Characterizing Late Roadblocks in Ribosome Assembly

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

- 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.
A genetic system to perturb large subunit biogenesis — in vivo

- Defined quantities of ribosomal protein L17 provides titratable population of assembling ribosomes.
Ribosomal protein (rpL17) depletion perturbs sucrose density gradient profiles.

Density-based separation

L17 strain + 5.0 nM HSL
L17 strain + 0.2 nM HSL

Slide by Dmitry Lyumkis
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Density-based separation

L17 strain + 5.0 nM HSL
L17 strain + 0.2 nM HSL

~45S

70S

30S

50S

cryoEM

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Disparate structures revealed through single-particle analysis

13 structures, ~4-5 Å resolution

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

Joey Davis, Yong Zi Tan, and Jamie Williamson
Theoretical density generated for each helix/protein from docked PDB.
For each map, calculated fraction of mature density occupied.

1. Helix (RNA) and protein occupancy differs between maps.
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1. Theoretical density generated for each helix/protein from docked PDB
2. For each map, calculated fraction of mature density occupied

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

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 and identify folding domain boundaries.
Folding blocks co-localize on tertiary structure
... but not in sequence space

- Blocks co-localize on tertiary structure and identify folding domain boundaries.
Folding blocks co-localize on tertiary structure … but not in sequence space

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

Slide by Dmitry Lyumkis
## State of current library of protein depletion strains

<table>
<thead>
<tr>
<th>Dataset</th>
<th>MotionCorr/CTF/etc</th>
<th>Initial 2D classification</th>
<th>Making a stack</th>
<th>gCTF</th>
<th>Relion 2D Classification</th>
<th>Relion 3D Classification</th>
<th>Frealign/Occ. Analysis</th>
<th>Hi-Res Model</th>
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L34 depletion, FrealignX 25-model single-particle classification
Occupancy analysis across strains
L17, L28, L32, L34 depletions, combined!
Harnessing cryo-EM to study macromolecular assembly

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