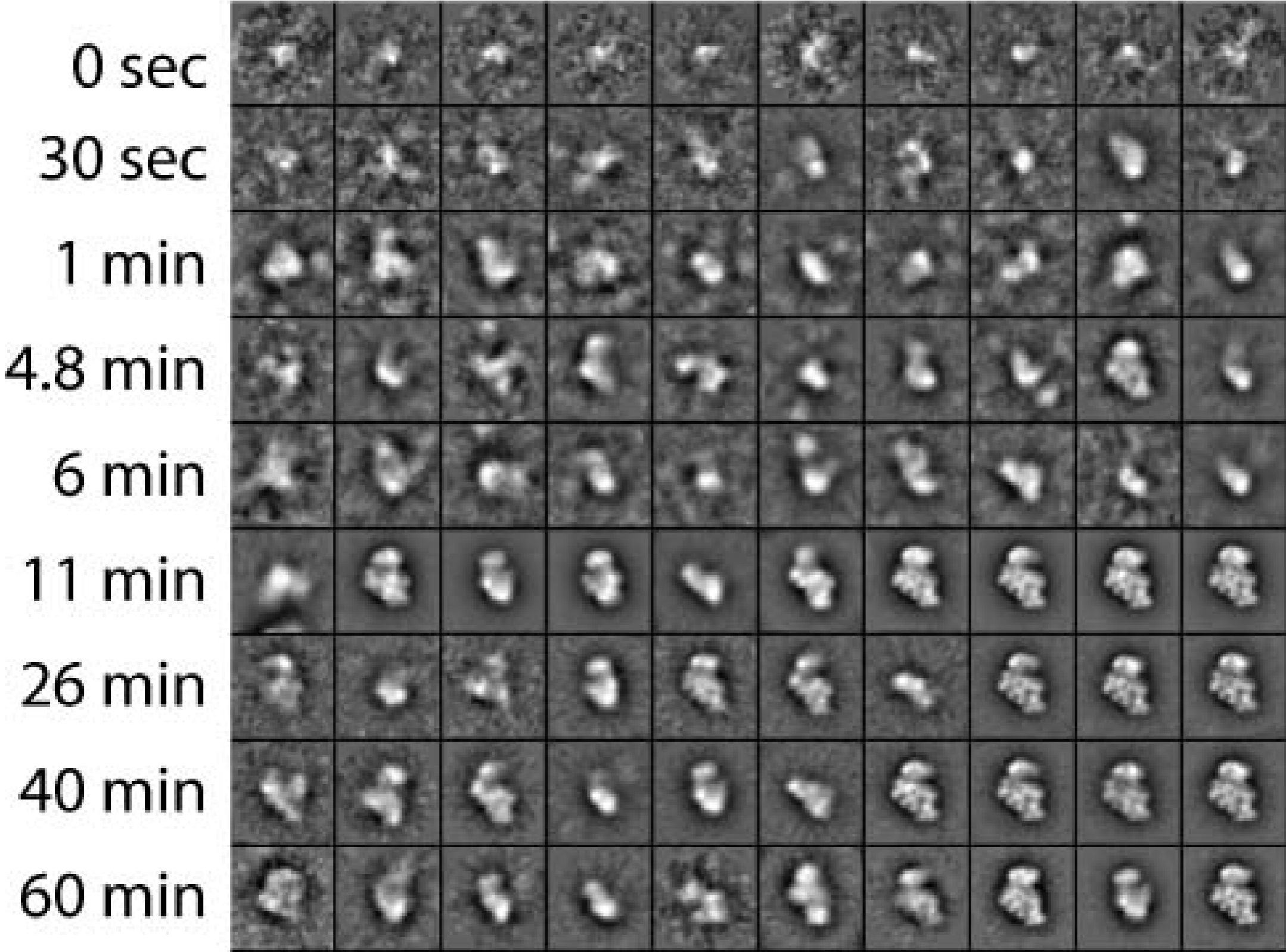
Classes from $t = 11$ min Time Point

Reference Free Alignment, 10 Classes

11 min

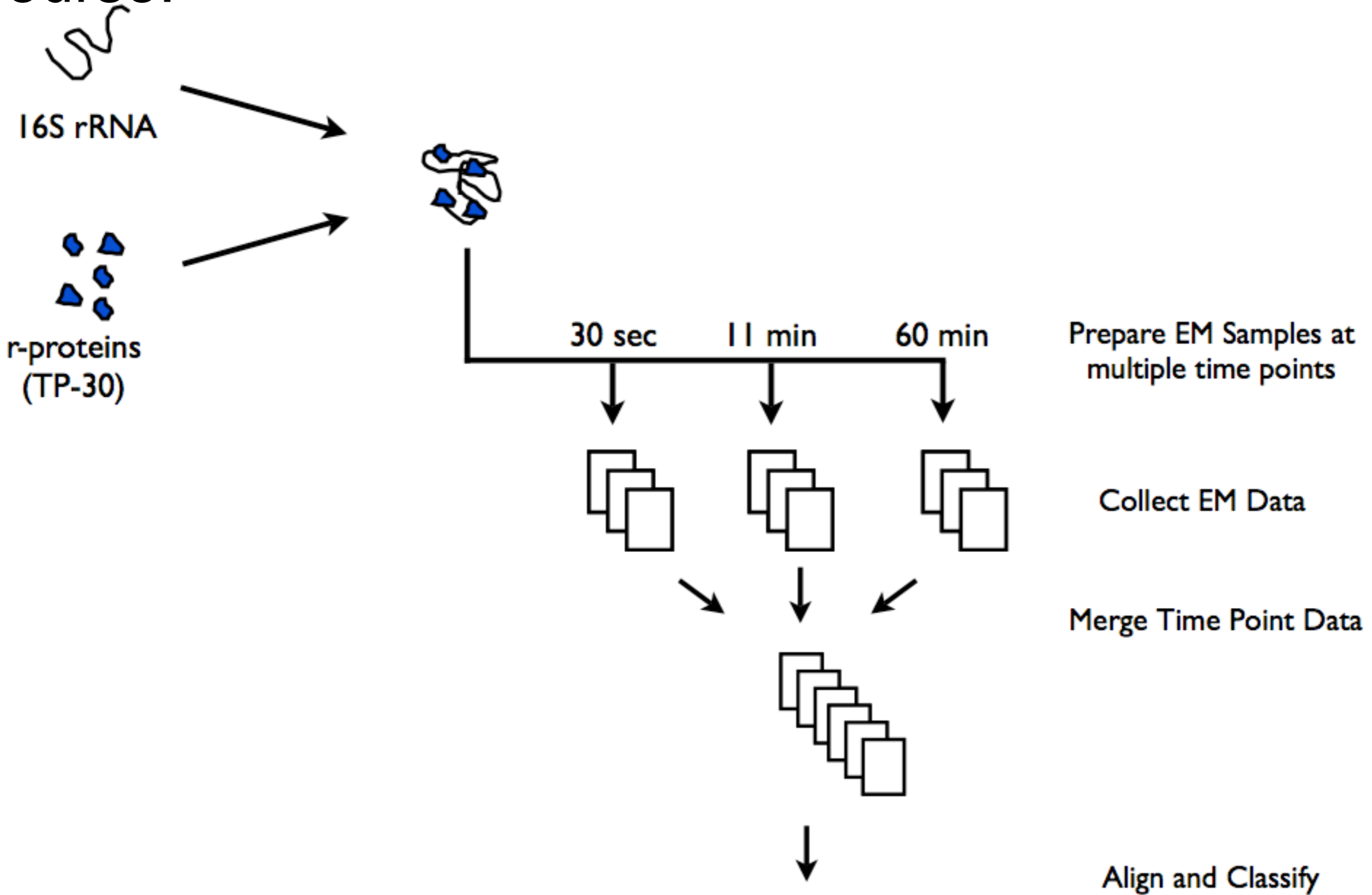


Particle Classes From All Time Points



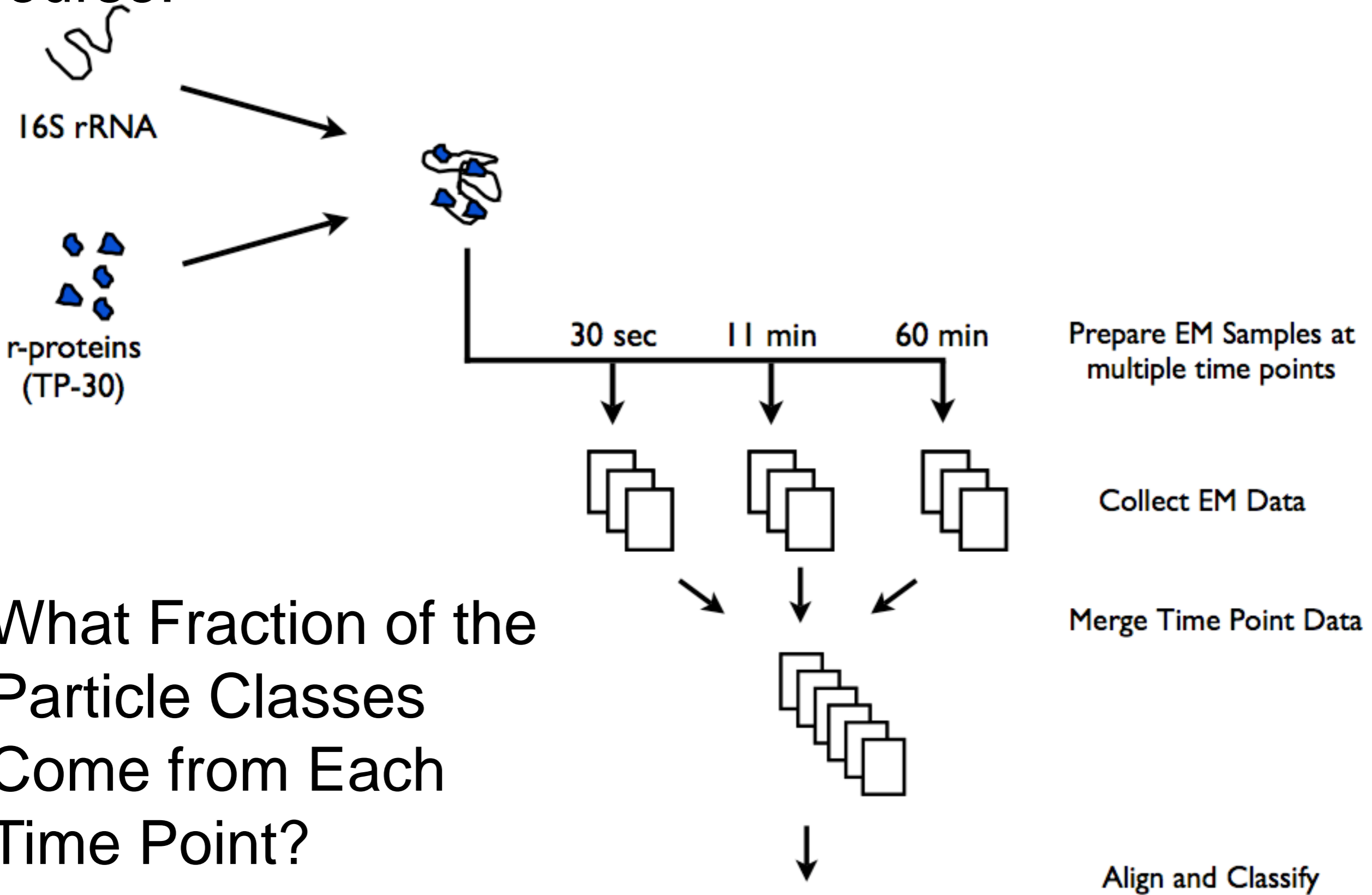
Classification of Particles from the Entire Time Course:

