Molecular architecture of the SEA complex, a modulator of the TORC1 pathway

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Please download the tutorial from github

`git clone https://github.com/salilab/Workshop_SEA.git`
Integrative determination of macromolecular structures for maximizing accuracy, resolution, completeness, and efficiency of structure determination

Use structural information from any source: measurement, first principles, rules; resolution: low or high resolution to obtain the set of all models that are consistent with it.
Integrating various sources of data

- NMR
- X-ray structures
- FRET
- crosslinking
- site-directed mutagenesis
- affinity purification
- bioinformatics
- cryo-EM
- SAXS

atom positions
residue positions
member orientations
member positions
Why Integrative Modeling?

1. Benefits from the **synergy** among the input data, maximizing accuracy, resolution, completeness, and efficiency of structure characterization.

2. Finds “**all**” models consistent with the data, not just one.

3. Facilitates **assessing** the input data as well as results in terms of precision and accuracy.

4. Provides feedback to **guide** future experiments (eg, “what if”, ...).
SEA (Seh1-associated) complex, a major regulator of the TORC1 pathway

The SEA (Seh1-associated) complex dynamically associates with (or localized around) the vacuole membrane.

The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress.

The SEA complex physically interacts with TORC1 and is an important regulator of its activity.

- S. Dokudovskaya et al, “A conserved coatomer-related complex containing Sec13 and Seh1 dynamically associates with the vacuole in Saccharomyces cerevisiae”. MCP, 2011.
- Algret et al, “Molecular Architecture and Function of the SEA Complex, a Modulator of the TORC1 Pathway”, MCP, 2014
SEA (Seh1-associated) complex

The SEA complex is dynamically associated with (or localized around) the vacuole membrane. Functional and genetic analyses are consistent with a role for the members of the SEA complex in membrane trafficking and autophagy.

- SEA complex proteins possess structural characteristics similar to the membrane coating complexes COPI, COPII, the nuclear pore complex.

SEA (Seh1-associated) complex

8 component complex

SEA1 (1584 residues)
SEA2 (1341 residues)
SEA3 (1148 residues)
SEA4 (1038 residues)
Npr2 (615 residues)
Npr3 (1140 residues)
Seh1 (349 residues)
Sec13 (297 residues)

- It contains the nucleoporin Seh1 and Sec13, the latter subunit of both the nuclear pore complex and the COPII coating complex.

Localization, inhibition, and activation of TORC1 depend on the SEA complex.

membrane-associated scaffold, needed for activation of TORC1

interacts with and inhibits TORC1

V-ATPase

Integrative structure determination of the SEA complex

Experimental data
- Residue-specific cross-linking: 45 inter-molecular and 143 intra-molecular cross-links
  - Inter- and intra-molecular distance restraints (residue level)
- Protein and domain interactions: 23 affinity purifications
  - Domain connectivity (Composites)
- Stoichiometry: 8 proteins
  - Number of subunits
- X-ray crystallography: 2 proteins / domains
  - Atomic structures

Statistical inference and physical principles
- Comparative modeling: 19 domains
  - Fold models
- Bioinformatics: 8 proteins
  - Excluded Volume

Gathering Data
Representing and Translating Data into Spatial Restraints
Sampling the Good Scoring Configurations
Analyzing and Assessing the ensemble

Random configurations
Initial sampling (Cross-links and selected composite restraints)
Refinement (Cross-links and ALL composite restraints)
Final structures

Model clustering
Protein and domain contact frequency
Protein and domain localization
Data 1: Residue-specific DSS chemical cross-links

45 inter-molecular and 143 intra-molecular DSS (Lys-Lys) cross-links
Data: Residue-specific DSS (Lys-Lys) crosslinks

45 inter-molecular (and 143 intra-molecular) DSS crosslinks

Two subunits

1. SEA1, Npr2, Npr3
2. SEA3/Sec13, SEA4/Seh1, SEA2

The interactions between SEA2, SEA3/Sec13 and SEA4/Seh1 strongly depend on the C-terminal RING domain.

The connection between these two subunits happens via Npr3/SEA1 and N-terminal part of SEA3.

