Molecular architecture and function of the SEA complex

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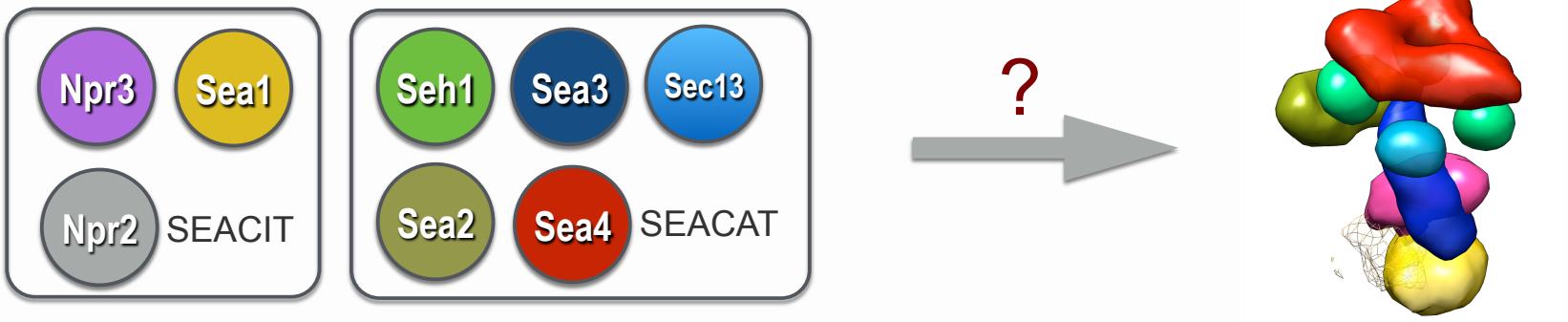
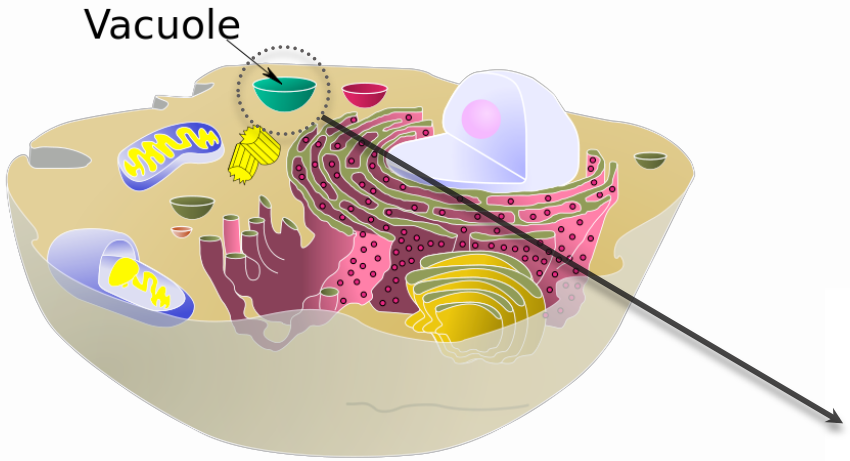
- From a proteomics screen, yeast Npr2 and Npr3 are regulators of TORC1 (Neklesa & Davis, 2009).
- The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress.
- The SEA complex in yeast also contains Sea1-4, Sec13 (COPII and NPC) and Seh1 (NPC) (Dokudovskaya et al, 2011).
- Mammalian analogs of SEA are GATOR1 and GATOR2, corresponding to SEACIT and SEACAT in yeast (Bar-Peled et al, 2013).
- Localized at vacuolar membrane; SEA proteins are predicted to contain alpha-solenoid and beta-propeller domains present in complexes that interact and curve membranes (eg, NPC, HOPS, CORVET, NPC, COPI, COPII, clathrin/adaptin).



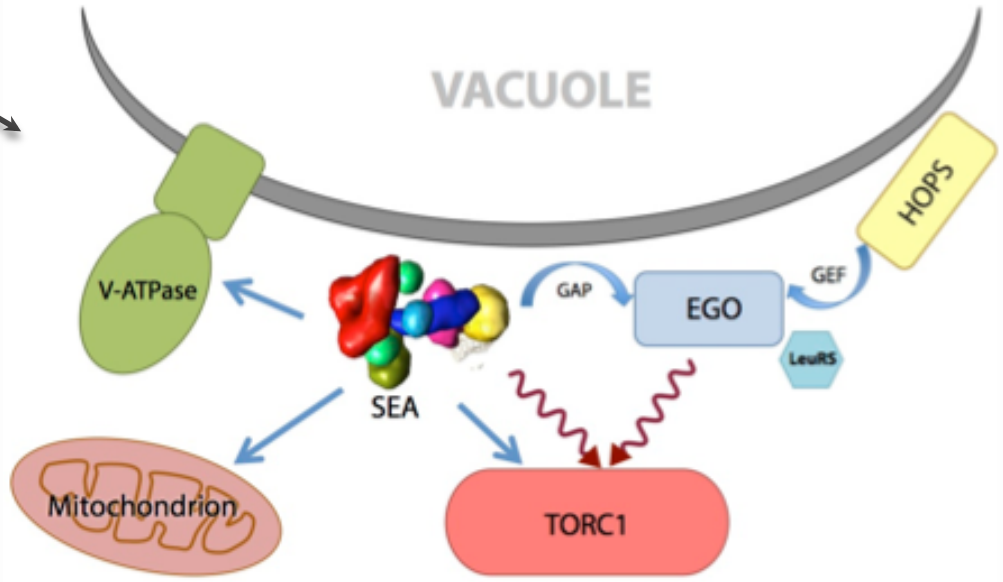
SEA (Seh1-associated) complex, a major regulator of the TORC1 pathway



The SEA complex is dynamically associated with (or localized around) the vacuole membrane.

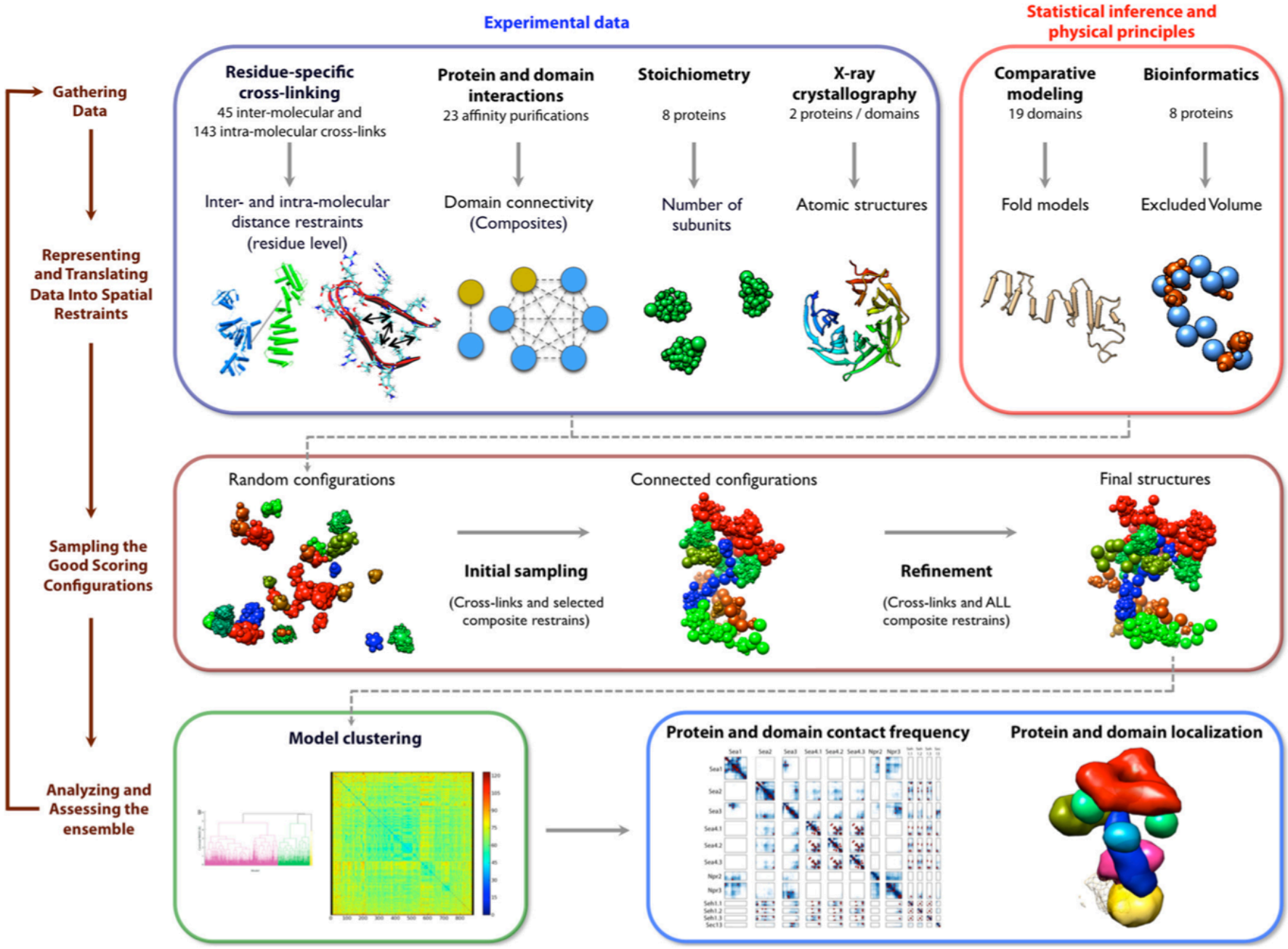
The TORC1 (Target of Rapamycin Complex 1) signaling pathway plays a major role in the control of cell growth and response to stress.