Course:



Classification of Particles from the Entire Time Course:

Course:



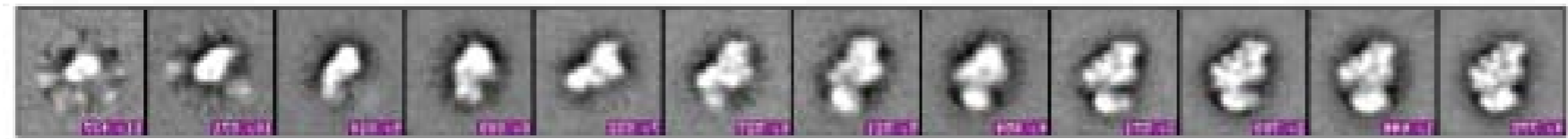
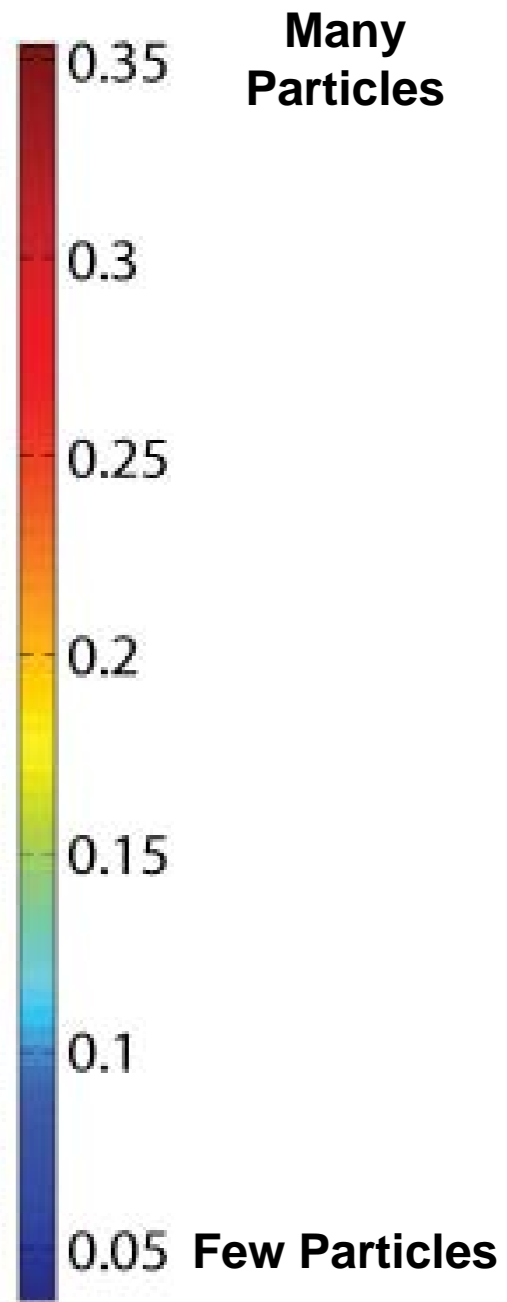
What Fraction of the Particle Classes Come from Each Time Point?

Classification of Particles from the Entire Time Course

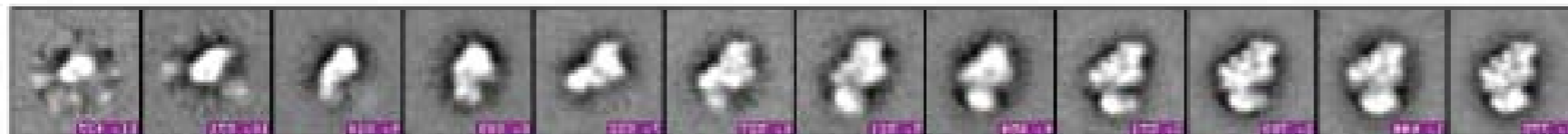
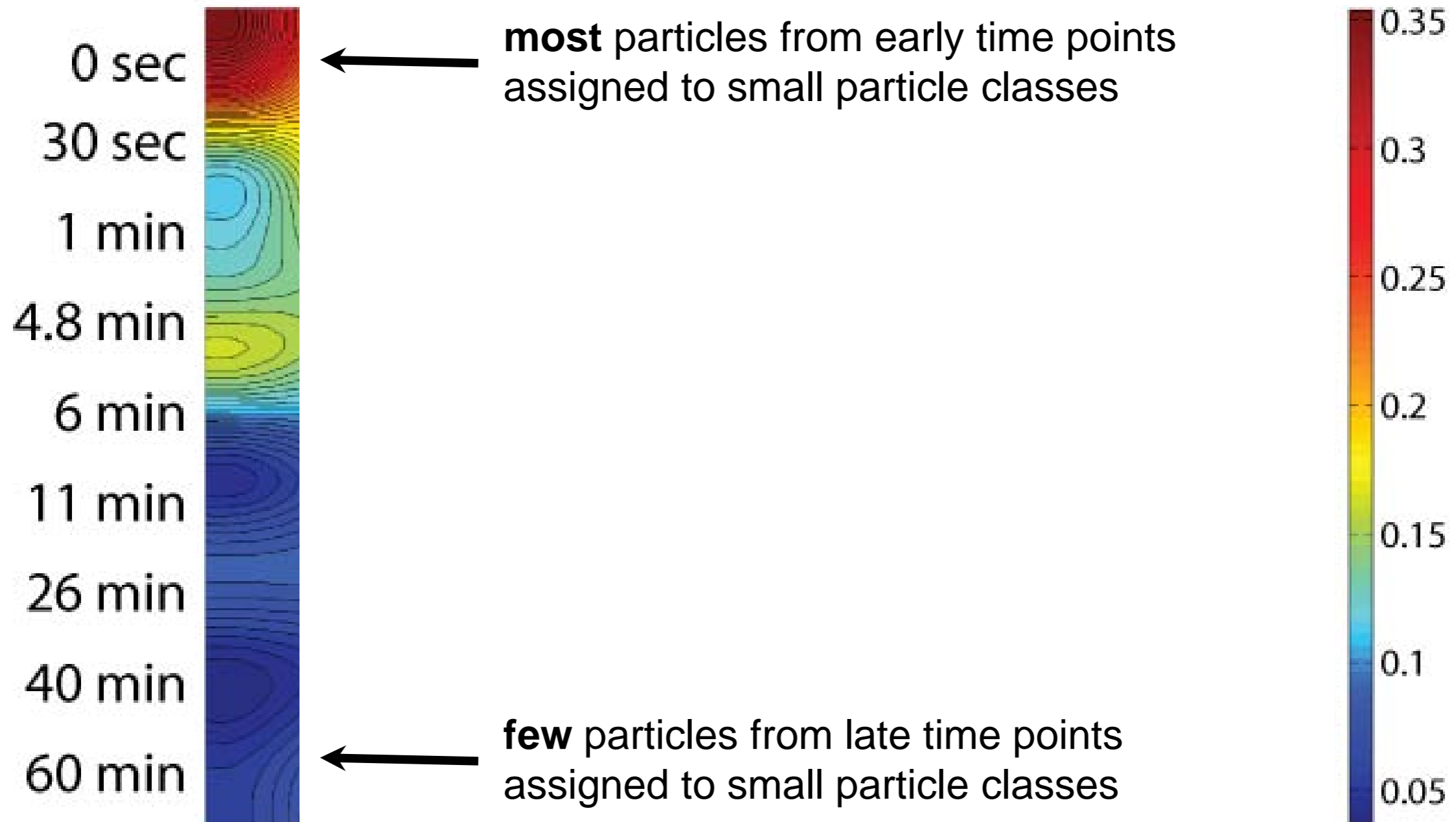


