Integrative Structural Biology Workshop

UCSF
University of California
San Francisco

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

Seung Joong Kim

Bioengineering & Therapeutic sciences

Andrej Šali Lab

University of California, San Francisco

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Please download the tutorial from github

git clone https://github.com/salilab/Workshop_SEA.git

Integrative determination of macromolecular structures

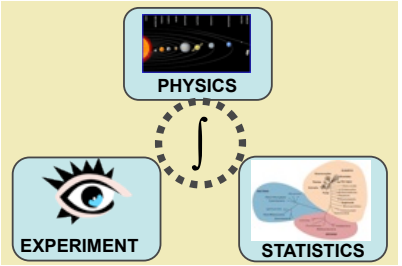
for maximizing accuracy, resolution, completeness, and efficiency of structure determination

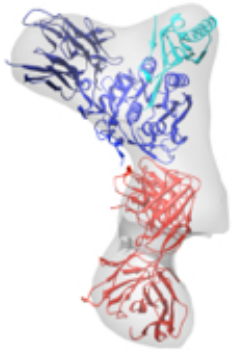
Use structural information from any

source: measurement, first principles, rules;

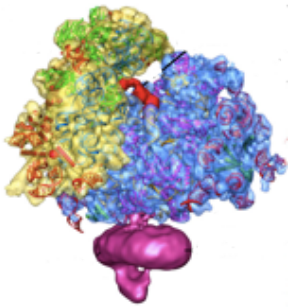
resolution: low or high resolution

to obtain the set of all models that are consistent with it.

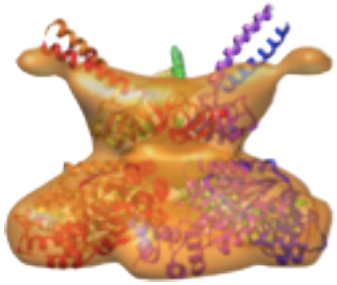




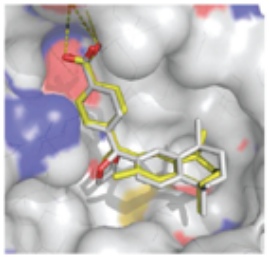
PCSK9-Fab



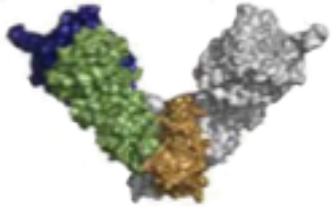
ribosome



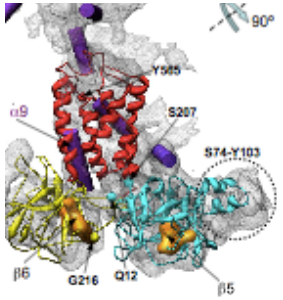
26S proteasome



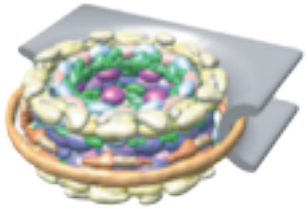
RXRa



HSP90



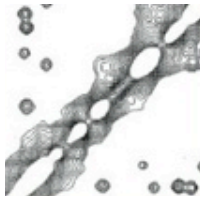
RyR1



NPC

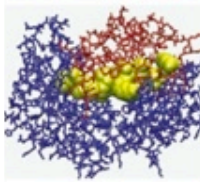
atom positions → residue positions → member orientations → member positions

Integrating various sources of data



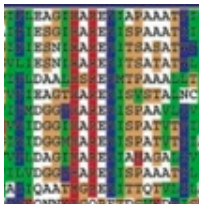
NMR

structure prediction

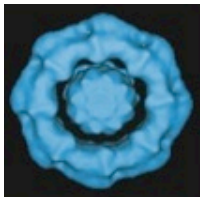


site-directed mutagenesis

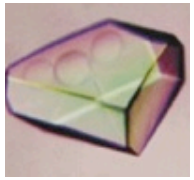
affinity purification



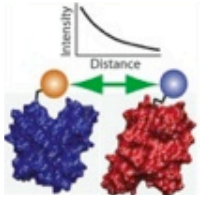
bioinformatics



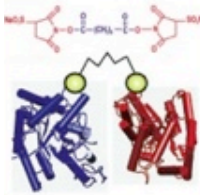
cryo-EM



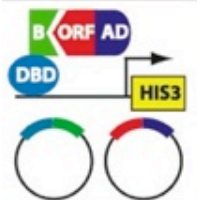
X-ray structures



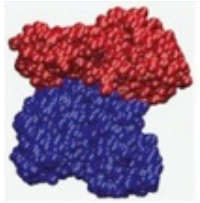
FRET



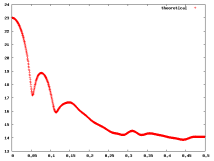
crosslinking



yeast two-hybrid



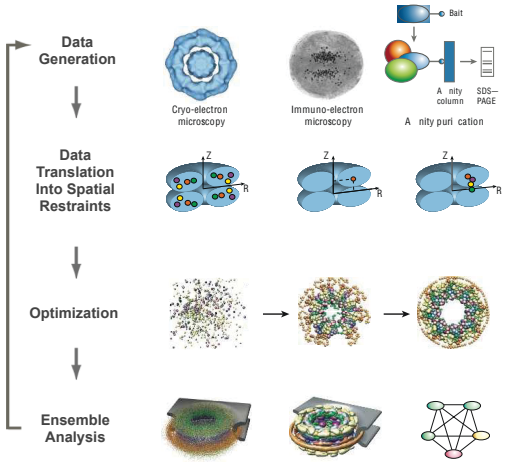
computational docking

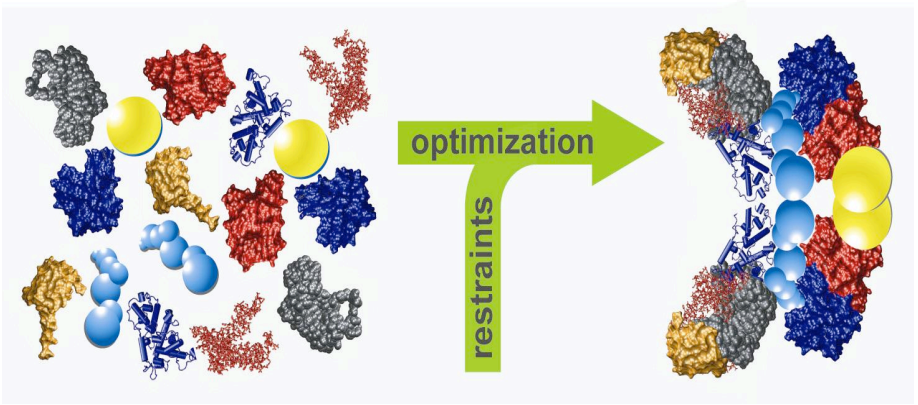


SAXS

atom positions → residue positions → member orientations → member positions

Why Integrative Modeling?





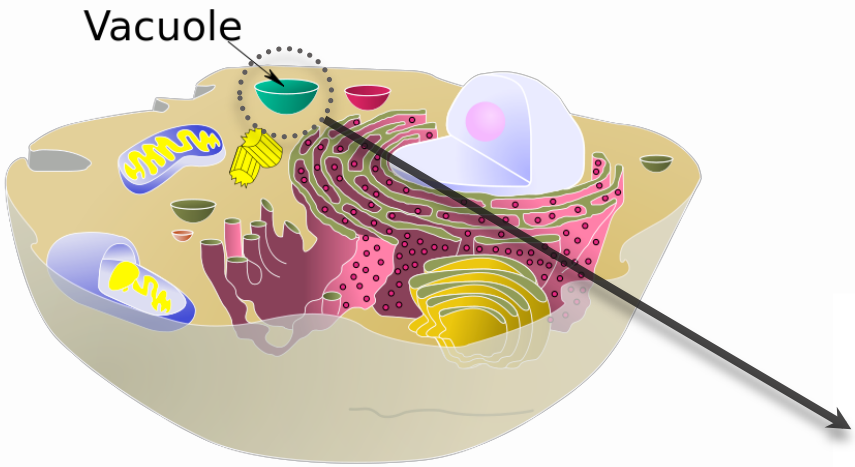
1. Benefits from the **synergy** among the input data, maximizing accuracy, resolution, completness, and efficiency of structure characterization.

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", ...).