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



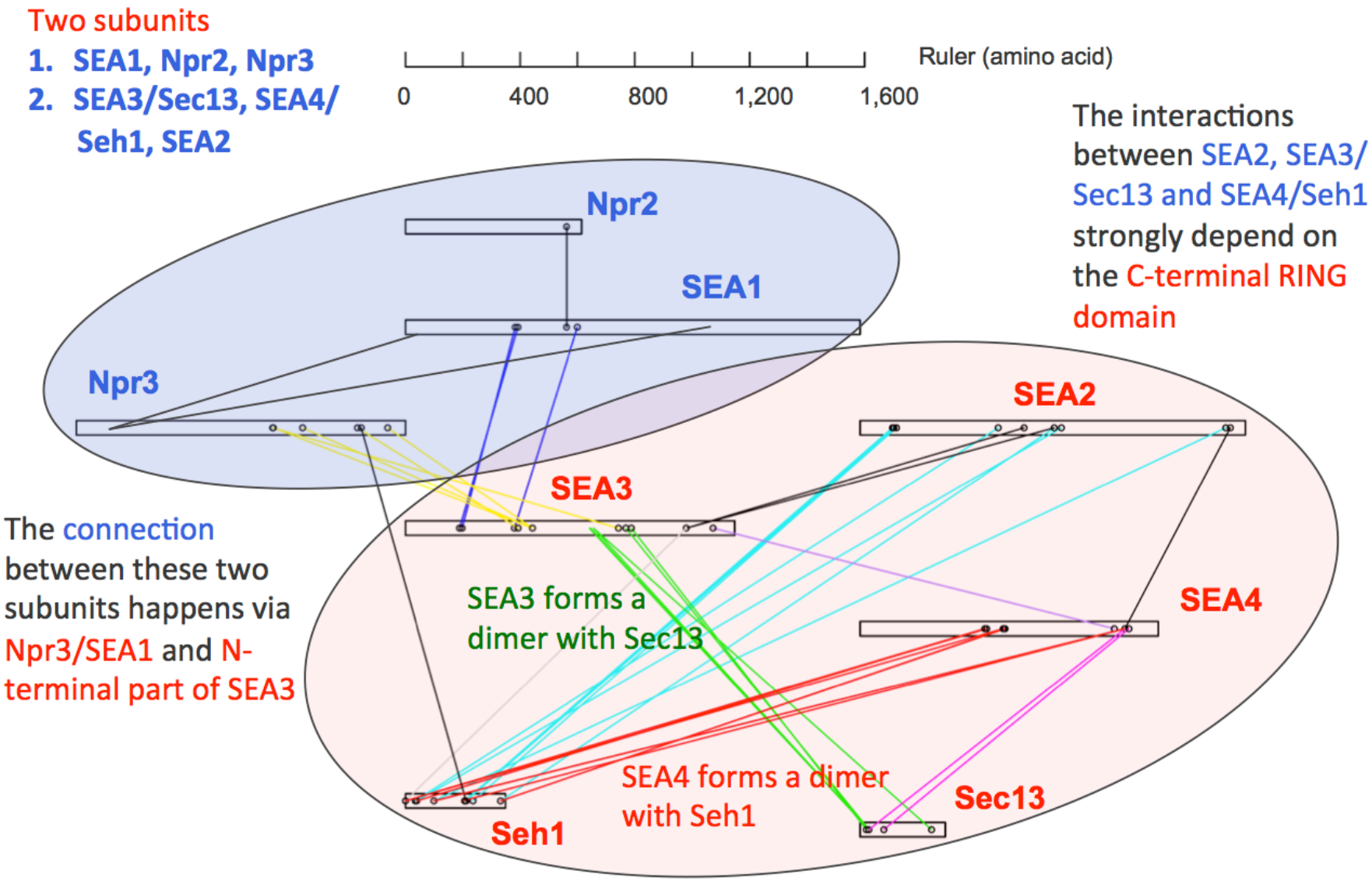
S. Dokudovskaya et al, "A conserved coatomer-related complex containing Sec13 and Seh1 dynamically associates with the vacuole in Saccharomyces cerevisiae". MCP, 2011.