Classification of Particles from the Entire Time Course

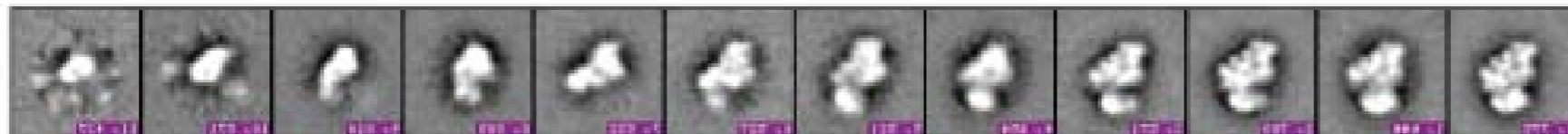
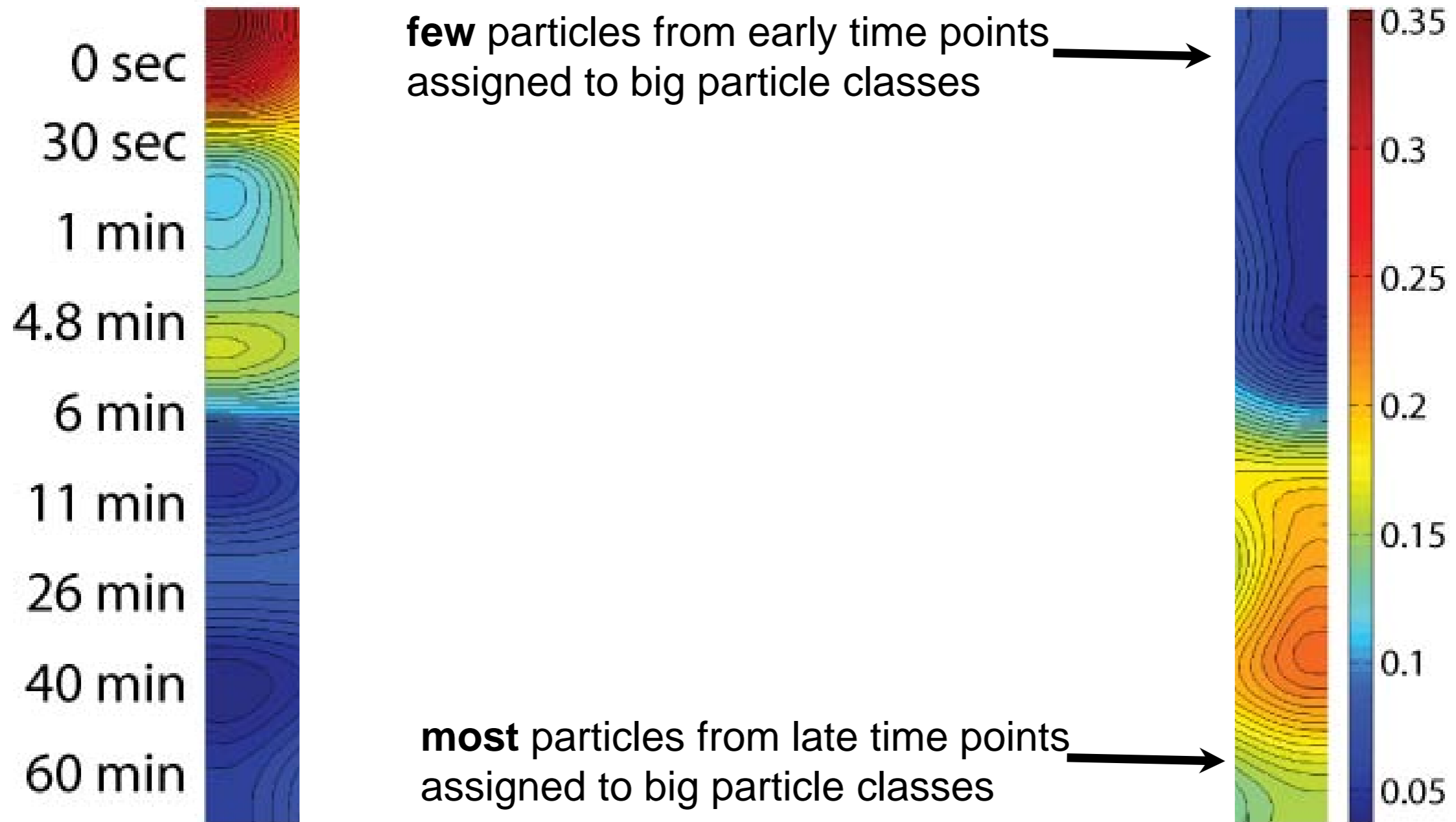
0 sec
30 sec
1 min
4.8 min
6 min
11 min
26 min
40 min
60 min



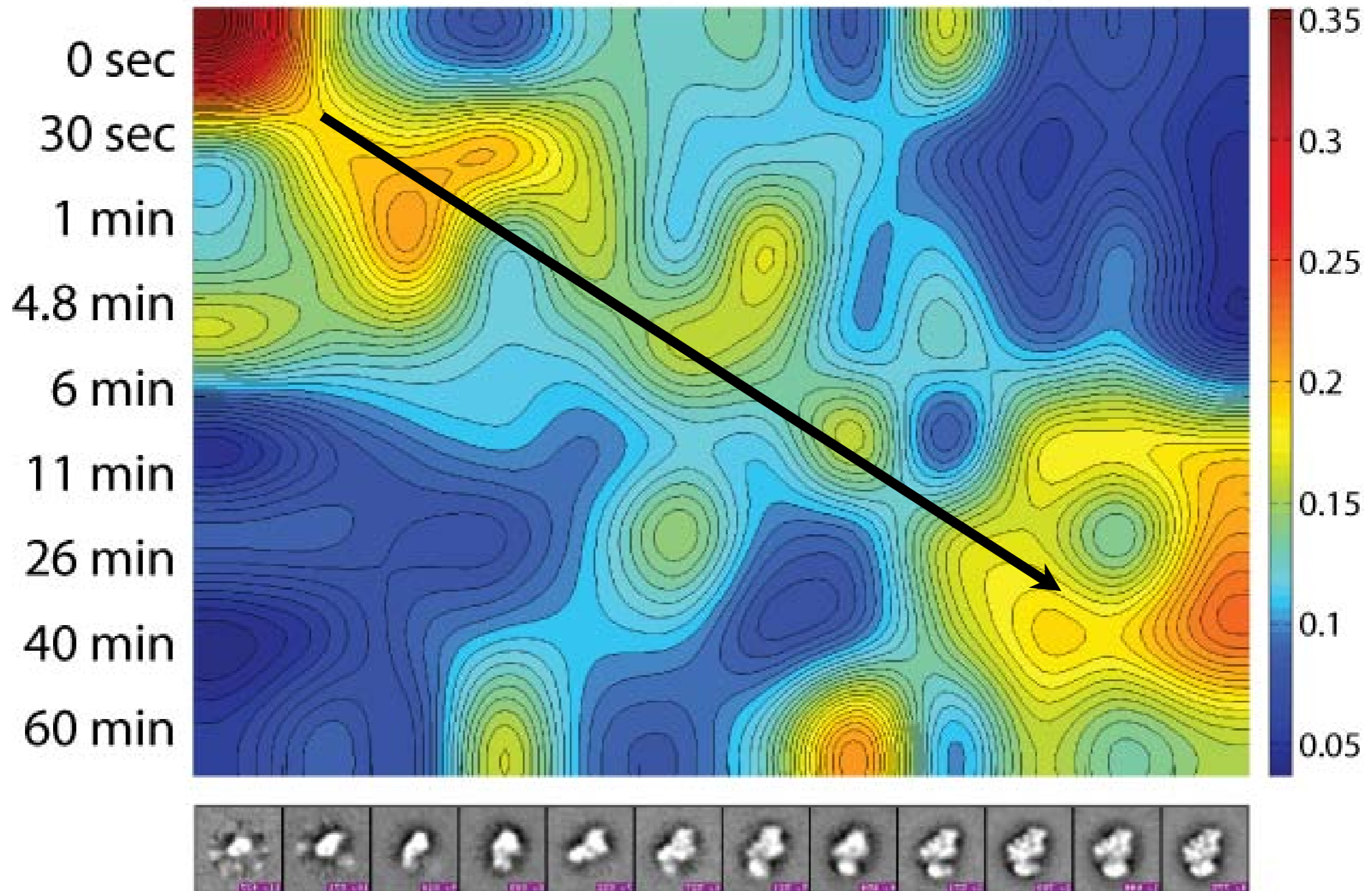
Fraction of Particles in Each Class Derived from Each Time Point



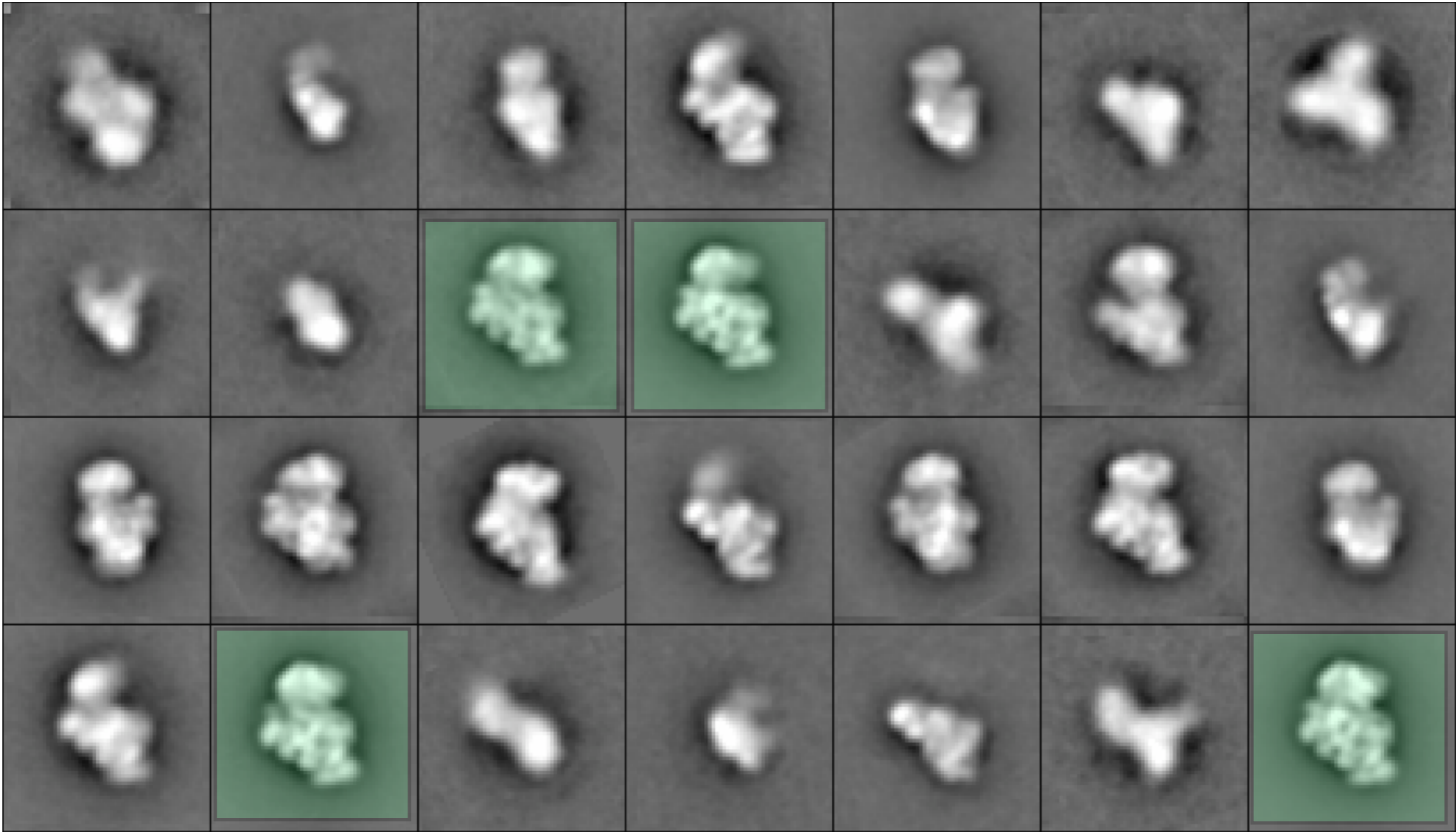
Fraction of Particles in Each Class Derived from Each Time Point