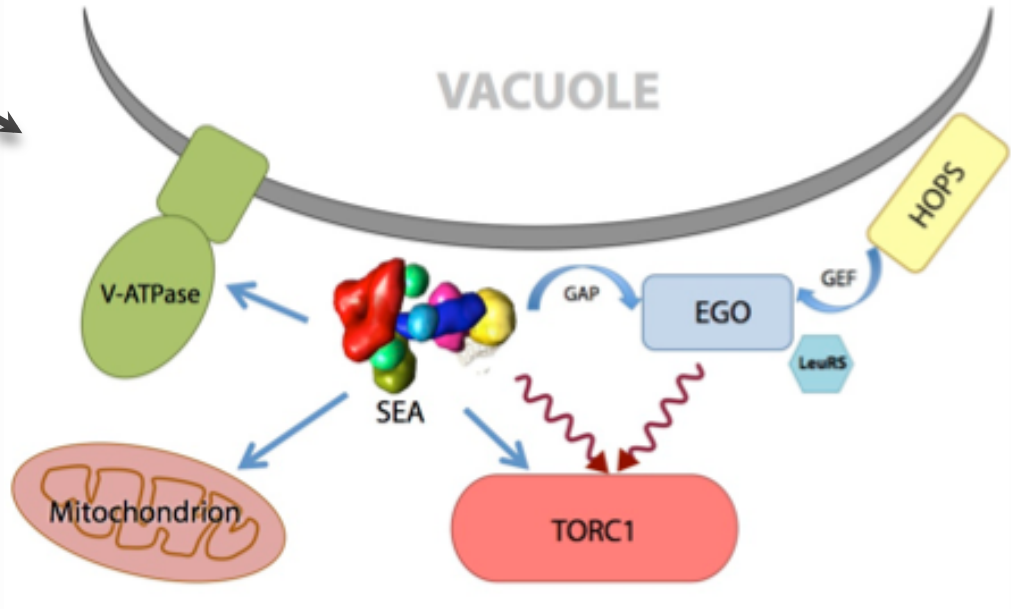
SEA (Seh1-asasociated) complex, a major regulator of the TORC1 pathway



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.

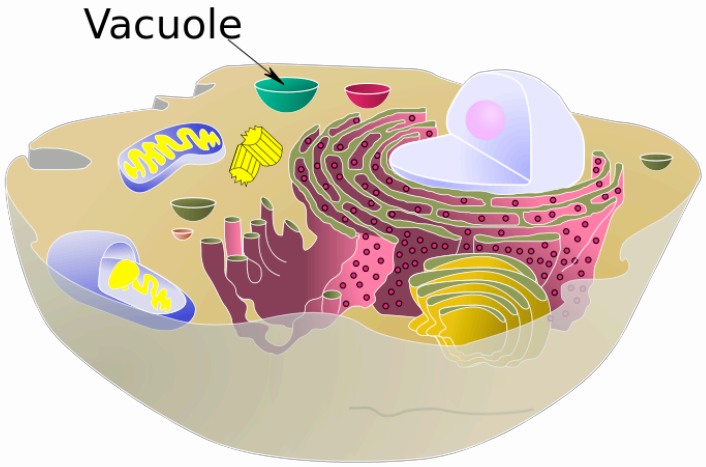
The SEA complex physically interacts with TORC1 and is an important regulator of its activity.



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

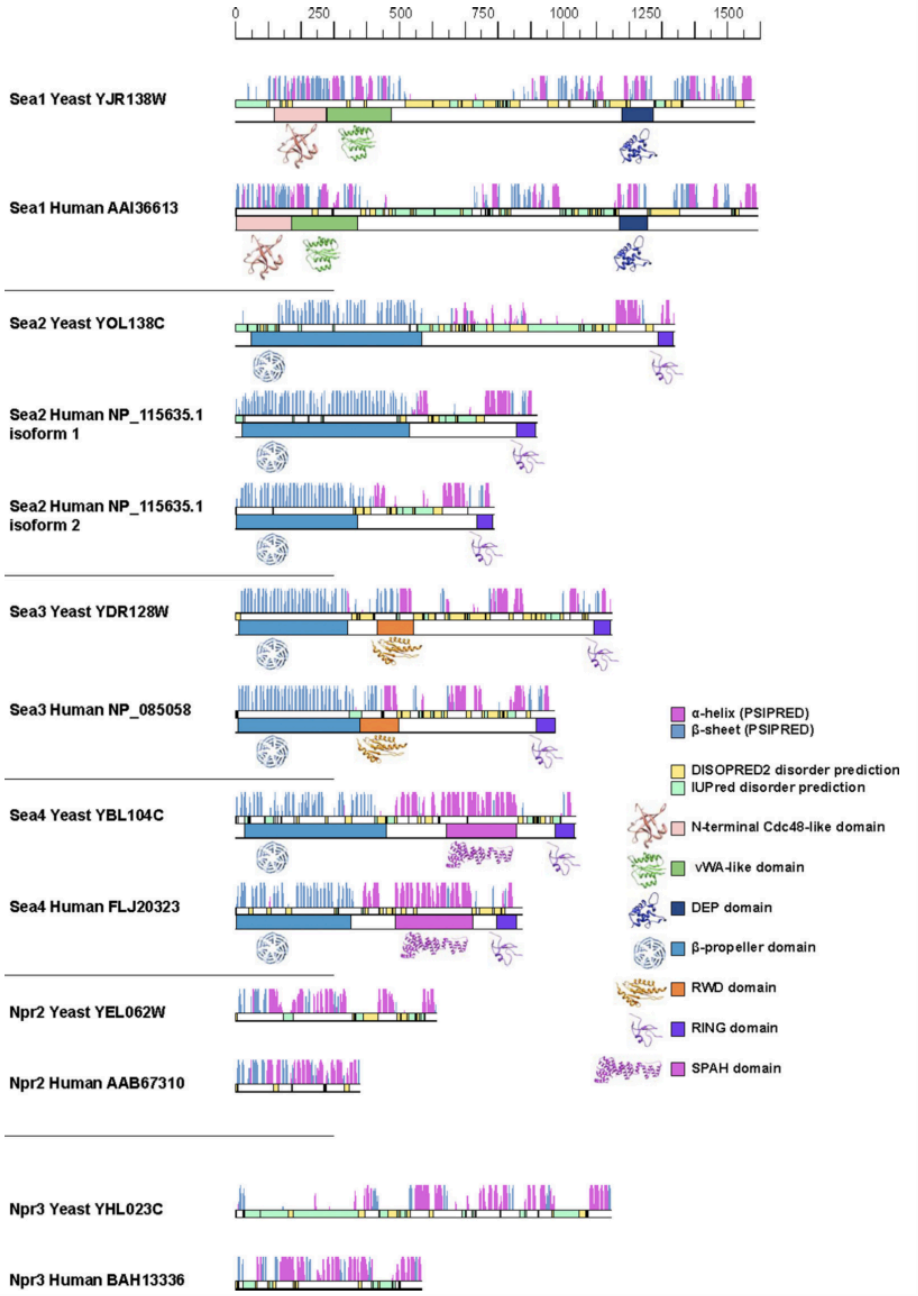
TABLE II

Summary of composition and domain architecture of various coating assemblies components. β-propeller (cyan), SPAH (magenta), and RING (purple) folds are represented schematically. Sea4 model was created by combination of ModWeb models for the β-propeller and the RING domains, and I-TASSER model for the SPAH domain (see Experimental procedures)

Complex	Proteins	Structure	Reference
COPI	α-COP		(5)
	β-COP		(5)
COPII	Sec31		(13-15, 23)
	Sec13		(15)
Clathrin	Clathrin heavy chain		(61)
NPC	Nup170, Nup157, Nup133, Nup120		(3, 4, 15, 16, 76)
	Nup192, Nup188, Nup145C, Nup85, Nup84		
	Sec13, Seh1		
IFT	Ift40, Osm4, Wdr19, Wdr35, Ift172		(17)
	Che2		
	Ift188		
HOPS/ CORVET	Vps3, Vps16		(19)
	Vps8, Vps11, Vps18, Vps41		
SEA	Sea2, Sea3		(This study)
	Sec13, Seh1		(16, 76)
	Sea4		(This study)

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

SEA (Seh1-associated) complex



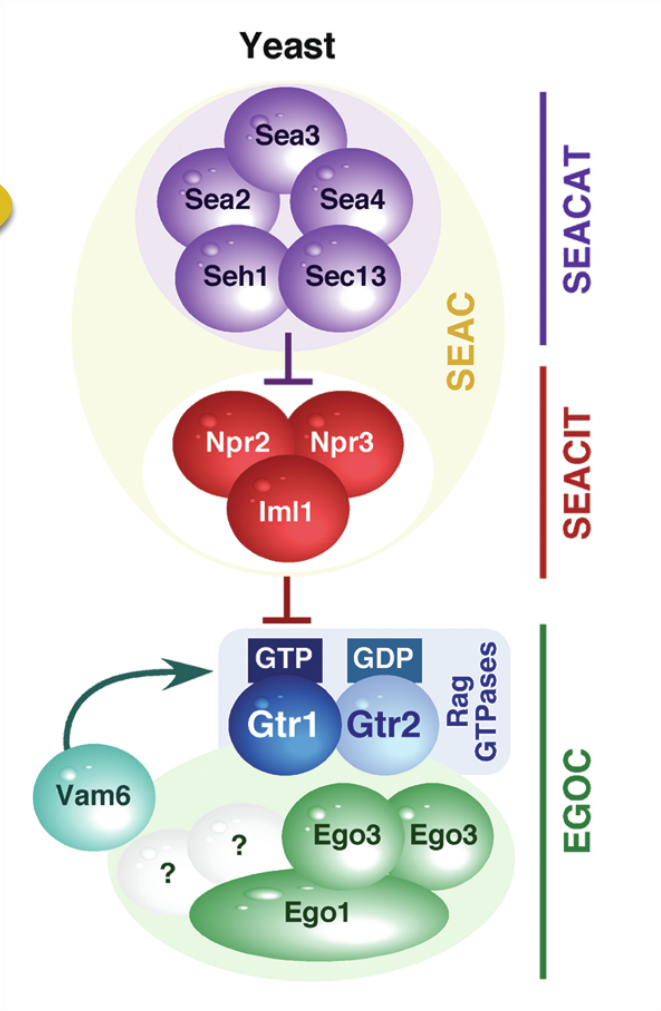
8 component complex

SEA1 (1584 residues)
SEA2 (1341residues)
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