Integrative structure determination of the SEA complex



Data: Residue-specific DSS (Lys-Lys) crosslinks

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



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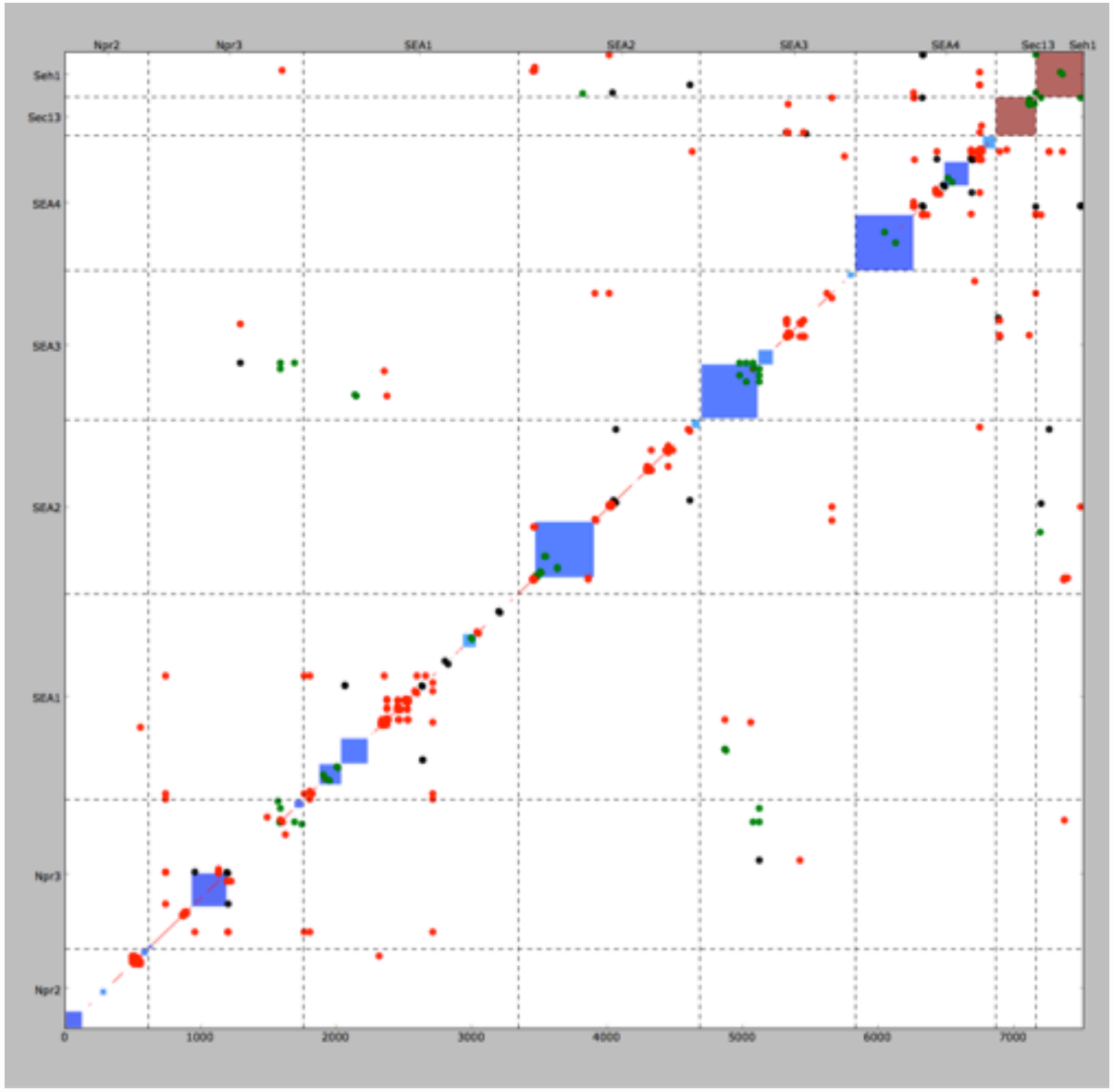
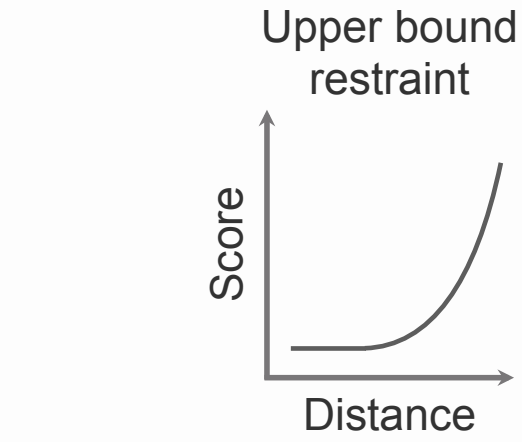
Data: Residue-specific DSS (Lys-Lys) crosslinks

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.

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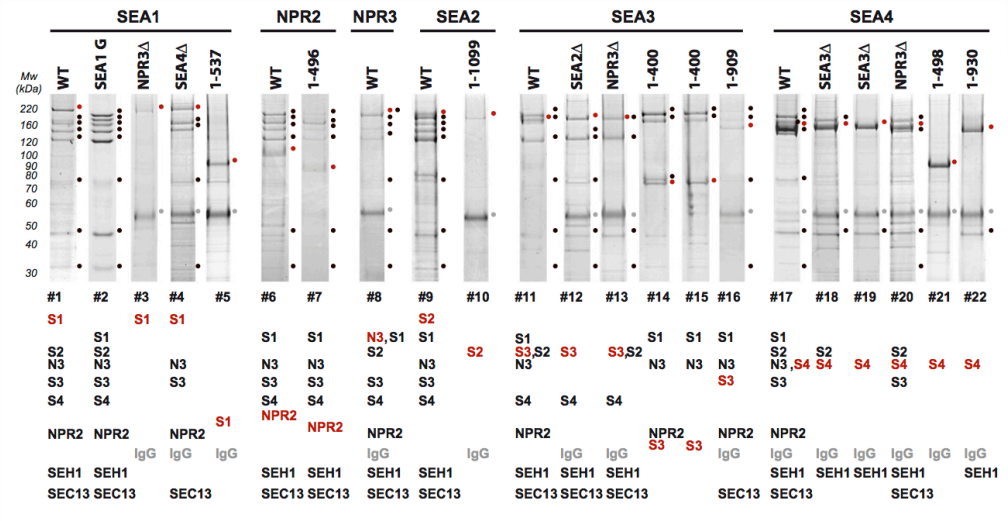
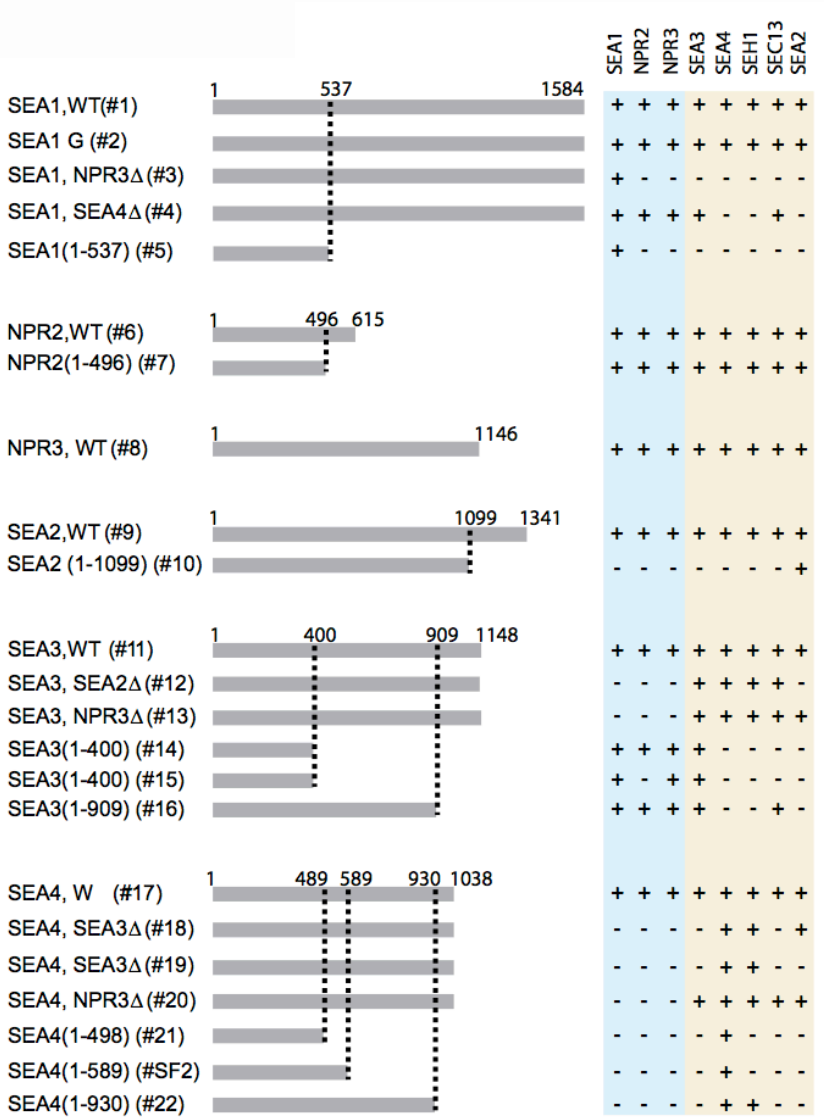
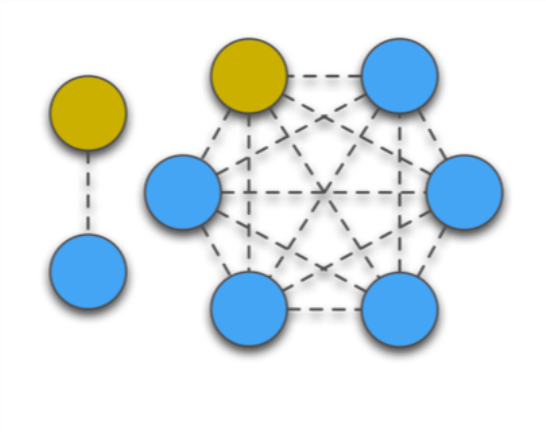
Data: Affinity co-purification