SEA3 forms a dimer with Sec13

SEA4 forms a dimer with Seh1

Yi Shi, Javier Fernandez-Martinez
Data: Residue-specific DSS (Lys-Lys) crosslinks

45 inter-molecular and 143 intra-molecular DSS (Lys-Lys) crosslinks (XLs)

- RED dot: XL in "DISORDERED" region.
- GREEN dot: XL in "STRUCTURED" region.
- BLACK dot: XL in "UNKNOWN" region.

Square box, template structure coverage:
- RED: 100% sequence identity.
- BLUE: 10~15% sequence identity.

Yi Shi, Javier Fernandez-Martinez
Data 2: Affinity co-purification

7 protein pullouts, 16 domain deletion pullouts

“Composite” restraint
Data 3: Relative stoichiometry by SYPRO Ruby staining

The SEA complex was isolated in 5-20% sucrose velocity gradients and the resulting 12 equal fractions were analyzed on 4%-12% Bis-Tris gels. Gels were stained with SYPRO Ruby (Molecular Probes) and digitized.

1:3 stoichiometry for Sea4 and Seh1

Benchmark with known proteins
Symmetry
Hierarchical model representation facilitates using imprecise information.
<table>
<thead>
<tr>
<th>Representative</th>
<th>1:1:1:1:1:1:1:1 Stoichiometry</th>
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<td><strong>S.cerevisiae</strong></td>
<td><strong>Domain</strong></td>
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<td>SEA1</td>
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<td>1083</td>
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<tr>
<td><strong>Total</strong></td>
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</table>
### Multi-scale Representation

- Resolution = 0 (atomic resolution)
- Resolution = 1 (1 bead / 1 residue)
- Resolution = 5 (1 bead / 5 residues)
- Resolution = 10 (1 bead / 10 residues)
- Resolution = 100 (1 bead / 100 residues)

Resolution = 100
~100 residues per bead

Domain mapping (composite) restraints shown is also a “Gaussian” envelope
Monte Carlo sampling with simulated annealing:
• Start with a random configuration of protein centers.
• Minimize violations of input restraints by Monte Carlo with simulated annealing.
• Obtain an “ensemble” of many independently calculated models (885 refined models).

Sampling is exhaustive
10% of models are already representative of the entire set.

Total score =
188 Harmonic Upper Bounds for Crosslinks (~35Å) +
23 Composite Restraints +
Linkers Between Beads +
Excluded Volume

885 best scoring models satisfy all restraints.
Clustering and the Localization probability map

Calculated from the structural superposition of the ensemble of models that satisfy all input restraints

Hierarchical clustering based on the RMSD distance matrix

Protein localization probability

Can see position of every SEA protein!

Algret et al., “Molecular Architecture and Function of the SEA Complex, a Modulator of the TORC1 Pathway”, MCP, 2014
• SEACAT and SEACIT sub-complexes interact via the Sec13 / Sea3 hub

• Cluster of beta-propellers from Seh1, Sec13, Sea4, and Sea2 (cf, COPI and COPII)

• RING domains of Sea2/3/4 interact with each other

• Stoichiometry of Sea4 and Seh1 is 3

• Can map mutations associated with diseases (eg, cancer) on structure.

• Can map transient protein-protein interactions to expand from this stable complex ...

• SEACAT activates TOR1; SEACIT inhibits TOR1

• Provides a starting point for X-ray, EM, more cross-linking
The proximities of any two residues in the topological map were measured by their relative “contact frequency”. A contact between a pair of residues is defined when their corresponding bead surfaces are less than 20 Å from each other.

Crosslinks were plotted as the red dots, and the residue contact frequency is indicated by a color ranging from white (0) to dark blue (1). Each box contains the contact frequency between the corresponding pair of the SEA complex proteins.
The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress. The SEA complex physically interacts with TORC1 and is an important regulator of its activity.

TORC1 inhibition changes the stability of SEA complex members

Sea1 is involved in the regulation of general autophagy

A number of functional data indicated a role for the SEA complex in intracellular trafficking, amino acid biogenesis, regulation of the TORC1 pathway and autophagy.
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