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

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

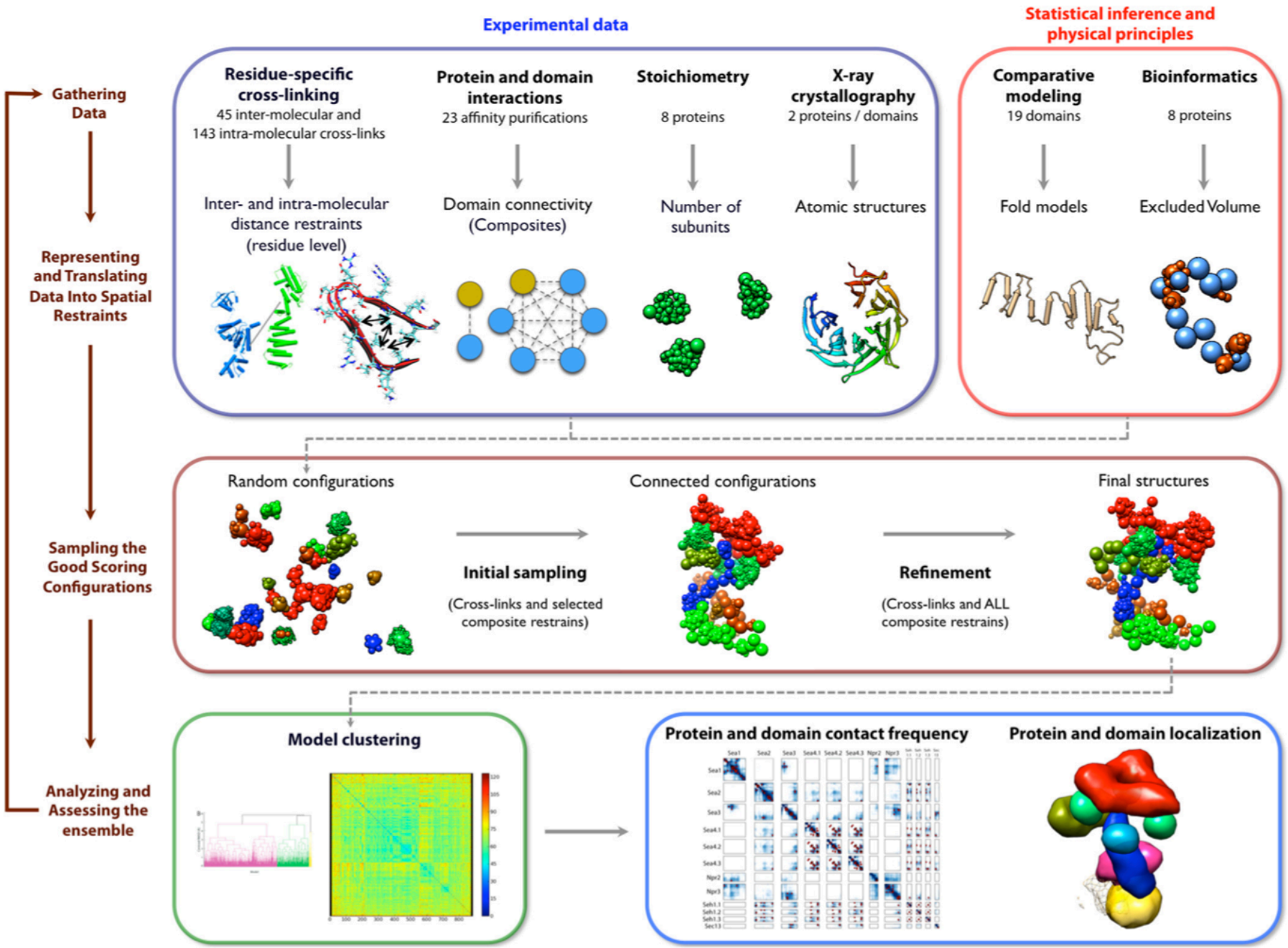


membrane-associated scaffold, needed for activation of TORC1

interacts with and inhibits TORC1

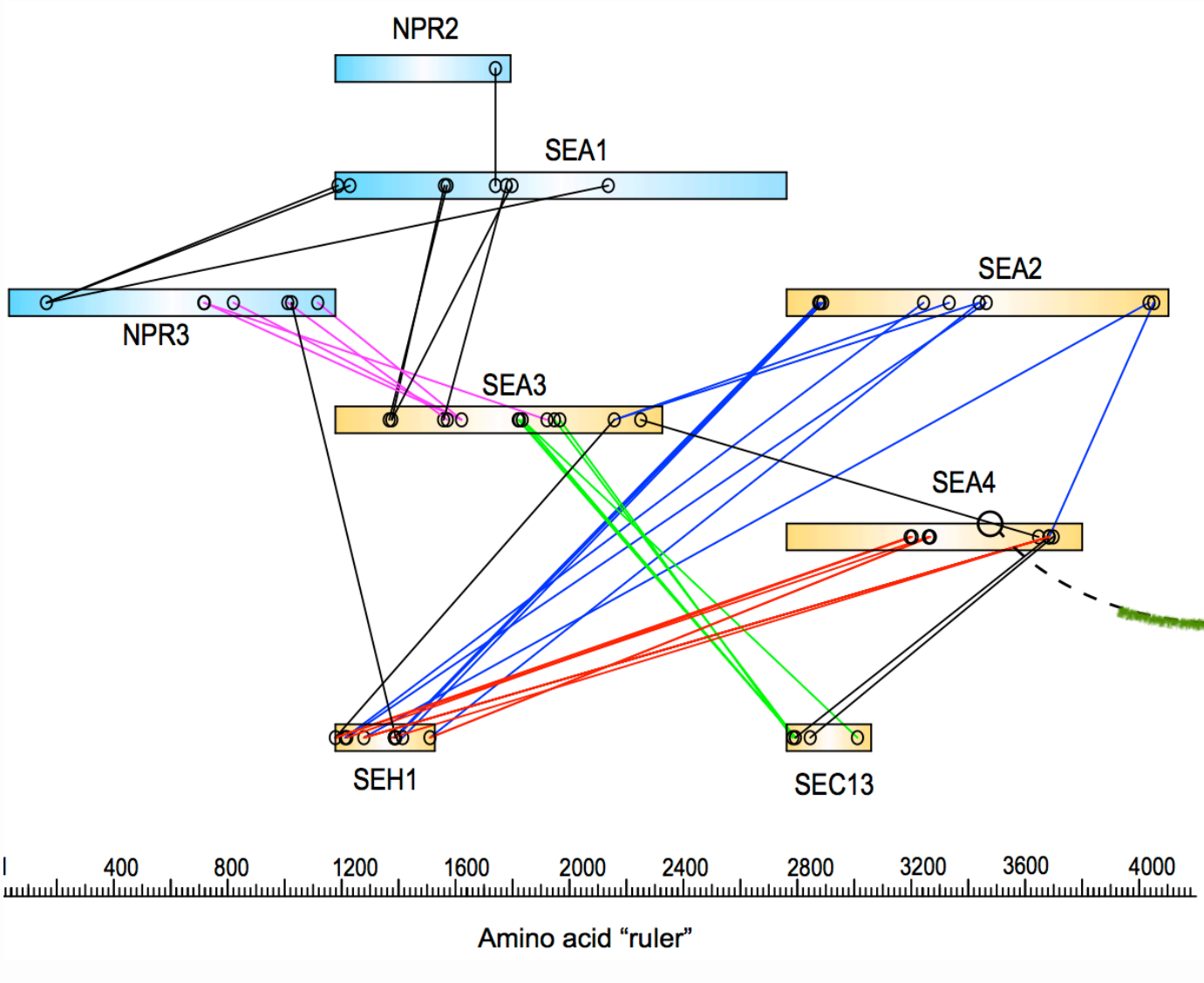
Panchaud et al. Cell Cycle 12, 2013.