7 protein pullouts, 16 domain deletion pullouts



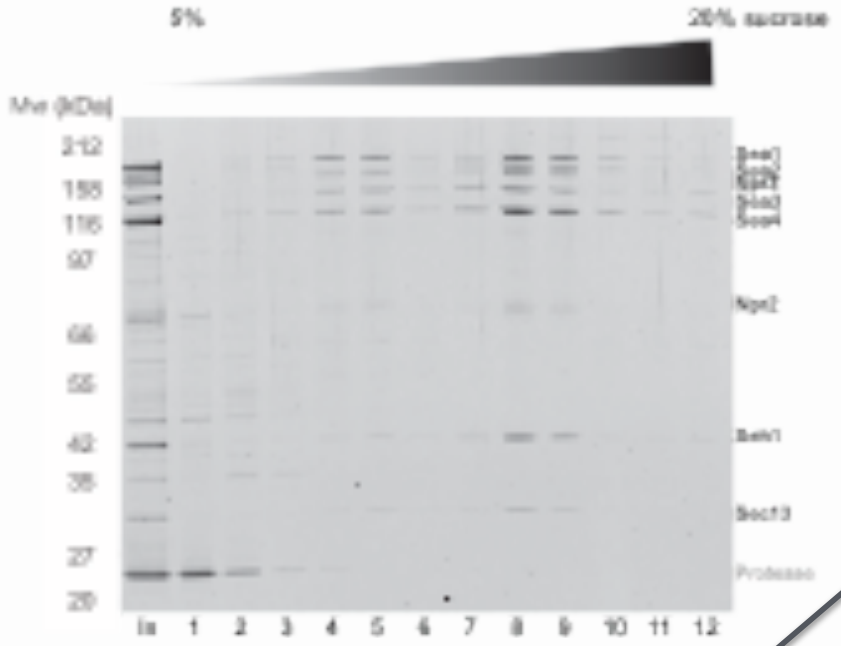


"Composite" restraint



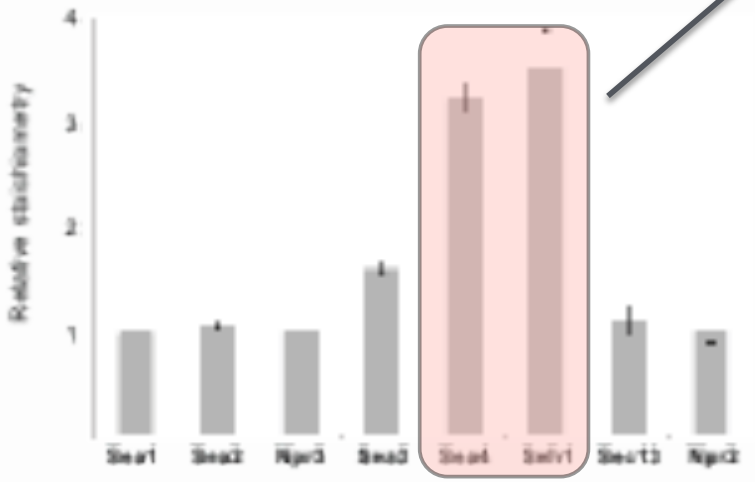
Romain Algret

Data: Relative stoichiometry by SYPRO Ruby staining

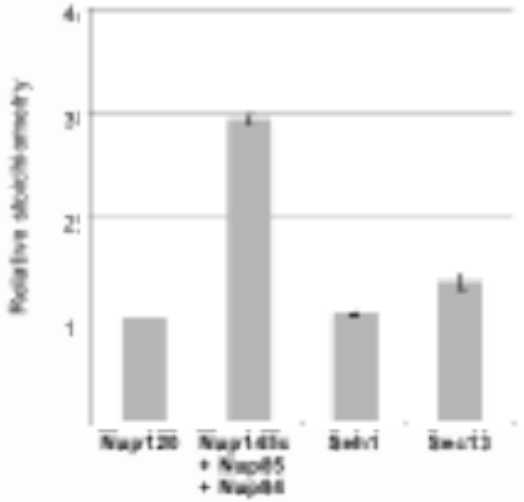


The SEA complex was isolated in 5- 20% sucrose velocity gradients and the resulting 12 equal fractions were analyzed on 4%–12% Bis-Tris gels. Gels were stained with SYPRO Ruby (Molecular Probes) and digitized.