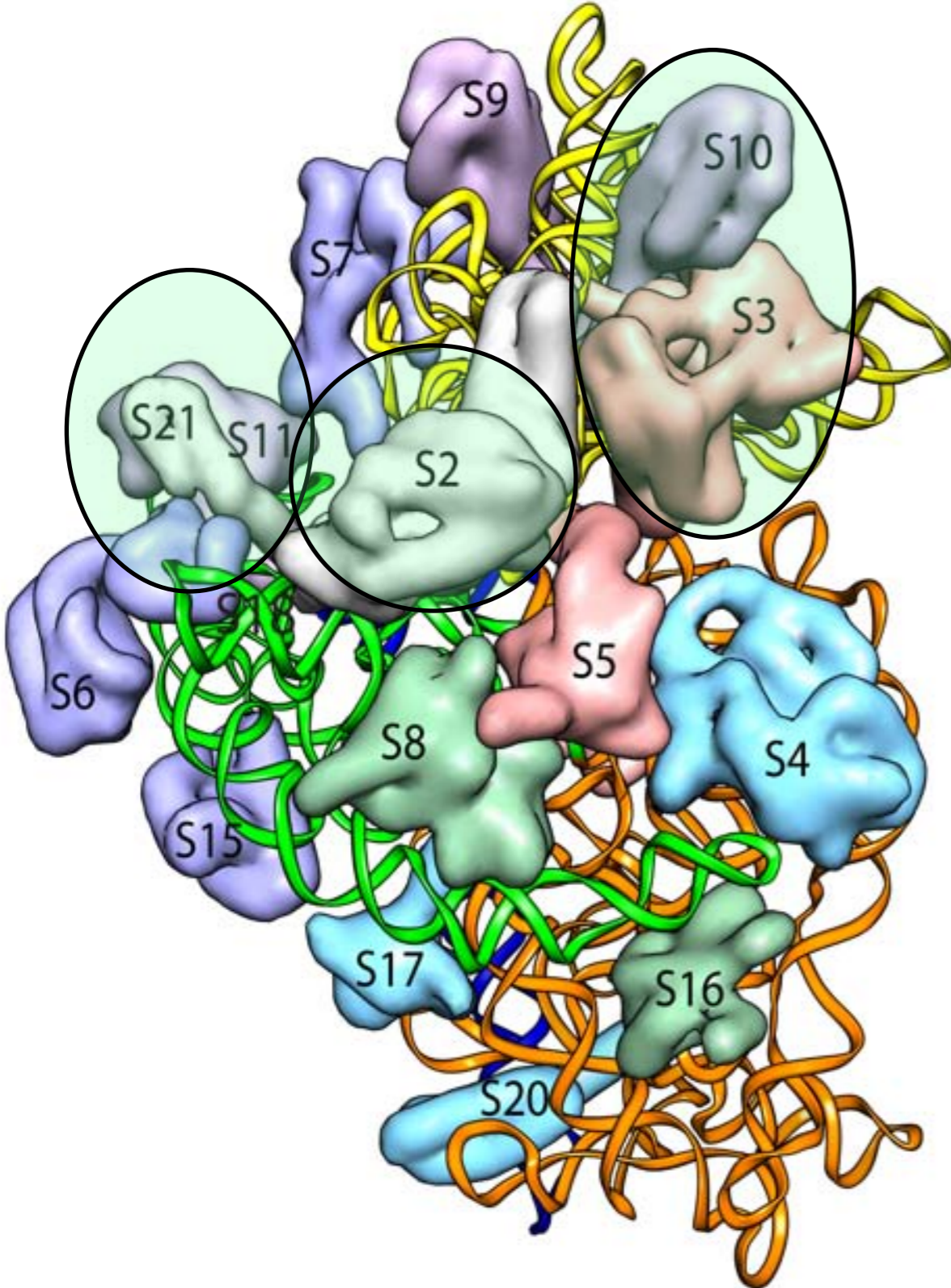
The Kinetics and Populations of Intermediates fall out from Classification of the Entire Dataset



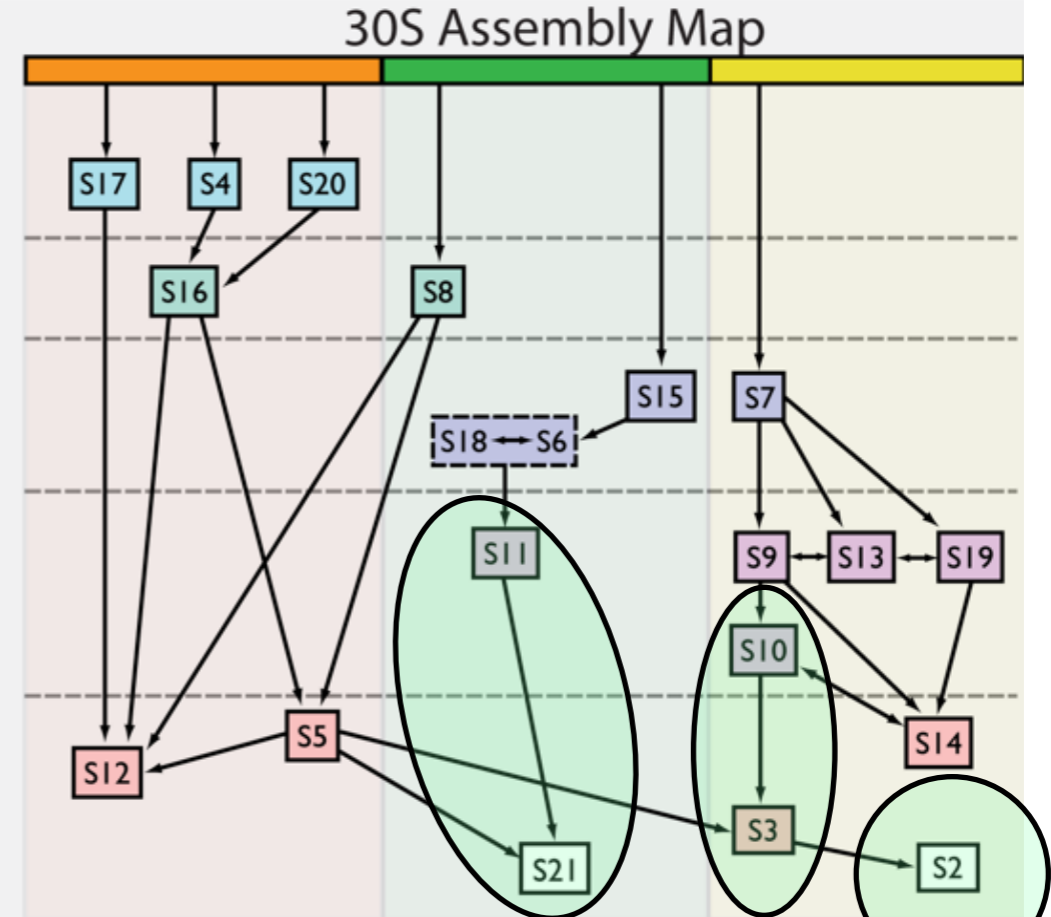
Organizing Sub-populations: Late Assembly