Integrative structure determination of the SEA complex

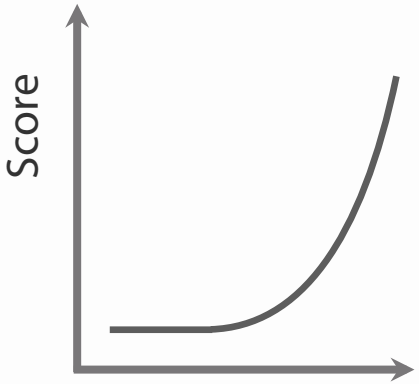


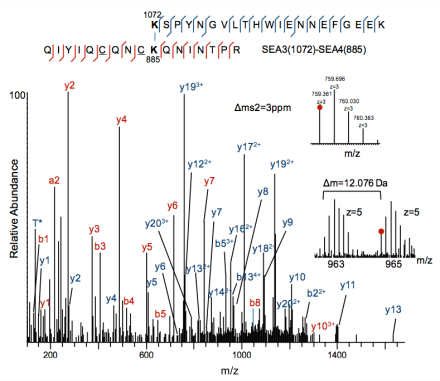
Data 1: Residue-specific DSS chemical cross-links

45 inter-molecular and 143 intra-molecular DSS (Lys-Lys) cross-links



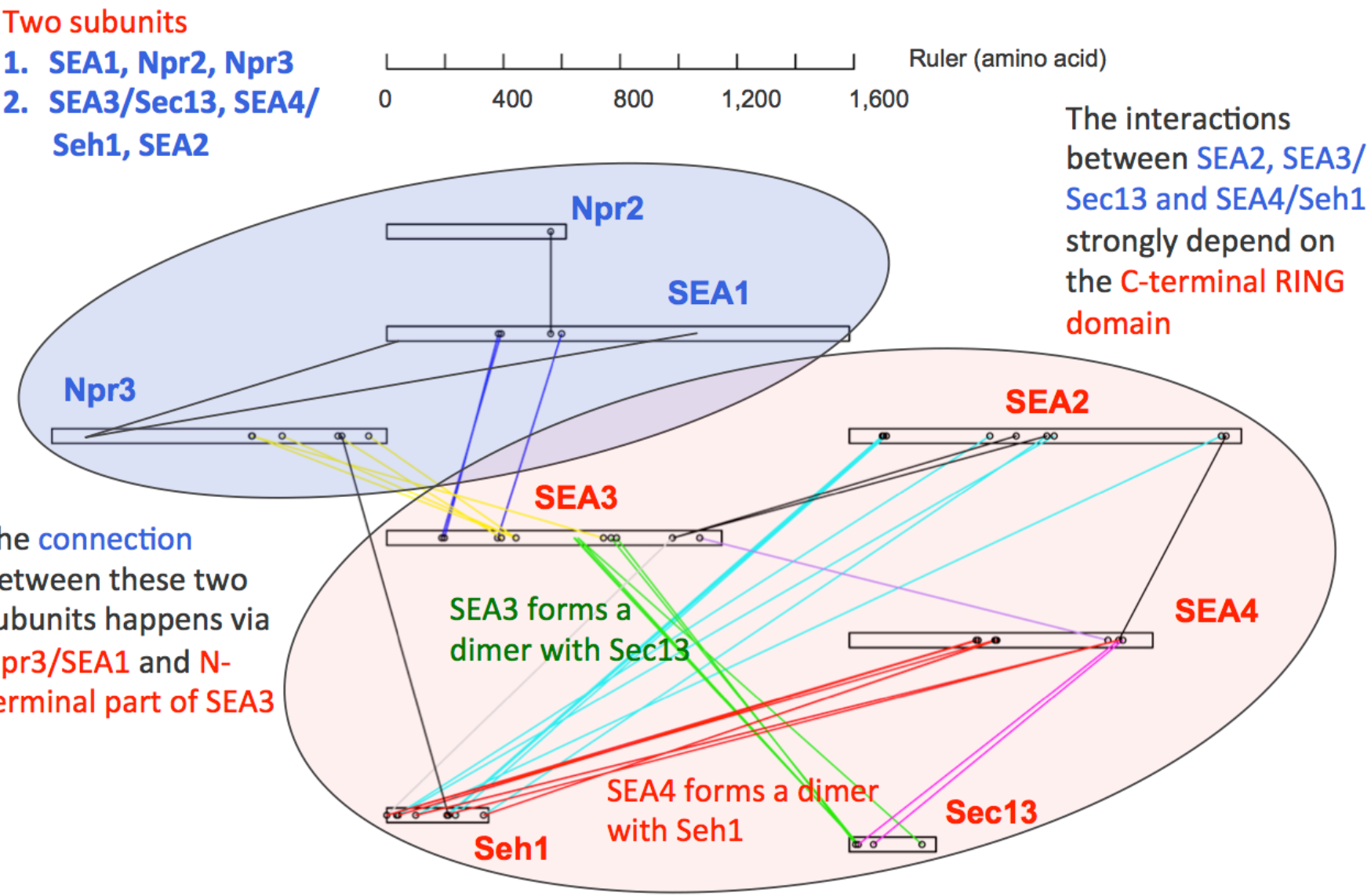
"Upper bound" distance restraints





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

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



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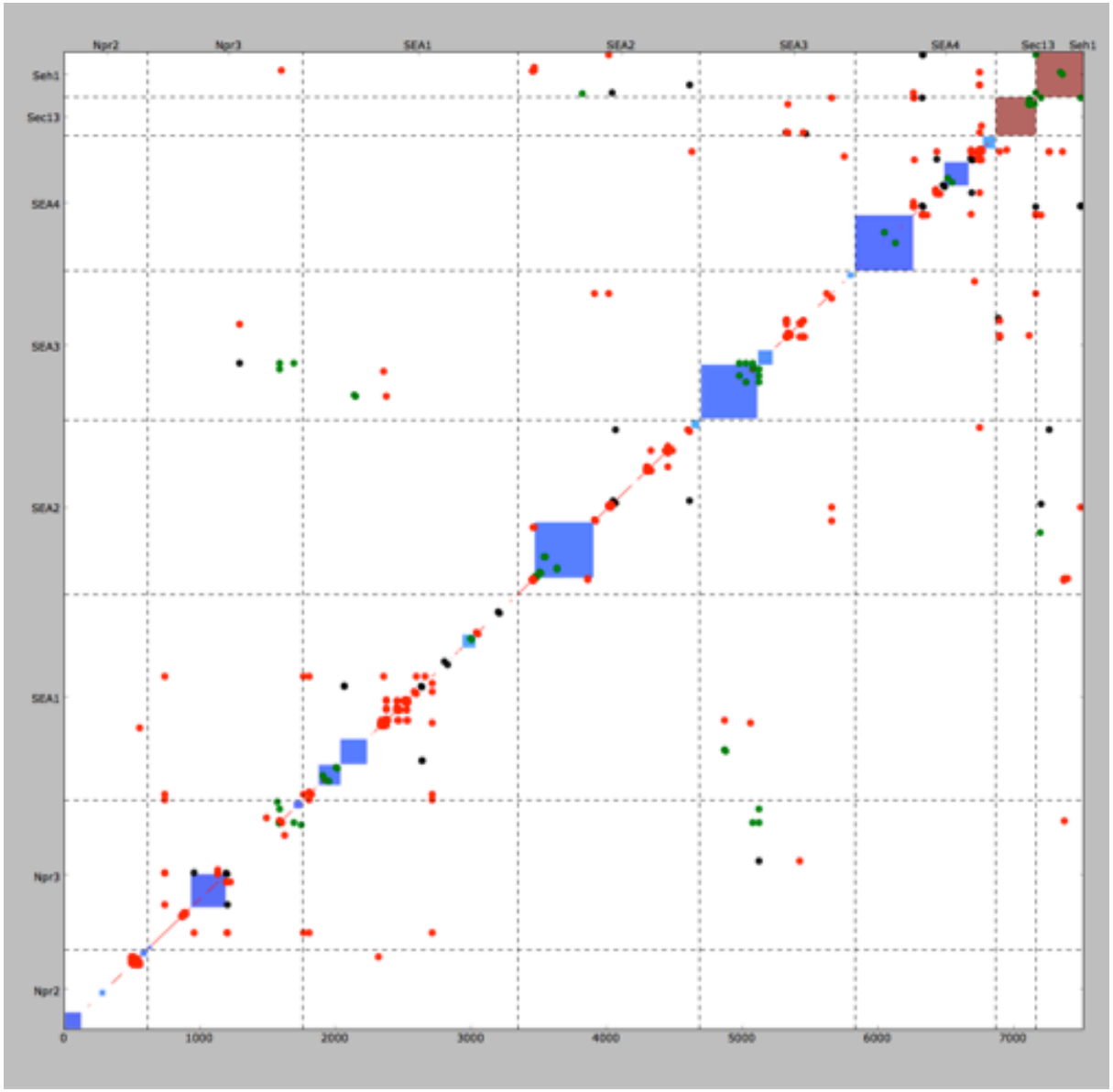
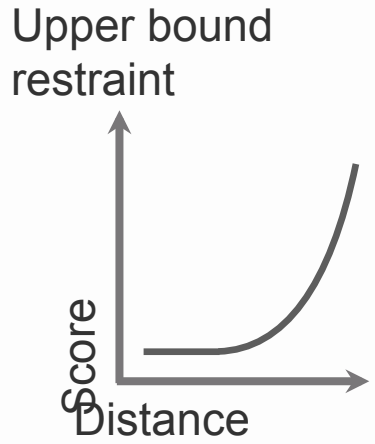
Data: Residue-specific DSS (Lys-Lys) crosslinks