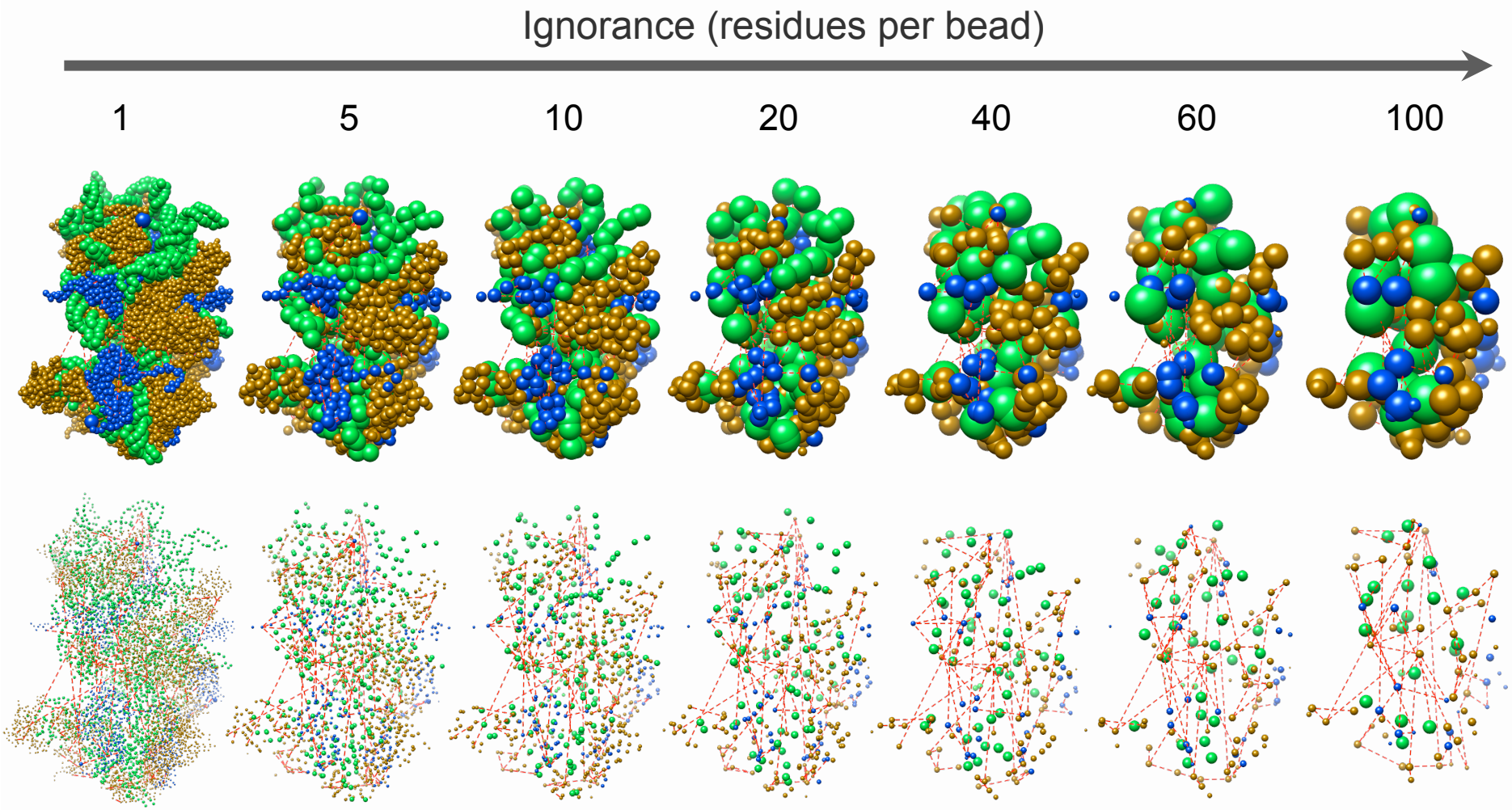
1:3 stoichiometry for Sea4 and Seh1



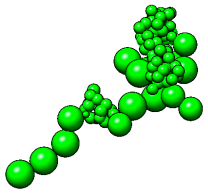
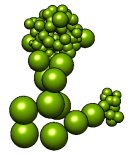
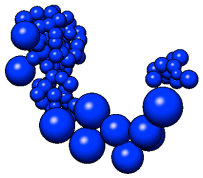
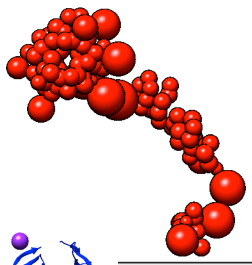
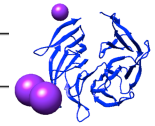
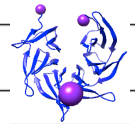
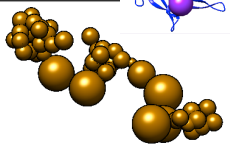
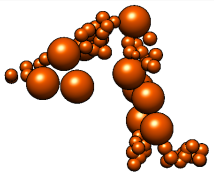
Benchmark, with known answers.



Hierarchical model representation facilitates using imprecise information



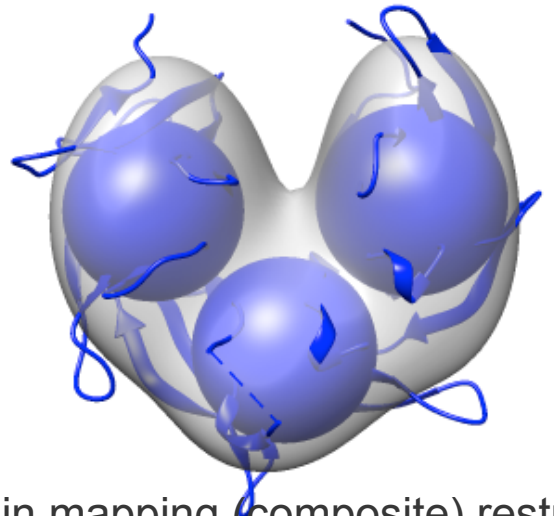
Representation of the SEA Complex

S.cerevisiae SEA proteins	Fold		8 Crystal Structure Fragments Begins at	8 Crystal Structure Fragments Ends at	52 Comparative Model Fragments Begins at	52 Comparative Model Fragments Ends at	85 Bead Fragments Begins at	85 Bead Fragments Ends at	85 Bead Fragments No. of Beads	85 Bead Fragments Residues per Bead	Template	Sequence Identity
Sea1		Disordered					1	100				
		N-terminal Cdc48-like			101	275						
		Linker					276	278				
		vWA-like			279	331						
							332	343				
					344	376						
							377	399				
					400	473						
		Unknown Structure					474	526				
		Disordered					527	859				
		Unknown Structure					860	1126				
		Disordered					1127	1177				
		DEP			1178	1273						
		Disordered					1274	1340				
		Unknown Structure					1341	1584				
Sea2		Disordered					1	126				
		Beta Propeller			127	172						
							173	200				
					201	319						
							320	337				
					338	403						
							404	433				
					434	520						
		Unknown Structure					521	563				
		Disordered / Unknown Folds					564	1155				
		Unknown Alpha Helices					1156	1279				
		RING			1280	1341						
Sea3		Unknown Structure					1	53				
		Beta Propeller			54	278						
							279	289				
					290	314						
							315					
					325	344						
							345					
					390	424						
							425					
		Linker					429					
		RWD			430	536						
		Disordered / Unknown Folds					537					
		Unknown Alpha Helices					717					
		Disordered					818					
		Unknown Alpha Helices					995					
		RING			1092	1139						
		Unknown Structure					1140					
Sea4.1 Sea4.2 Sea4.3		Unknown Structure										
		Beta Propeller			45	87						
							88					
					124	130						
							131					
					149	272						
							273					
					285	333						
							334					
					356	426						
							427					
		Disordered					429					
		Unknown Alpha Helices					490					
					659	782						
		SPAH					783					
					809	835						
		Unknown Structure					836					
		Disordered					889					
		RING			942	963						
		Unknown Structure					1030					
Seh1.1 Seh1.2 Seh1.3		Beta Propeller					249					
			288	346								
							347					
Sec13			2	158								
		Beta Propeller					159					
			166	296								
							297					
Npr2		Disordered										
		Longin			9	127						
		Unknown Structure					128					
		3B73_A			257	327						
		Unknown Structure					328					
		Disordered					350					
		Unknown Structure					410					
		Disordered					490					
		1TC3_C			563	610						
		Unknown Beta Strand					611					
Npr3		Longin			1	31						
		Disordered					32					
		Longin			322	351						
							352	399	1	48	3TW8_A	13%
					400	438						
		Disordered					439	530	1	92		
		Longin			531	577					3TW8_A	10%
		Unknown Alpha Helices					578	949	4	93		
		4F54_A			950	988					4F54_A	18%
		Disordered					989	1082	1	94		
		3HUG_A			1083	1140					3HUG_A	11%
		Unknown Beta Strand					1141	1146	1	6		

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

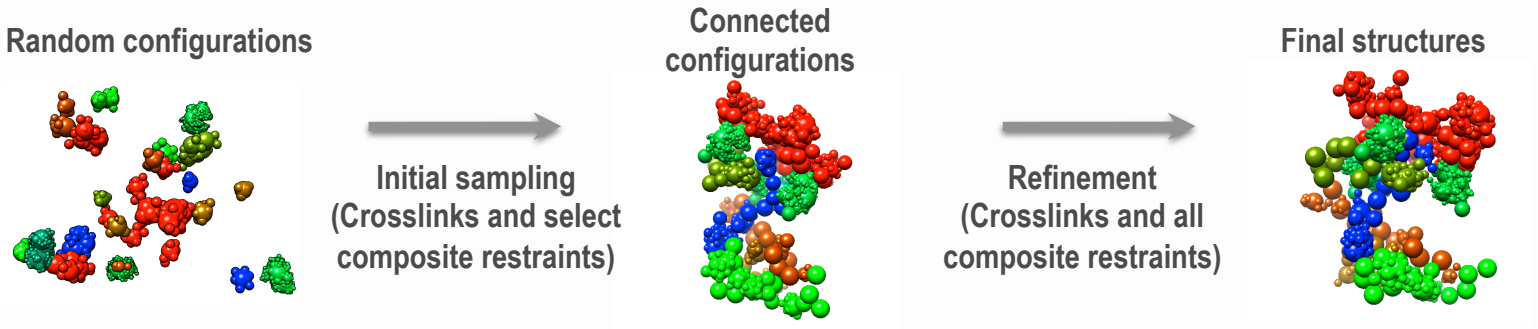


Domain mapping (composite) restraints
shown is also a “Gaussian” envelope

Sampling good scoring models

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 =

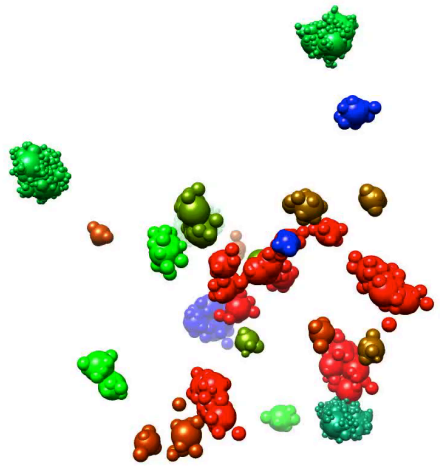
188 Harmonic Upper Bounds for Crosslinks (17Å) +