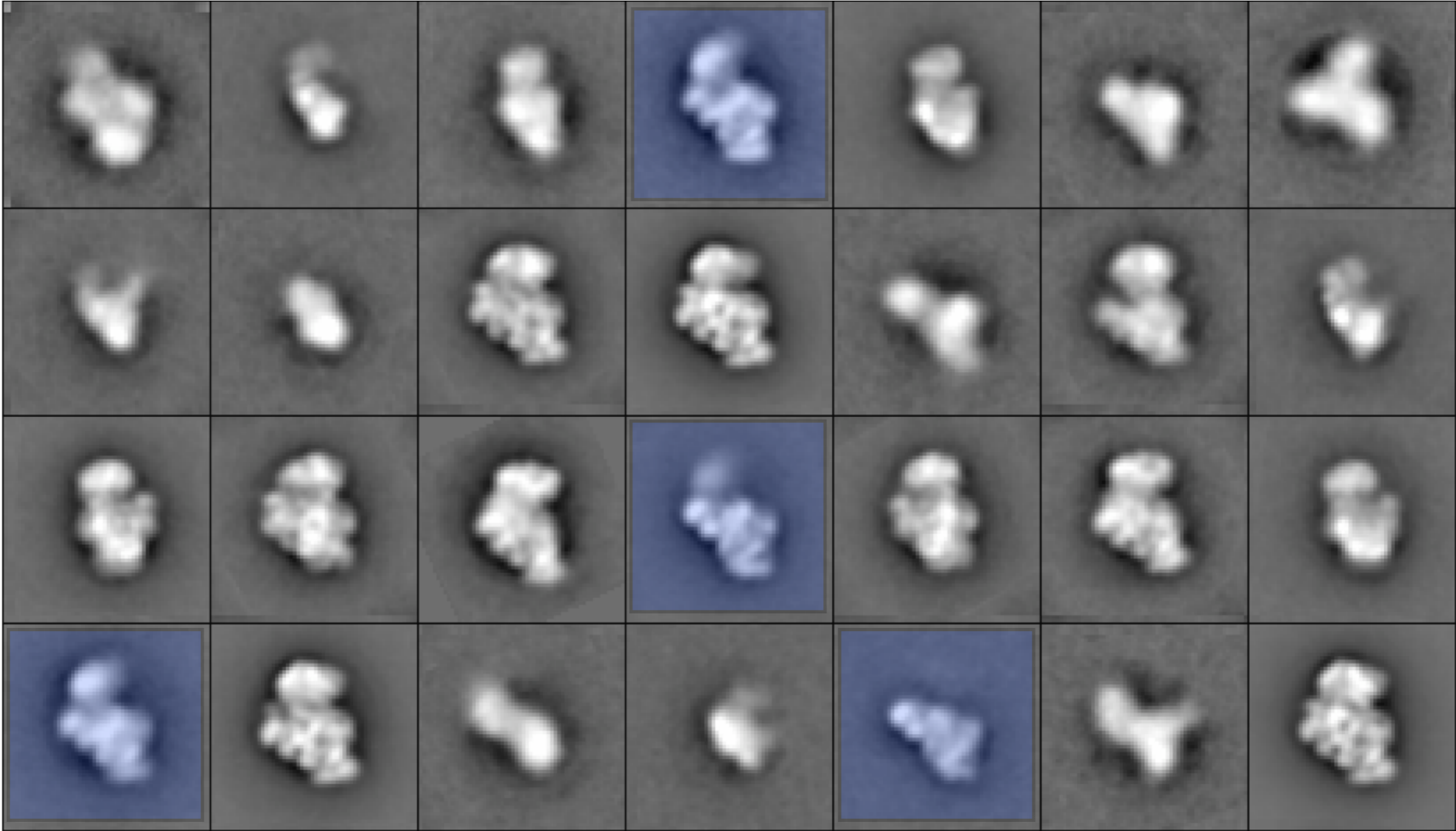
Potential Late Assembly IMIs



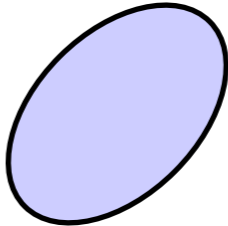
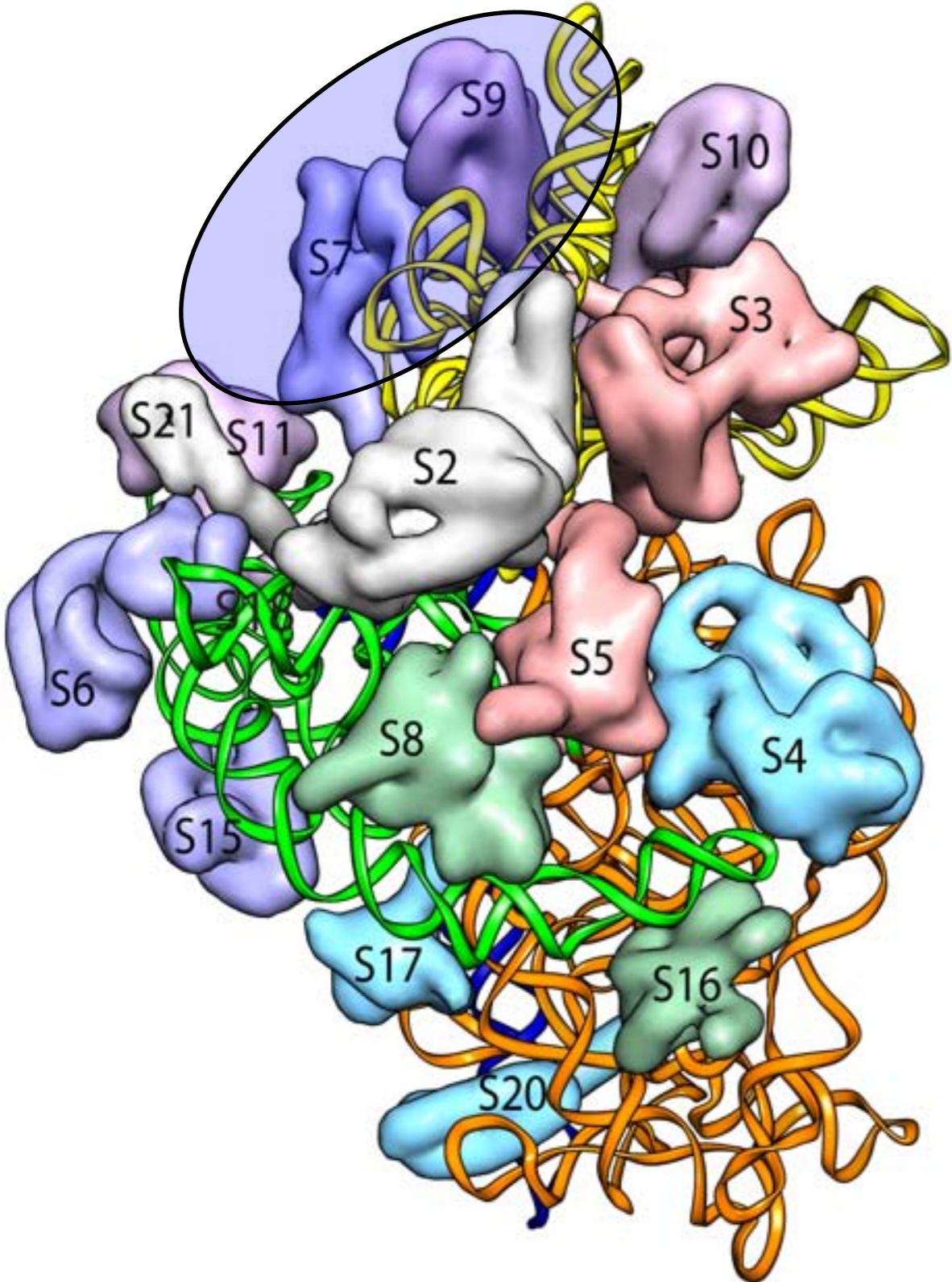
QuickTime™ and a
MPEG-4 Video decompressor
are needed to see this picture.



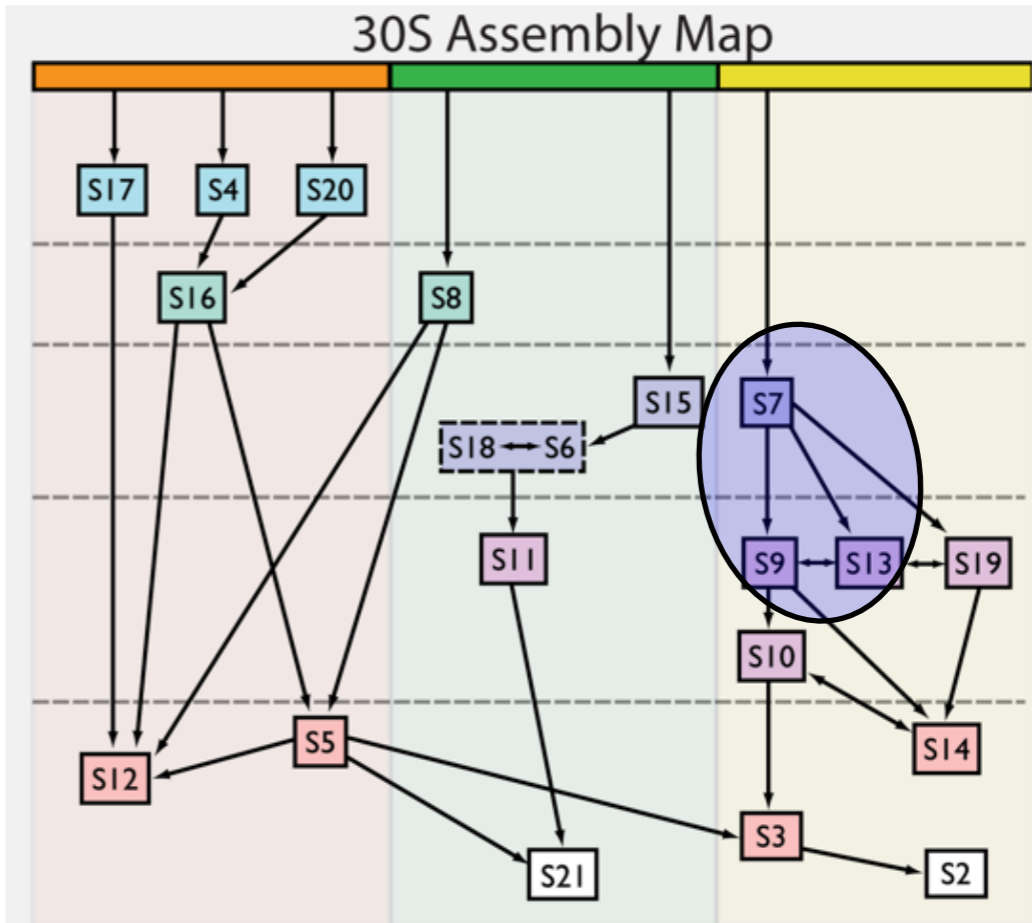
Organizing Sub-populations: Mid Assembly