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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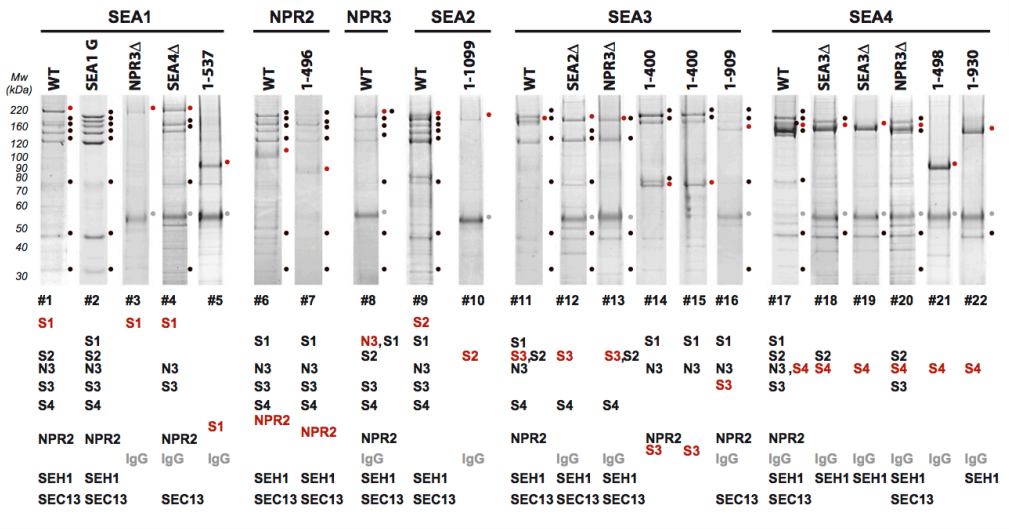
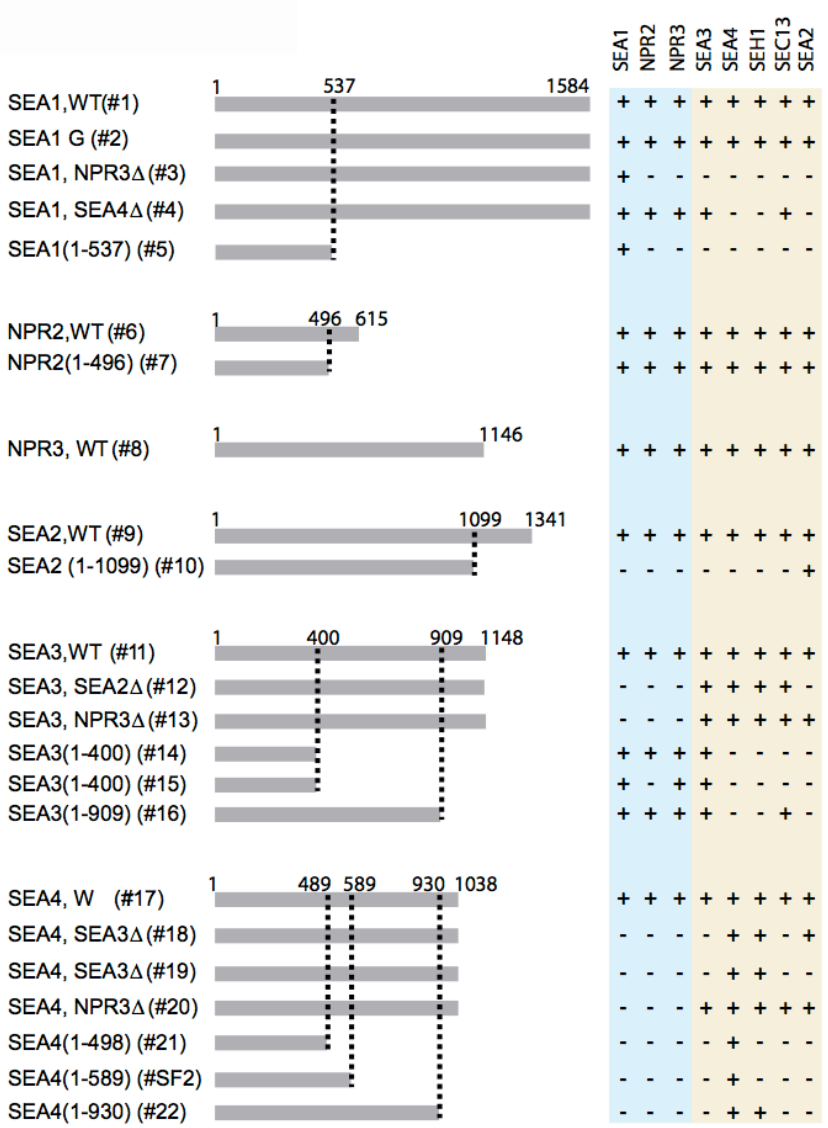
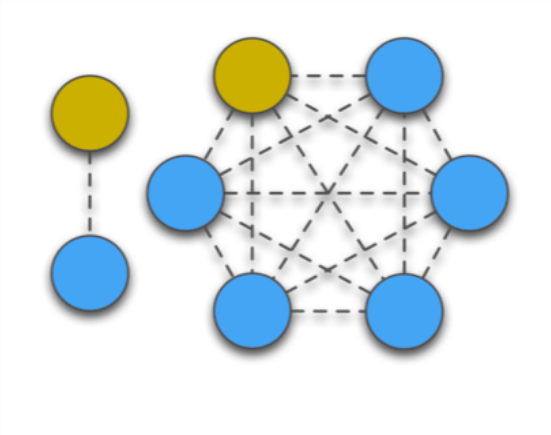
Data 2 : Affinity co-purification