23 Composite Restraints +

Linkers Between Beads +

Excluded Volume

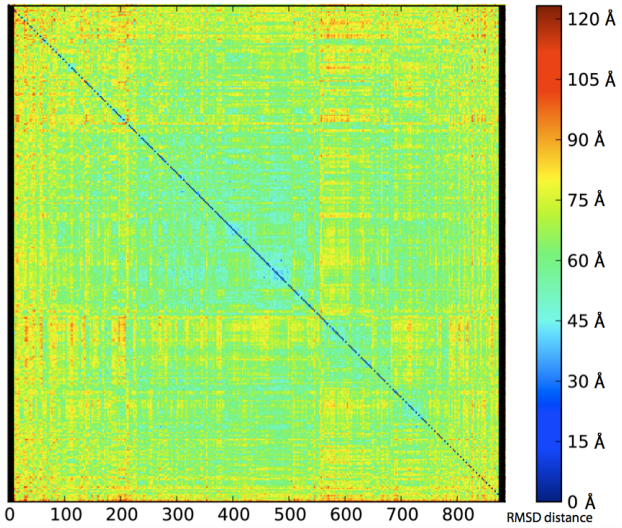
885 best scoring models satisfy all restraints.



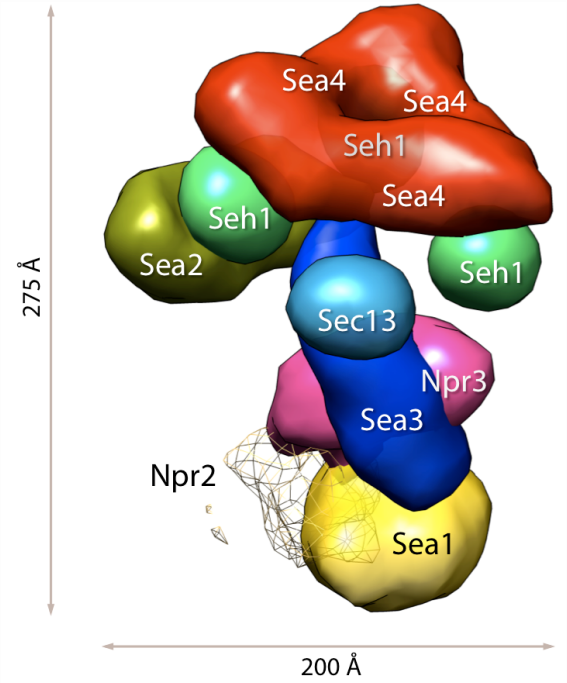
Protein Localization Probability and Volume

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

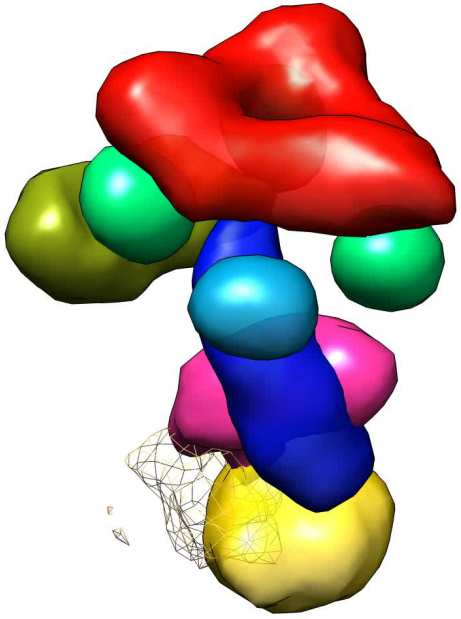
Hierarchical clustering based on the RMSD distance matrix



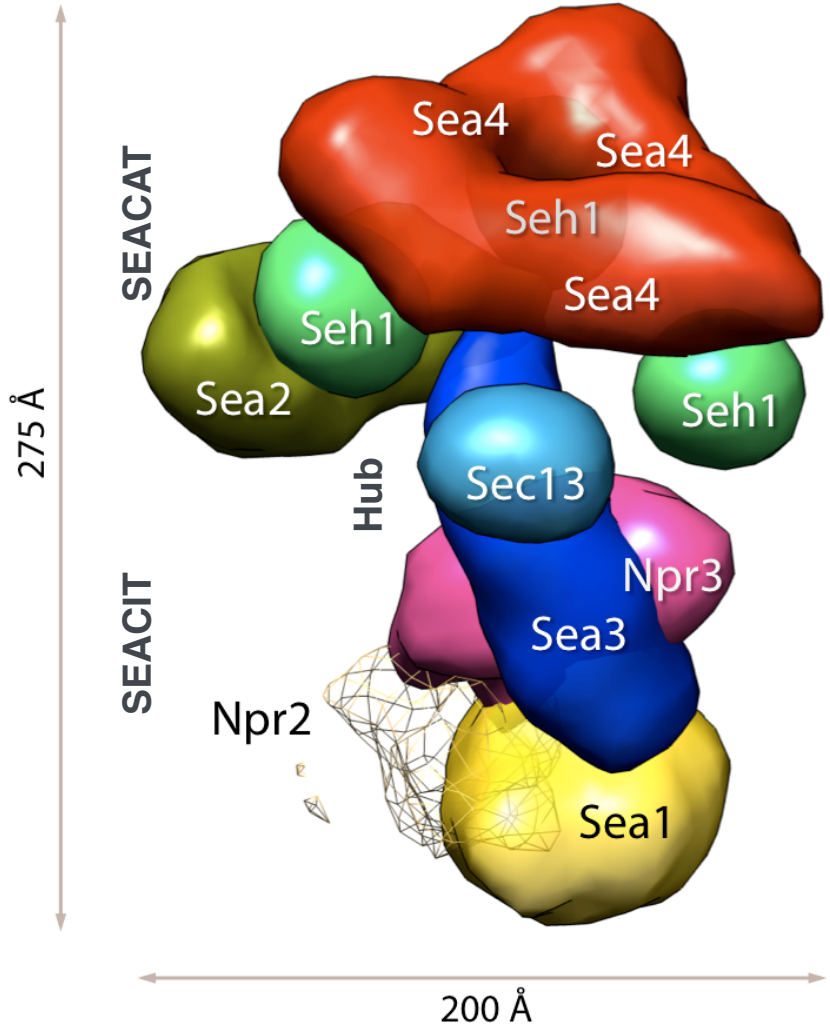
Protein localization probability (contoured to get 1.5 times protein volume)