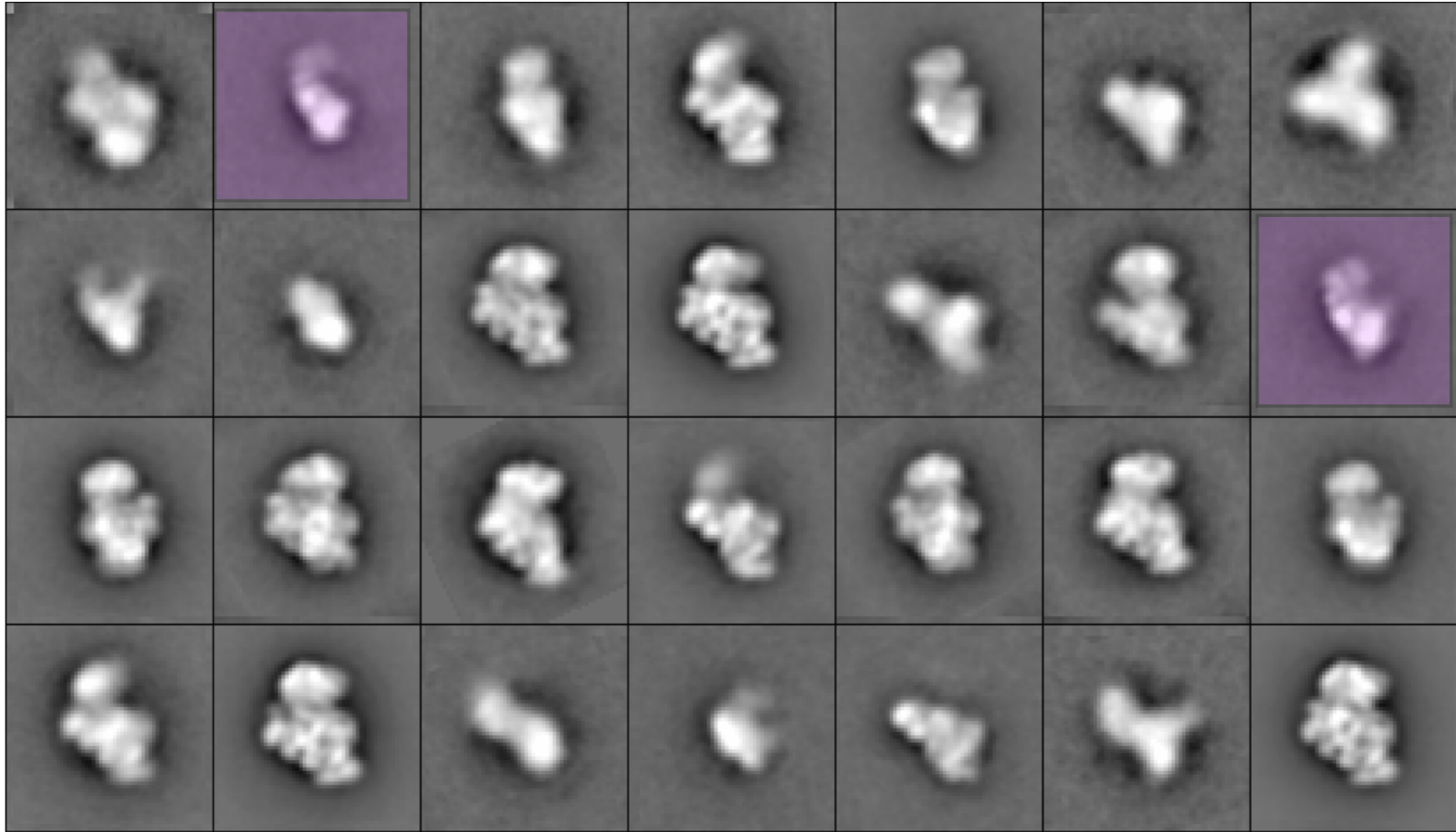
Potential Mid Assembly IMs



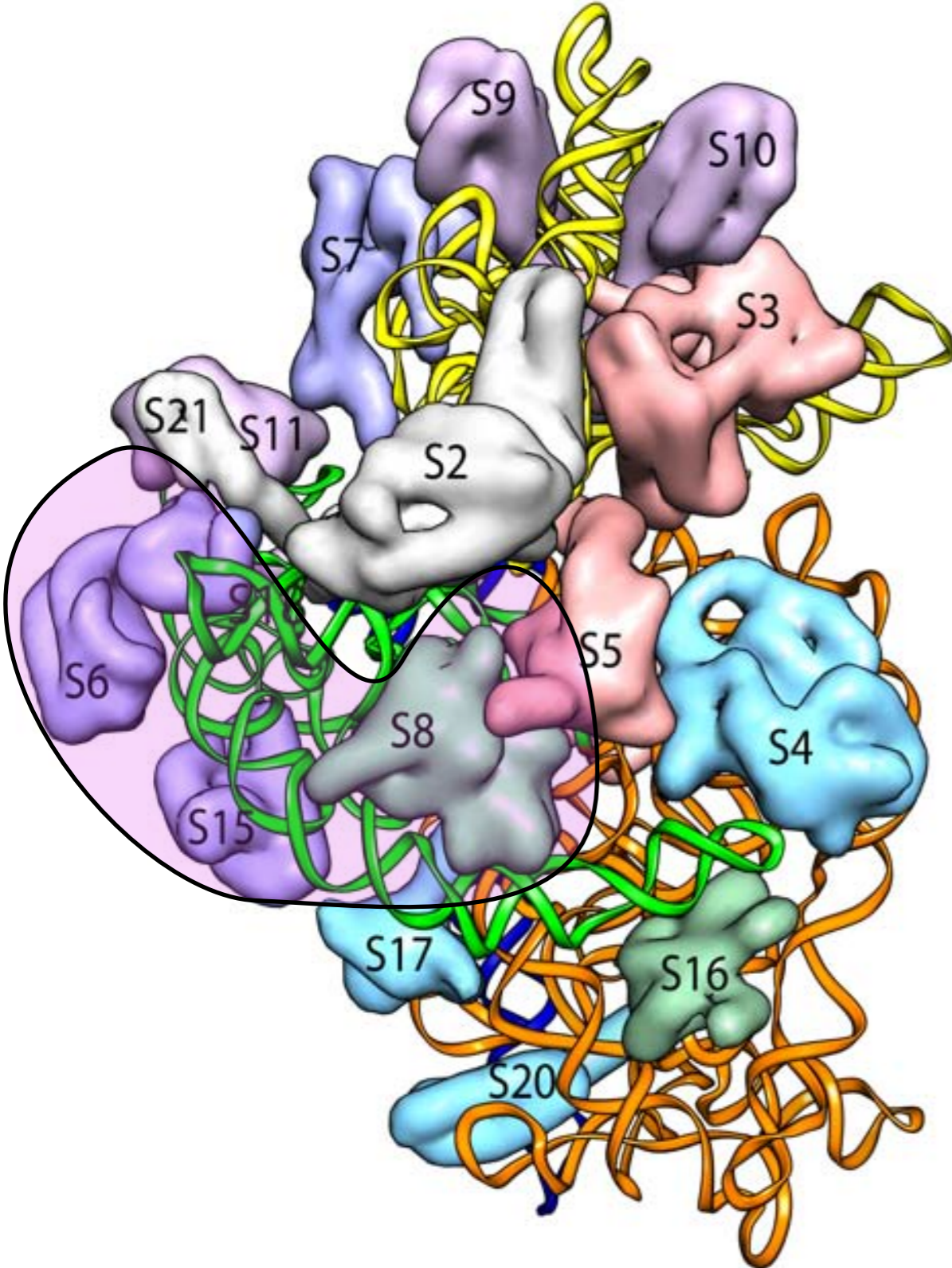
QuickTime™ and a
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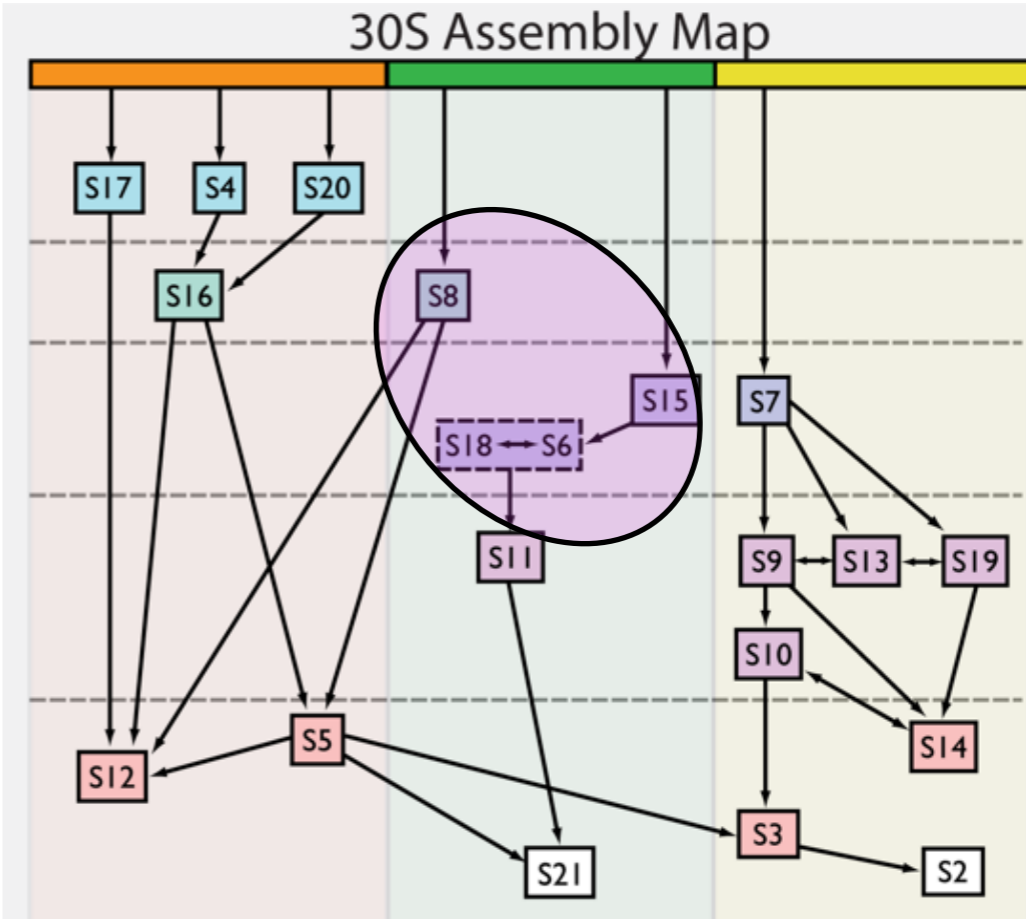
Organizing Sub-populations: Early Assembly