7 protein pullouts, 16 domain deletion pullouts

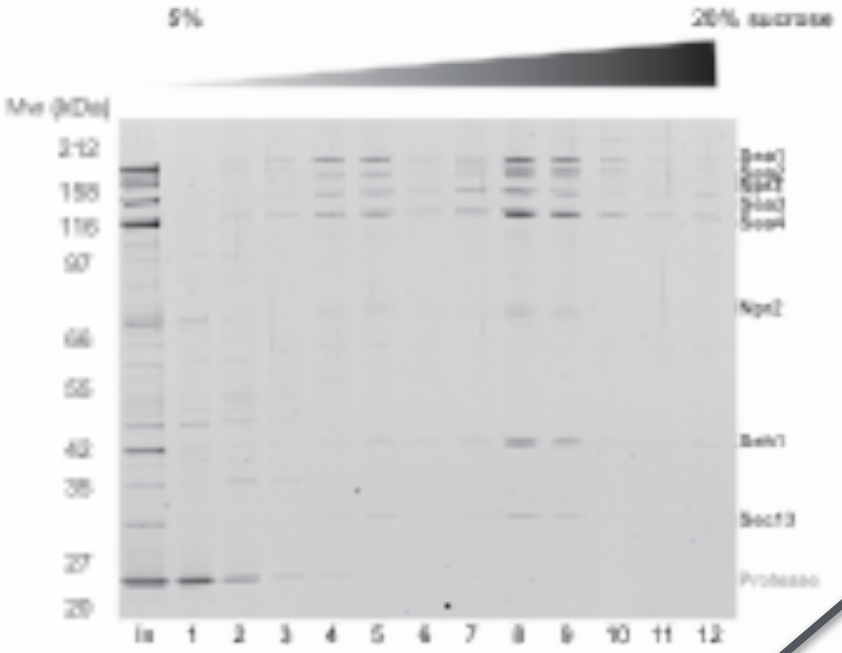


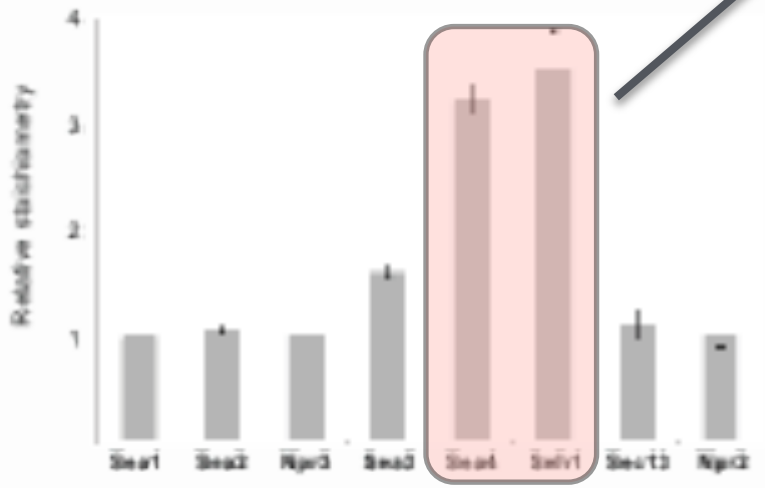


"Composite" restraint



Data 3 : Relative stoichiometry by SYPRO Ruby staining

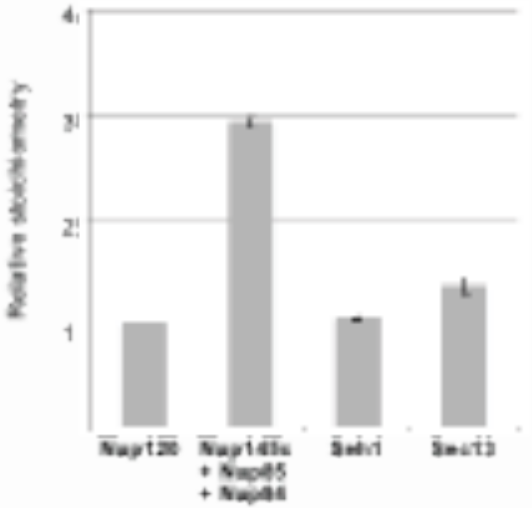




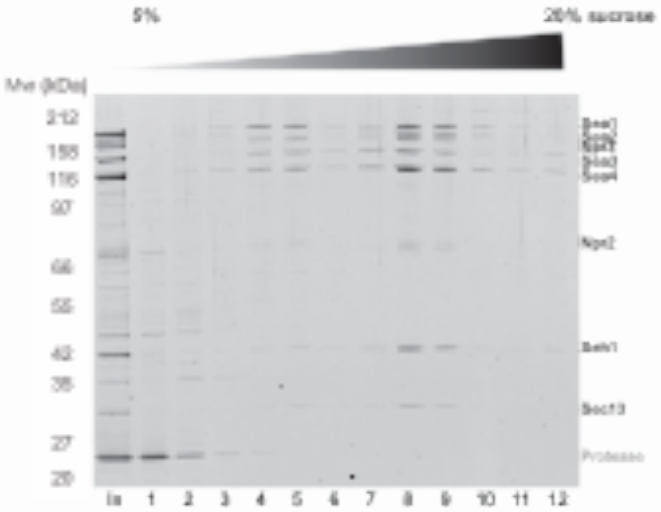
The SEA complex was isolated in 5- 20% sucrose velocity gradients and the resulting 12 equal fractions were analyzed on 4%–12% Bis-Tris gels. Gels were stained with SYPRO Ruby (Molecular Probes) and digitized.

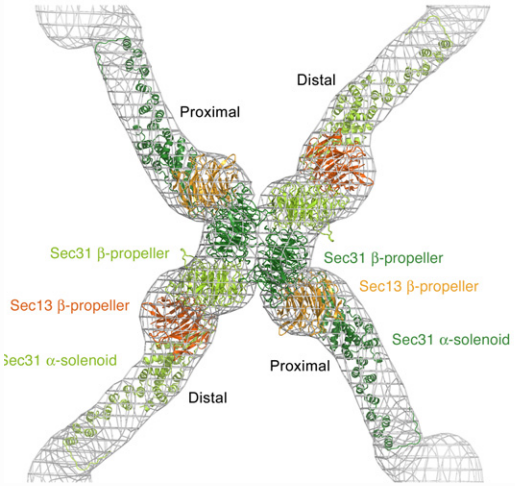
1:3 stoichiometry for Sea4 and Seh1

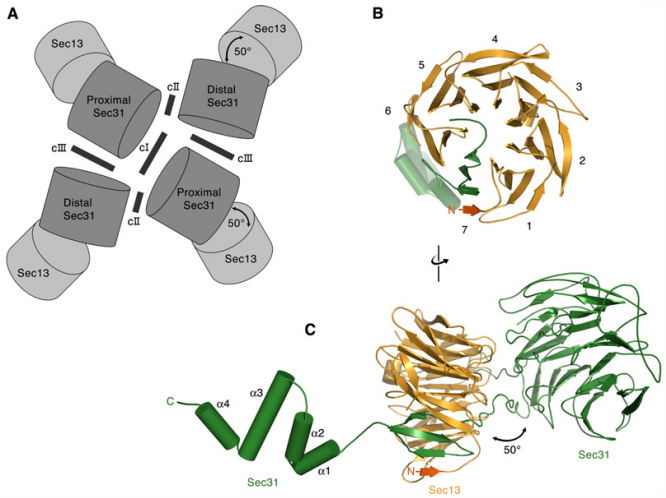
Benchmark with known proteins

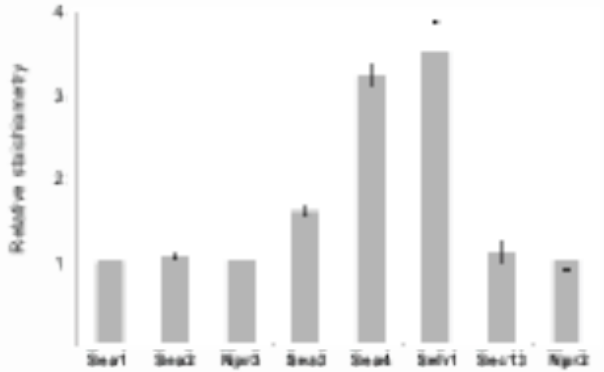


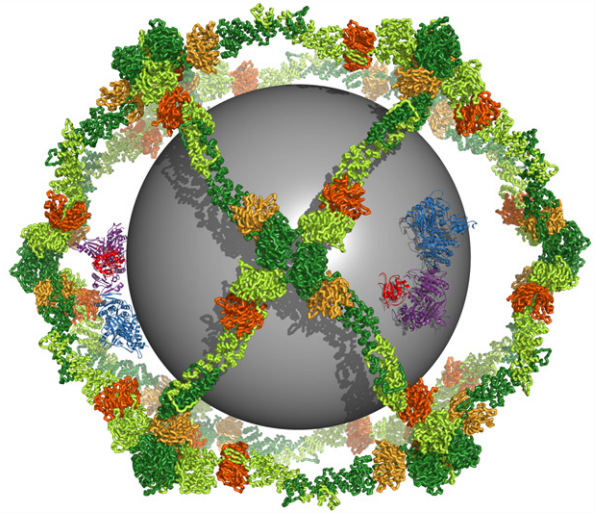
Symmetry


5%
Mw (kDa)
Nup2
Sec13
Protease
In 1 2 3 4 5 6 7 8 9 10 11 12

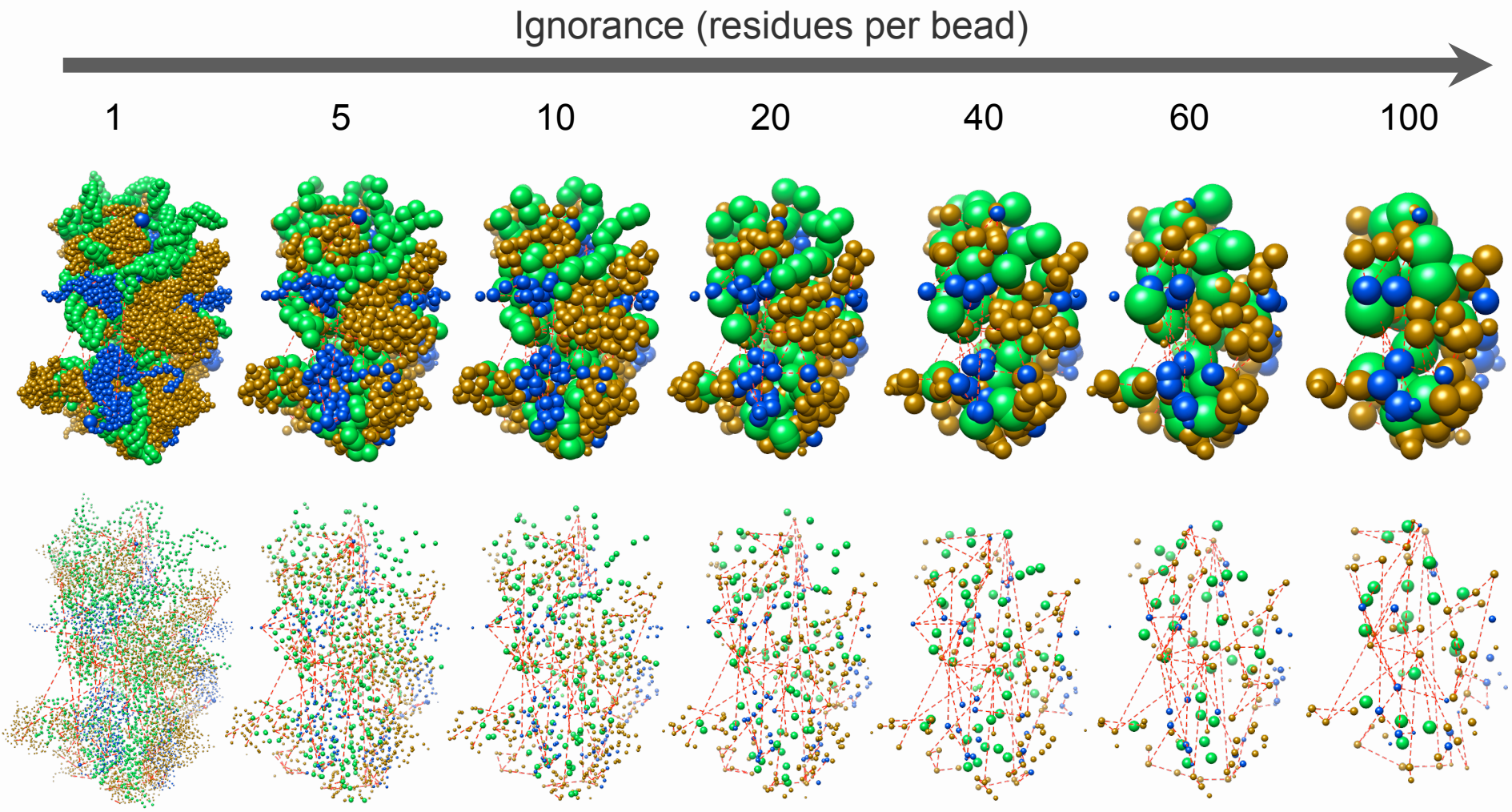

Proximal
Distal
Sec31 β-propeller
Sec13 β-propeller
Sec31 α-solenoid
Sec13 β-propeller
Sec31 α-solenoid
Proximal
Distal