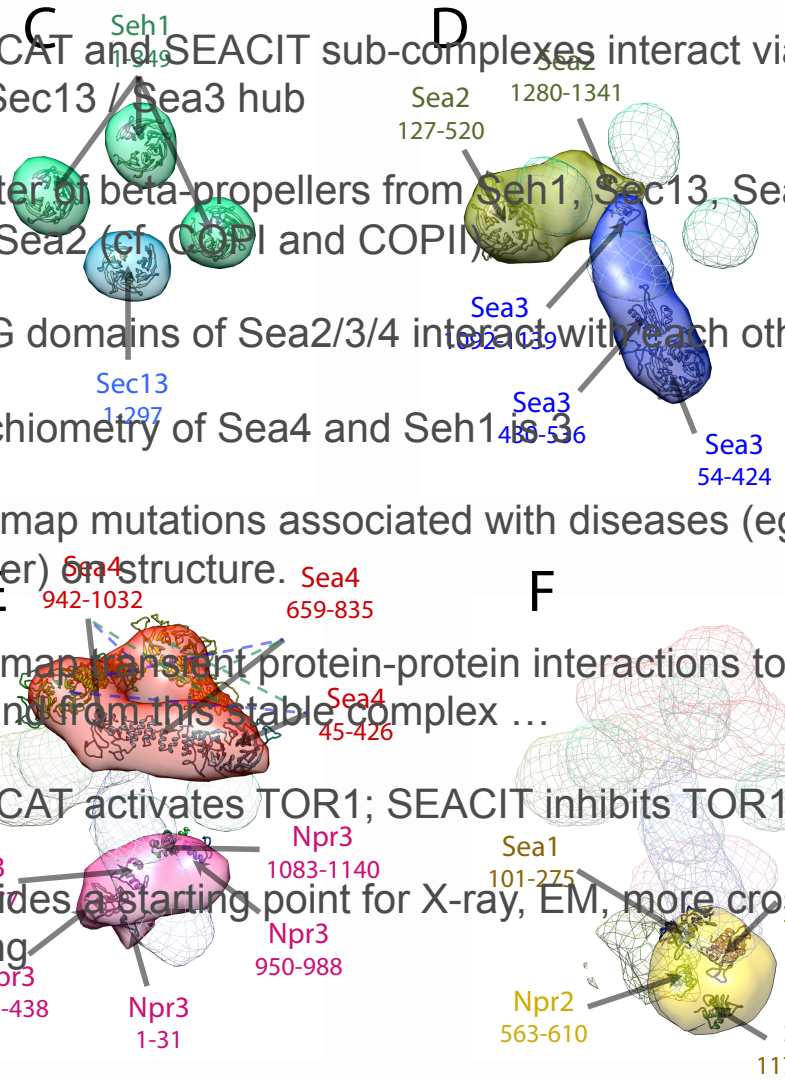
Can see position of every SEA protein!



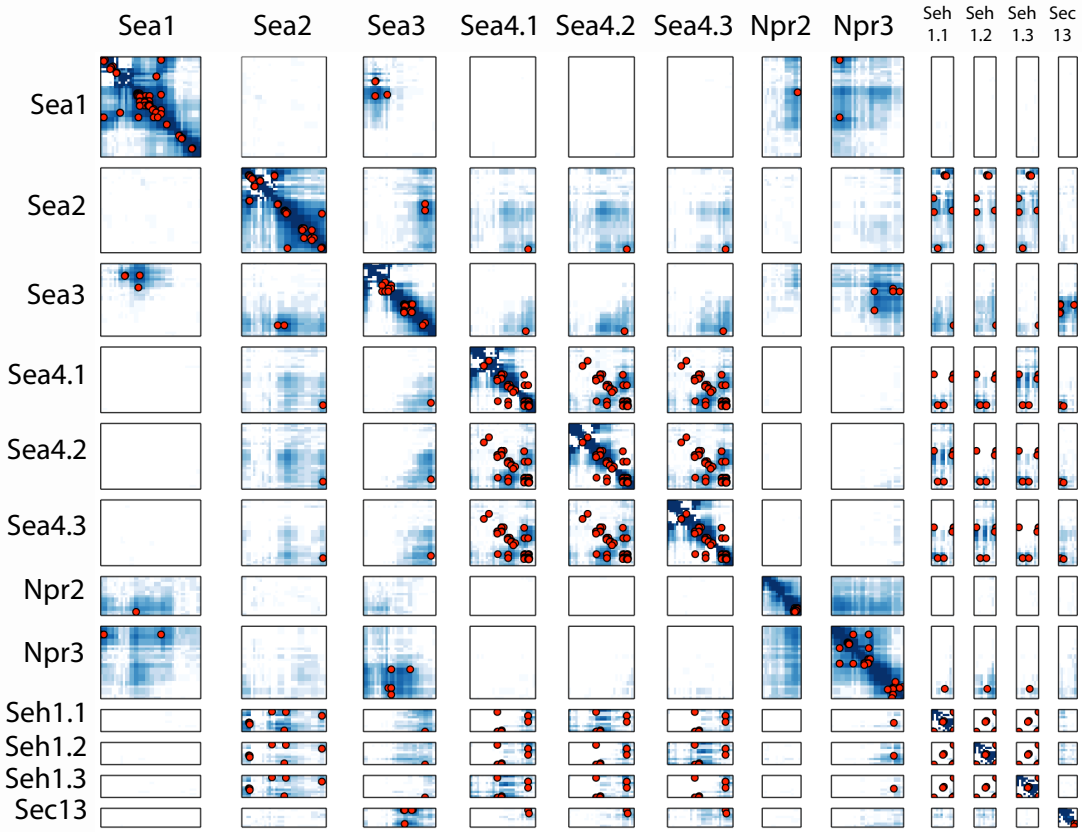
Molecular architecture of the SEA complex



- 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



Contact frequency map and crosslinks

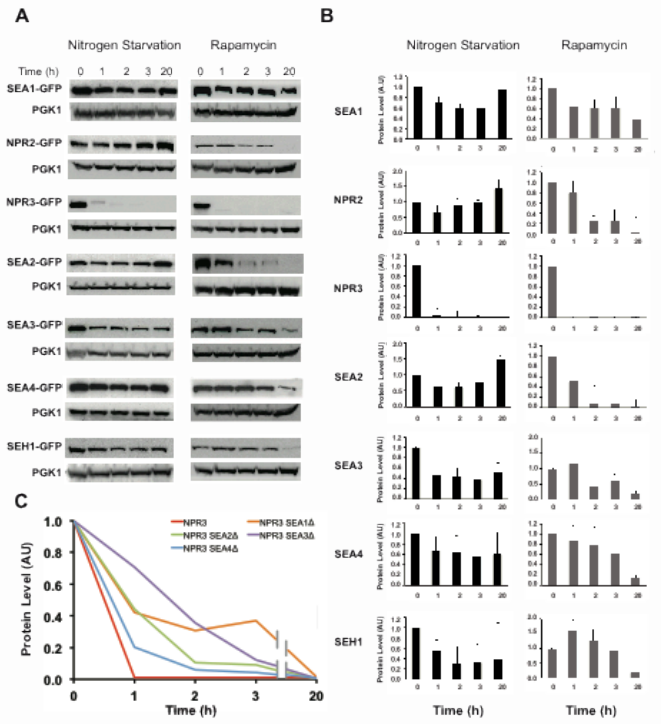


The proximities of any two residues in the topological map were measured by their relative "contact frequency". A contact between a pair of residues is defined when their corresponding bead surfaces are less than 20 Å from each other.

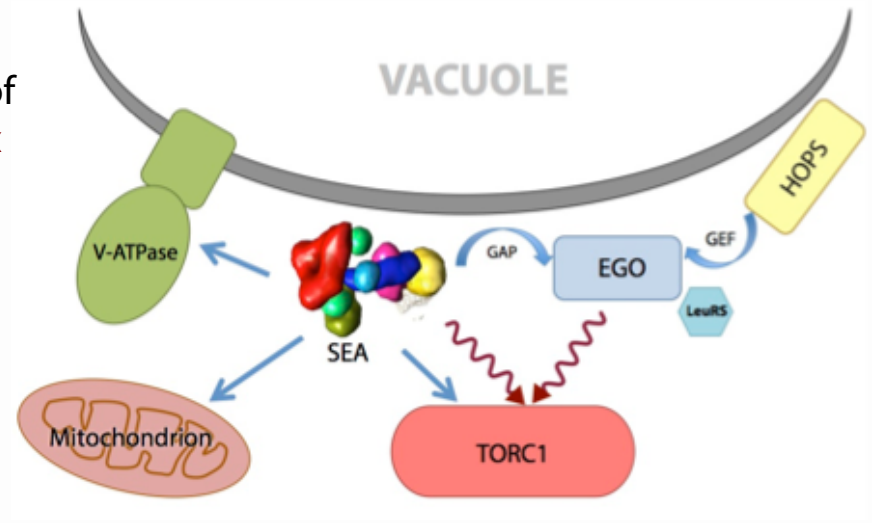
Crosslinks were plotted as the red dots, and the residue contact frequency is indicated by a color ranging from white (0) to dark blue (1). Each box contains the contact frequency between the corresponding pair of the SEA complex proteins.