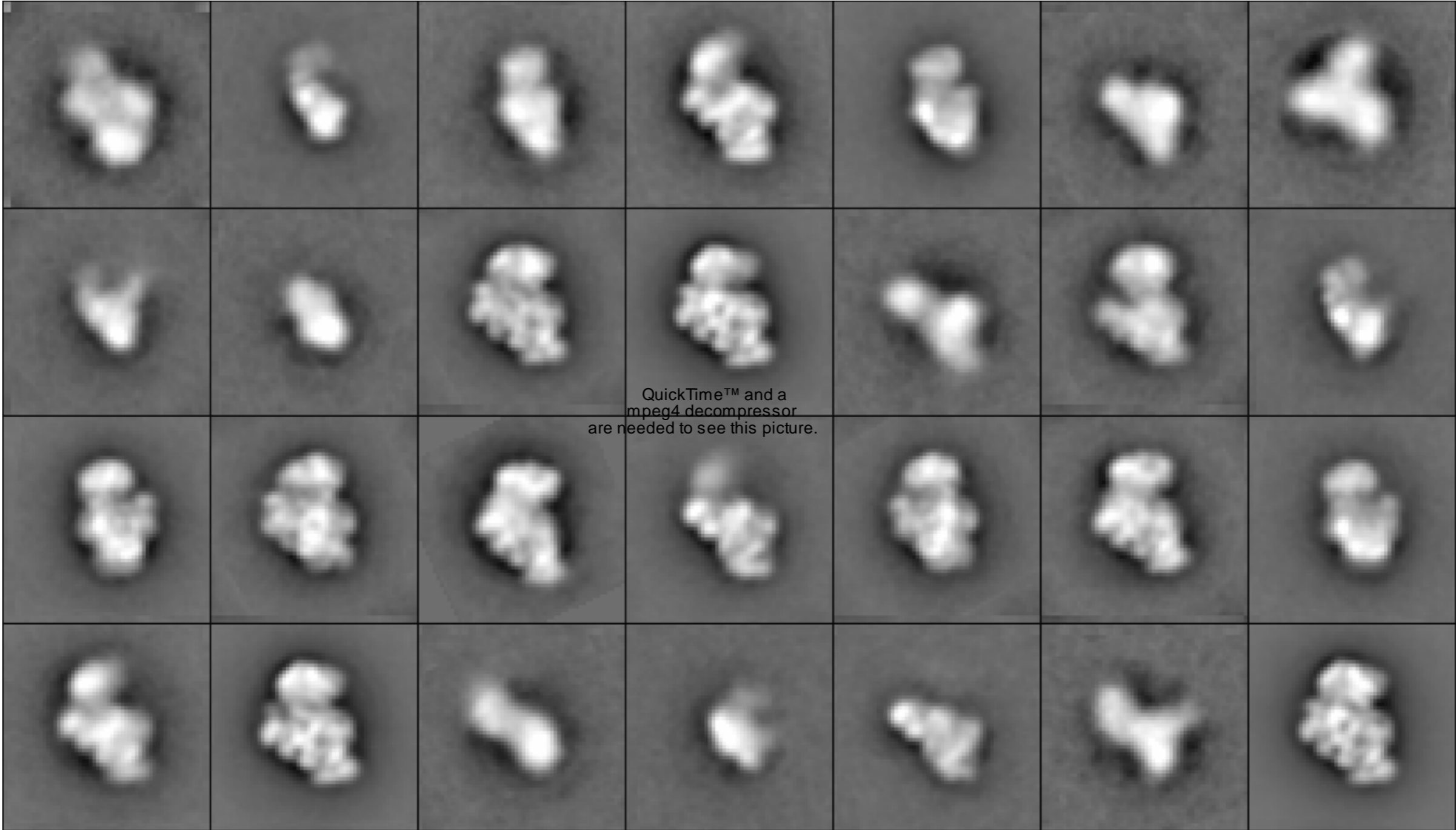
Potential Early Assembly IMs



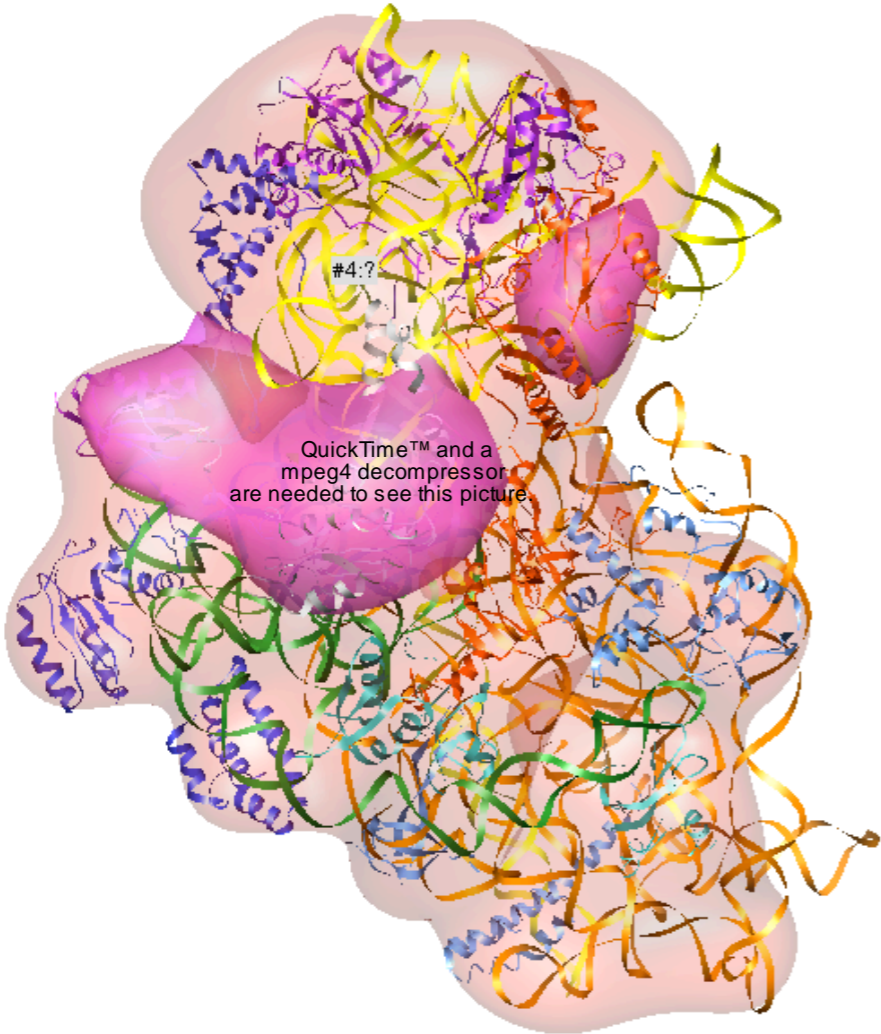
QuickTime™ and a
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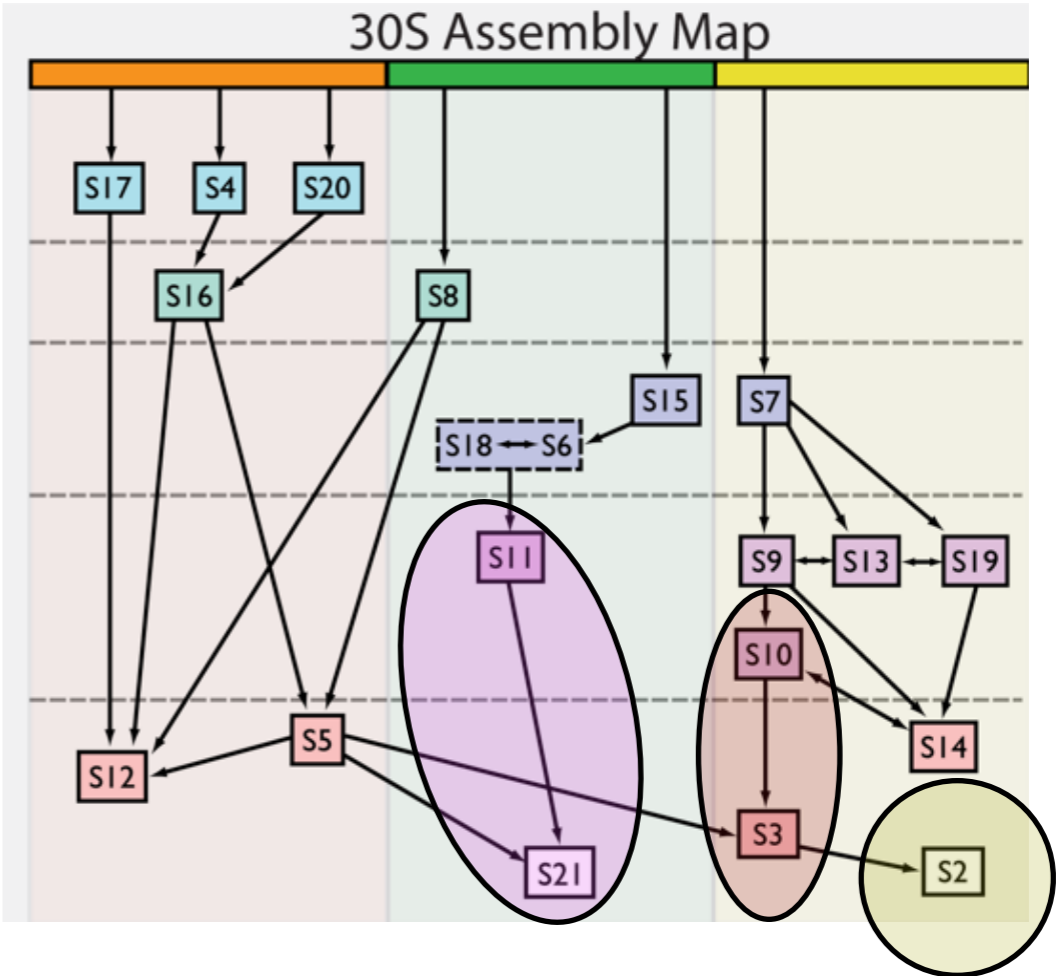
Potential Assembly Intermediates in 3D