A
Sec13
Proximal Sec31
Distal Sec31
cI
cII
cIII
50°
B
C
α1
α2
α3
α4
Sec31
Sec13
N
C


Relative stoichiometry

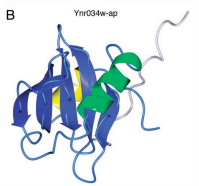


Hierarchical model representation facilitates using imprecise information



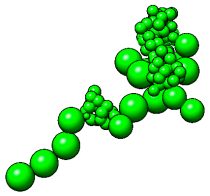
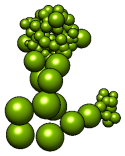
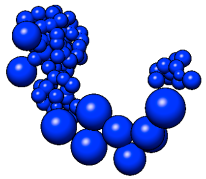
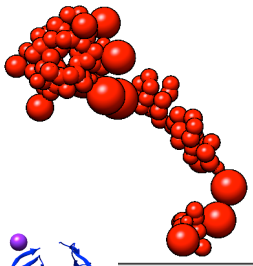
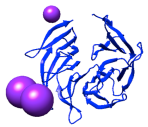
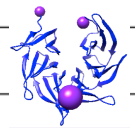
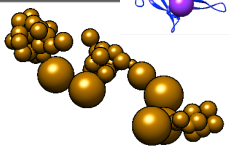
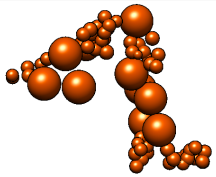
Representation

S.cerevisiae	Domain	Begins at	Ends at	Folds	Representation	# of beads	Residues per bead	Template	Coverage from	Coverage to	Sequence Identity	Predicted by
SEA1	1	1	100	Disordered	Beads	2	50					
	2	101	275	N-terminal Cdc48-like	Bead	1	175	1WLF_A	118	275	15%	HHpred
	3	276	474	vWA-like	Bead	1	199	1JEY_B	279	473	15%	HHpred
	4	476	1175	Disordered / Unknown	Beads	7	100					
	5	1176	1272	DEP	Bead	1	97	1FSH_A	1175	1273	23%	HHpred
	6	1273	1584	Disordered / Unknown	Beads	3	104					
SEA2	1	1	126	Disordered	Beads	2	63					
	2	127	557	Beta Propeller	Bead	1	431	2YMU_A	127	557	16%	HHpred
	3	558	1271	Disordered / Unknown	Beads	7	102					
	4	1272	1341	RING	Bead	1	70	2ECL_A	1280	1340	24%	HHpred
SEA3	1	1	429	Beta Propeller	Bead	1	429	1NR0_A	10	429	15%	HHpred
	2	430	549	RWD	Bead	1	120	2EMB_A	430	536	19%	HHpred
	3	550	1069	Disordered / Unknown	Beads	5	104					
	4	1070	1148	RING	Bead	1	79	2ECM_A	1092	1139	23%	HHpred
SEA4	1	1	458	Beta Propeller	Bead	1	458	1NR0_A	2	426	13%	HHpred
	2	459	642	Disordered / Unknown	Beads	2	92					
	3	643	856	SPAH	Beads	2	107	2PM7_A	659	835	13%	HHpred
	4	857	940	Disordered / Unknown	Bead	1	84					
	5	941	1038	RING	Bead	1	98	2ECL_A	942	1032	23%	HHpred
Seh1	1	1	349	Beta Propeller	3F3F_A	1	349	3F3F_A	1	349	100%	HHpred
Sec13	1	1	297	Beta Propeller	2PM7_B	1	297	2PM7_B	1	297	100%	HHpred
Npr2	1	1	140	longin (variation of a DENN	Bead	1	140	3TW8_A	9	127		Zhang 2012
	2	141	260	Disordered / Unknown	Bead	1	120					
	3	261	306	3K9T_A	Bead	1	46	3K9T_A	262	306	20%	HHpred
	4	307	615	Disordered / Unknown	Beads	3	103	1TC3_C	563	610	17%	HHpred
Npr3	1	1	31	longin (variation of a DENN	Bead	1	31	3TW8_A	1	31	23%	HHpred
	2	32	319	Disordered	Beads	3	96					
	3	320	577	longin (variation of a DENN	Bead	1	258	3TW8_A	322	577		Zhang 2012
	4	578	895	Disordered / Unknown	Beads	3	106					
	5	896	988	Disordered / 4F54_A	Bead	1	93	4F54_A	950	988	18%	HHpred
	6	989	1082	Disordered / Unknown	Bead	1	94					
	7	1083	1140	3HUG_A	Bead	1	58	3HUG_A	1083	1140	11%	HHpred
Total						60						



1:1:1:1:1:1:1:1 Stoichiometry

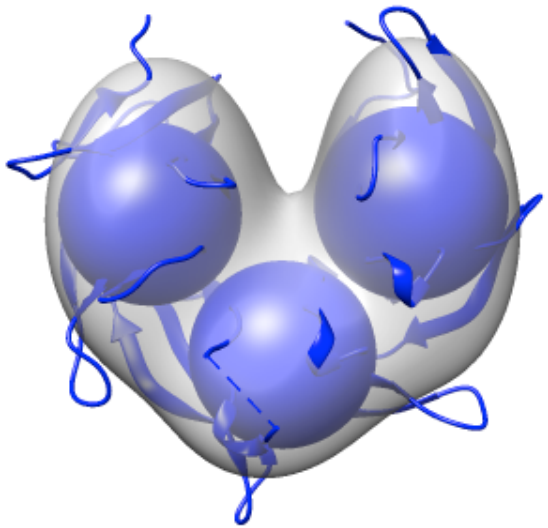
Multi-scale Representation

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	No. of Beads	Residues per Bead	Template	Sequence Identity
Sea1		Disordered					1	100	2	50		
		N-terminal Cdc48-like			101	275					1WLF_A	12%
		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						
							31					
					325	344						
							34					
					390	424						
		Linker					42					
		RWD			430	536						
		Disordered / Unknown Folds					53					
		Unknown Alpha Helices					71					
		Disordered					83					
		Unknown Alpha Helices					95					
		RING			1092	1139						
		Unknown Structure					114					
Sea4.1 Sea4.2 Sea4.3		Unknown Structure										
		Beta Propeller			45	87						
							8					
					124	130						
							13					
					149	272						
							27					
					285	333						
							33					
					356	426						
		Disordered					42					
		Unknown Alpha Helices					49					
		SPAH			659	782						
							70					
					809	835						
		Unknown Structure					81					
		Disordered					86					
		RING			942	963						
		Unknown Structure					103					
Seh1.1 Seh1.2 Seh1.3		Beta Propeller	288	346								
							24					
							34					
Sec13		Beta Propeller	2	158								
			166	296								
							15					
							25					
Npr2		Disordered										
		Longin			9	127						
		Unknown Structure					12					
		3B73_A			257	327						
		Unknown Structure					32					
		Disordered					35					
		Unknown Structure					43					
		Disordered					48					
		1TC3_C			563	610						
		Unknown Beta Strand					61					
Npr3		Longin			1	31						
		Disordered										
		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

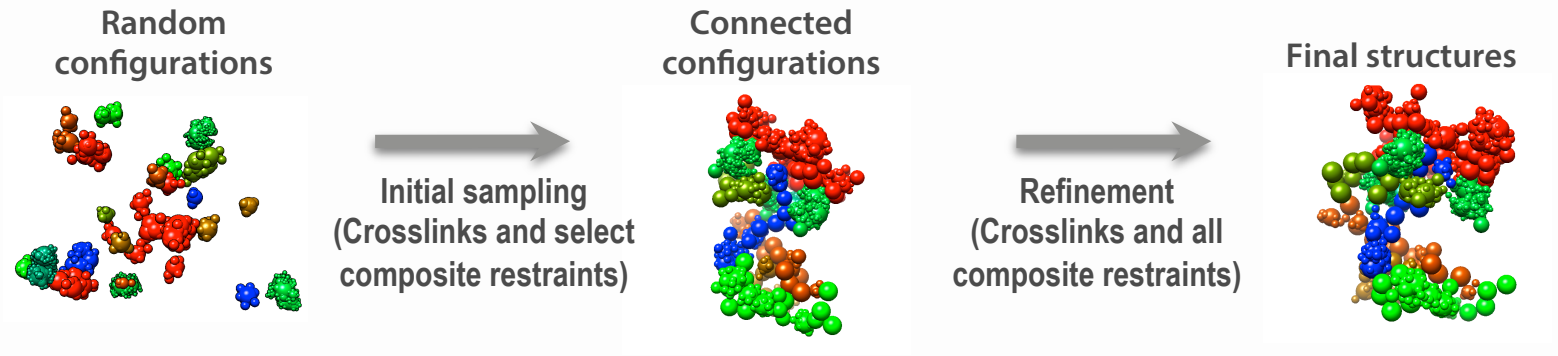


Domain mapping (composite) restraints
shown is also a “Gaussian” envelope

Montel Carlo Sampling

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.