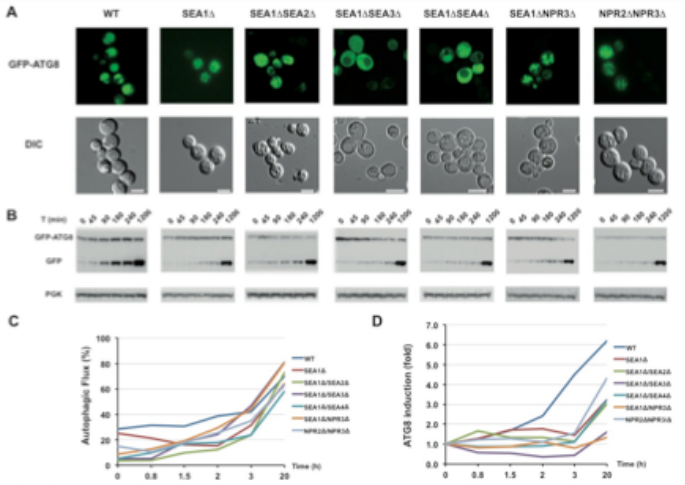
Functional implications

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

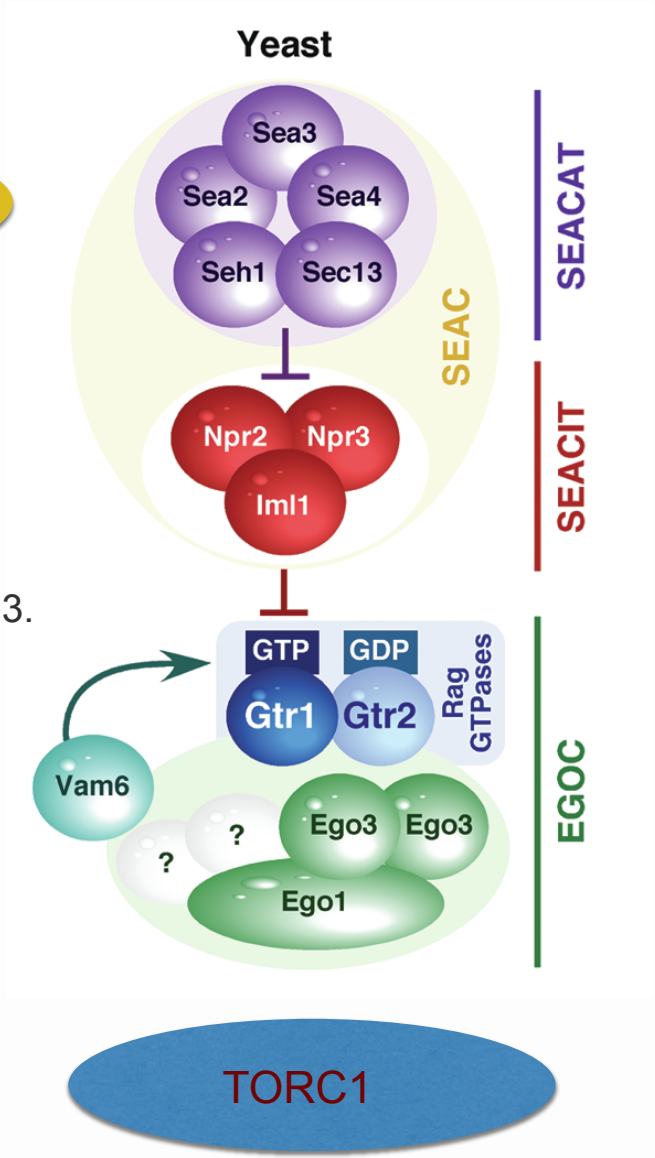




Sea1 is involved in the regulation of general autophagy

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.

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

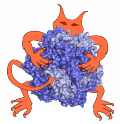


membrane-associated scaffold, needed for activation of TORC1

interacts with and inhibits TORC1

Adapted from
Panchaud et al. Cell Cycle 12, 2013.

Python Modeling Interface (PMI)



PMI provides repository of python classes and libraries to represent, score, sample and analyze models based on IMP.

Easy-to-use python libraries.

Create hierarchies, rigid bodies, and flexible parts for bead representations

In [ ]:
```
################################################
# Create hierarchies and rigid bodies and flexible parts
# for bead representations
################################################
m = IMP.Model()
simo = representation.SimplifiedModel(m,upperharmonic=True,disorderedlength=True)

# SEA1
tmp_color=0.5
simo.add_component_name("SEA1")
ds=[(1,50),(51,100)]; simo.add_component_beads("SEA1", ds,colors=[tmp_color])
simo.add_component_pdb("SEA1", '../pdb/SEA1_101-275.pdb', "A", resolutions=res, color=tmp_color)
ds=[(276,278)]; simo.add_component_beads("SEA1", ds,colors=[tmp_color])
simo.add_component_pdb("SEA1", '../pdb/SEA1_279-473.pdb', "A", resolutions=res, color=tmp_color, resrange=(279,331))
ds=[(332,343)]; simo.add_component_beads("SEA1", ds,colors=[tmp_color])
simo.add_component_pdb("SEA1", '../pdb/SEA1_279-473.pdb', "A", resolutions=res, color=tmp_color, resrange=(344,376))
ds=[(377,399)]; simo.add_component_beads("SEA1", ds,colors=[tmp_color])
simo.add_component_pdb("SEA1", '../pdb/SEA1_279-473.pdb', "A", resolutions=res, color=tmp_color, resrange=(400,473))
ds=[(474,526),\
    (527,609),(610,692),(693,775),(776,859),\
    (860,948),(949,1037),(1038,1126),\
    (1127,1177)]; simo.add_component_beads("SEA1", ds,colors=[tmp_color])
simo.add_component_pdb("SEA1", '../pdb/SEA1_1178-1273.pdb', "A", resolutions=res, color=tmp_color)
ds=[(1274,1340),\
    (1341,1421),(1422,1502),(1503,1584)]; simo.add_component_beads("SEA1", ds,colors=[tmp_color])
simo.setup_component_sequence_connectivity("SEA1", res_cry)
```

Cross-link restraint

In [ ]:
```
################################################
# Cross-link restraint
# sample format: "ssl1 ssl1 196 200"
################################################
res_XL = res_cry
xl = restraints.ConnectivityCrossLinkMS(prot, inputs.XL_input, expdistance=17., resolution=res_XL)

xl.add_to_model()
#xl.set_weight(0.1)
outputobjects.append(xl)
```

Excluded Volume Restraint

In [ ]:
```
################################################
# Restraints setup
# Excluded Volume restraint
################################################
ev = restraints.ExcludedVolumeSphere(prot, resolution=res_ev)
ev.add_to_model()
outputobjects.append(ev)
```

Monte Carlo Samplig and Optimization

In [ ]:
```
################################################
# Monte Carlo
################################################
mc = samplers.MonteCarlo(m,sampleobjects, 0.5)
mc.set_simulated_annealing(0.5, 10.0, 100, 50)
outputobjects.append(mc)

sw = tools.Stopwatch()
outputobjects.append(sw)

output = output.Output()
################################################
# the fields rmf_file and rmf_frame_index will be printed into the
# stat file, so that you'll keep track of the rmffile and the frame
# corresponding to that entry in the stat file
################################################
output.init_stat2(inputs.stat_output, outputobjects,\
                  extralabels=["rmf_file","rmf_frame_index"],\
                  listofsummedobjects=[(partialscore1,"PartialScore1"),(partialscore2,"PartialScore2")])
```

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