Potential Late Assembly IMs, 3D

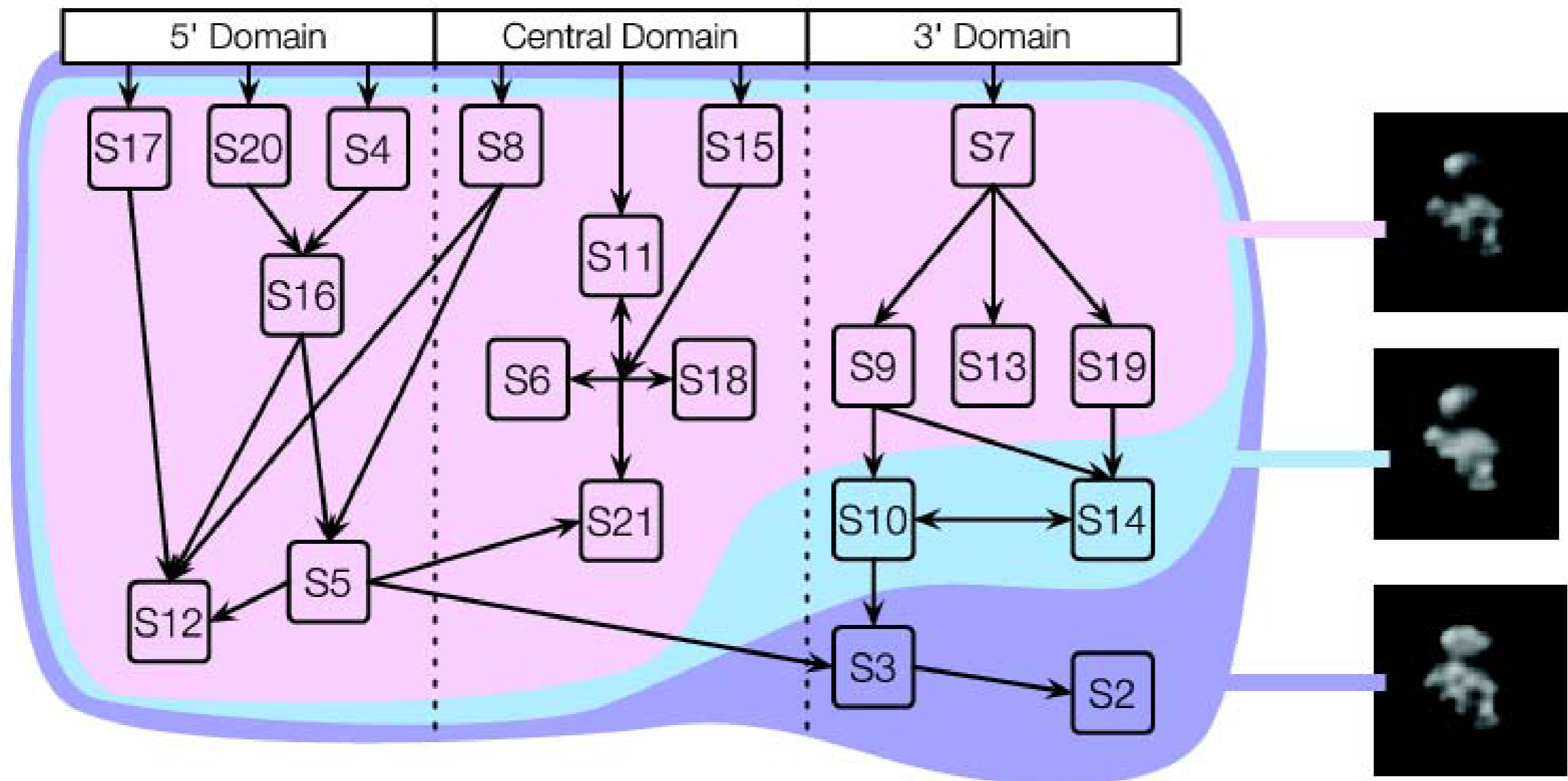


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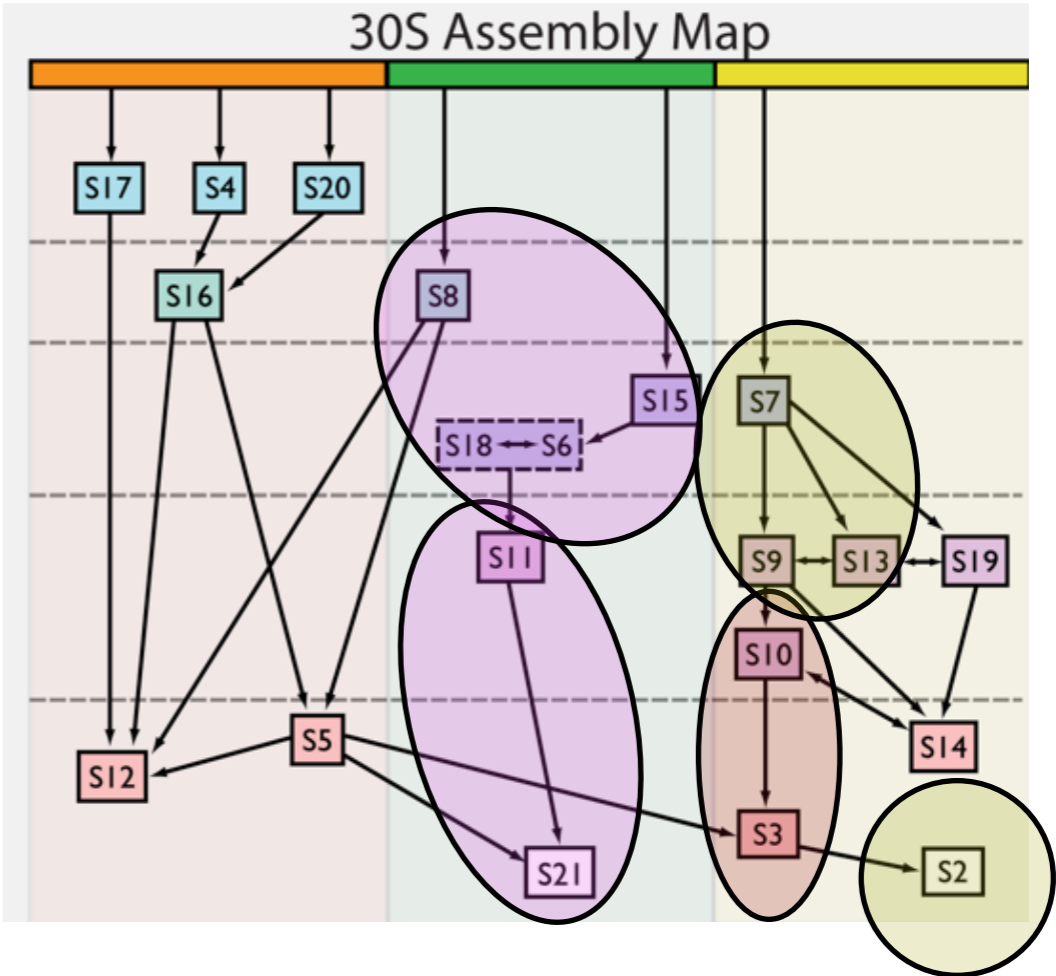


Identification of potential assembly intermediates

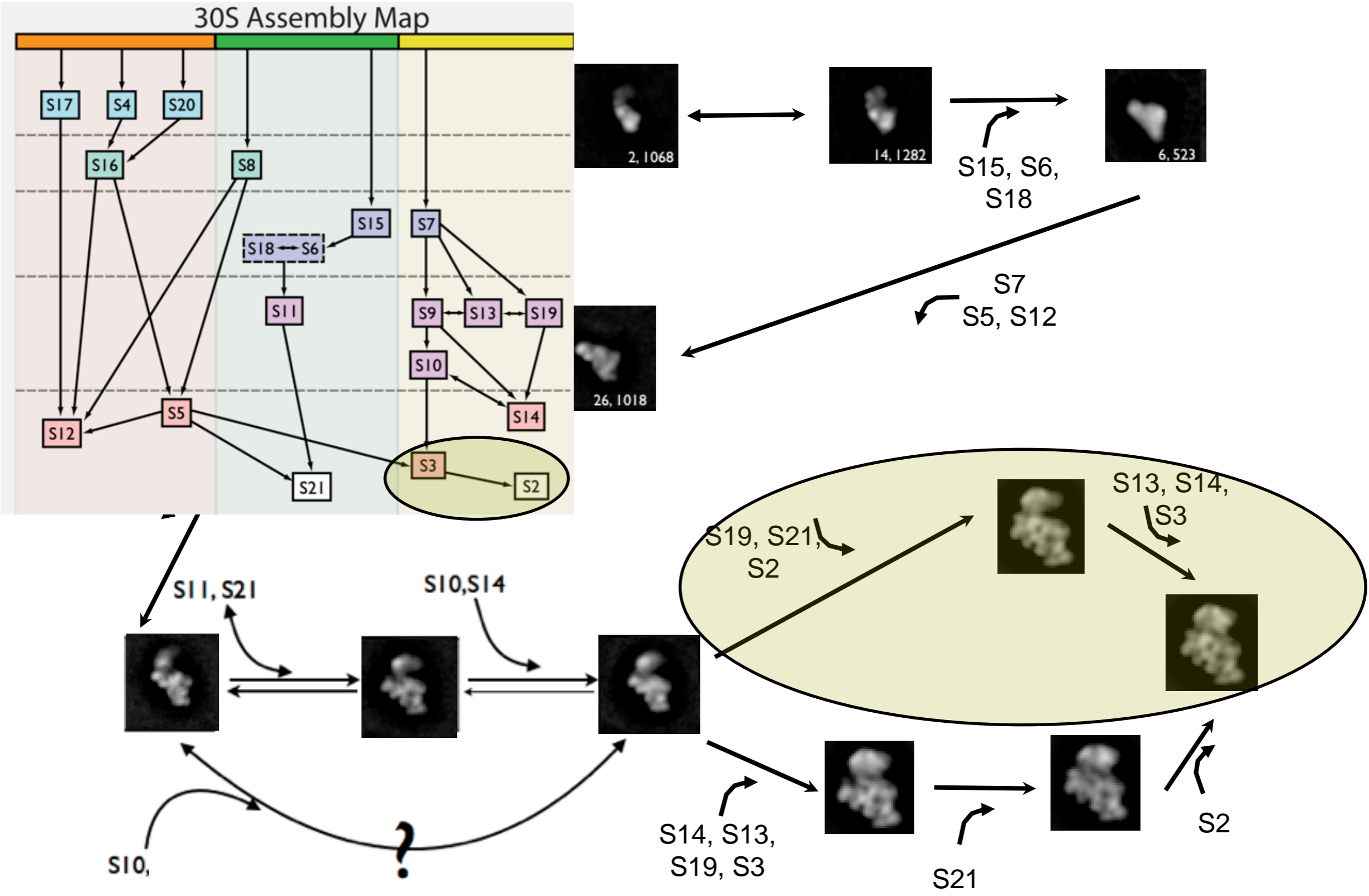
-- reconstitution of defined subcomplexes



From “Interactome” to ...



From "Interactome" to Parallel Assembly Pathways



Acknowledgements

Automated Molecular Imaging Group

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