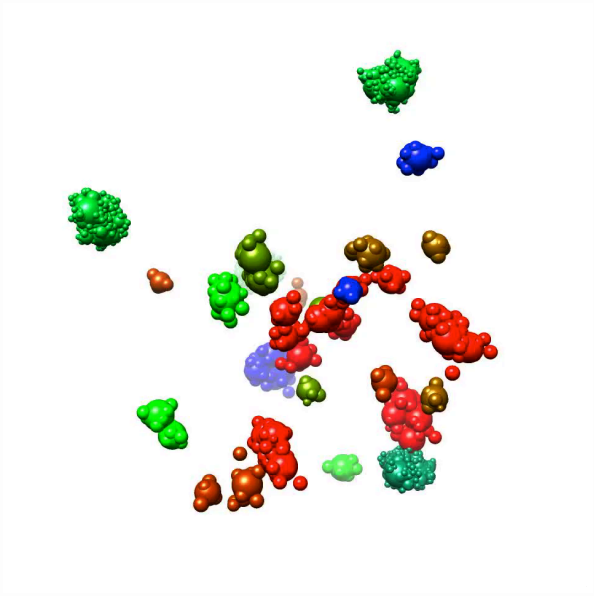
Total score =

188 Harmonic Upper Bounds for Crosslinks (~35Å) +

23 Composite Restraints +

Linkers Between Beads +

Excluded Volume

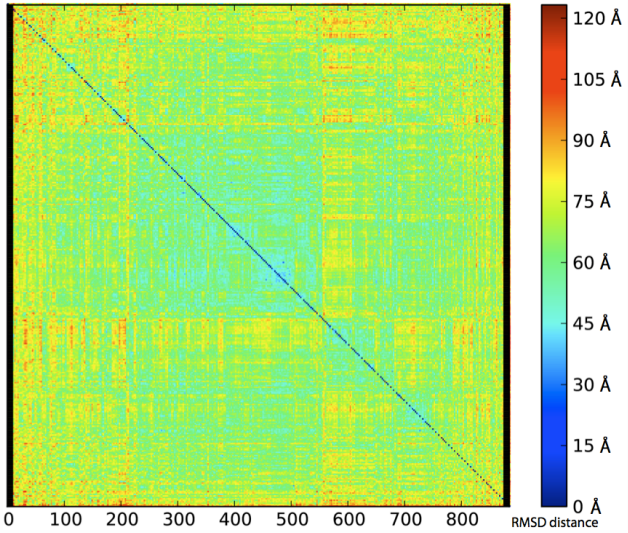


885 best scoring models satisfy all restraints.

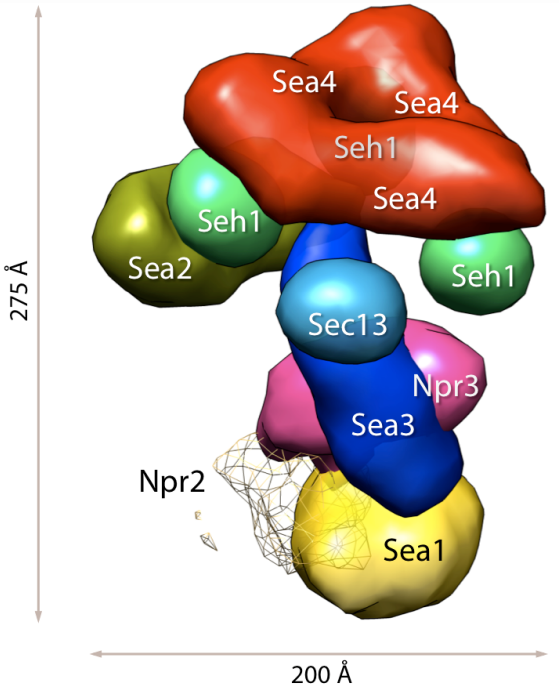
Clustering and the Localization probability map

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

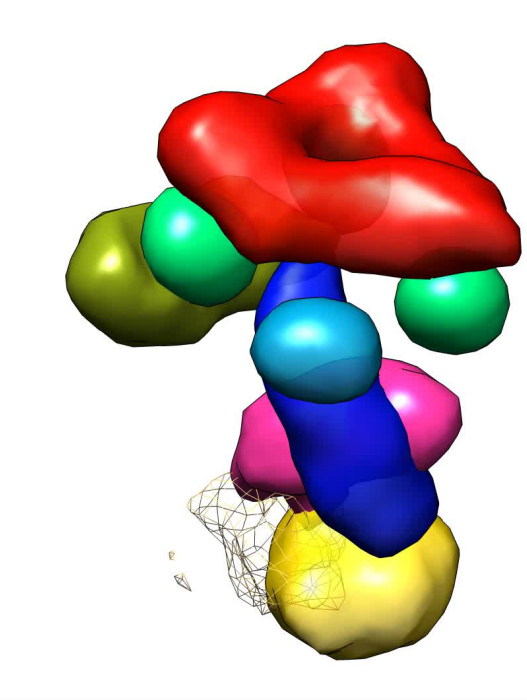
Hierarchical clustering based on the RMSD distance matrix



Protein localization probability

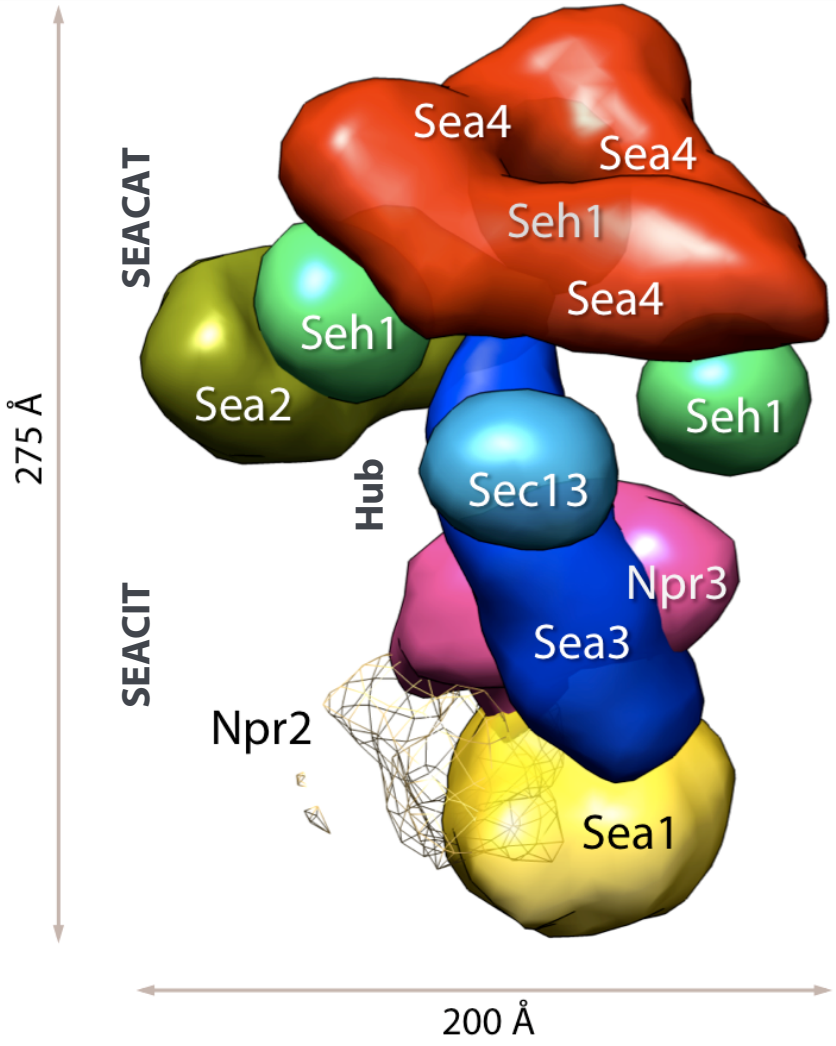


Can see position of every SEA protein !

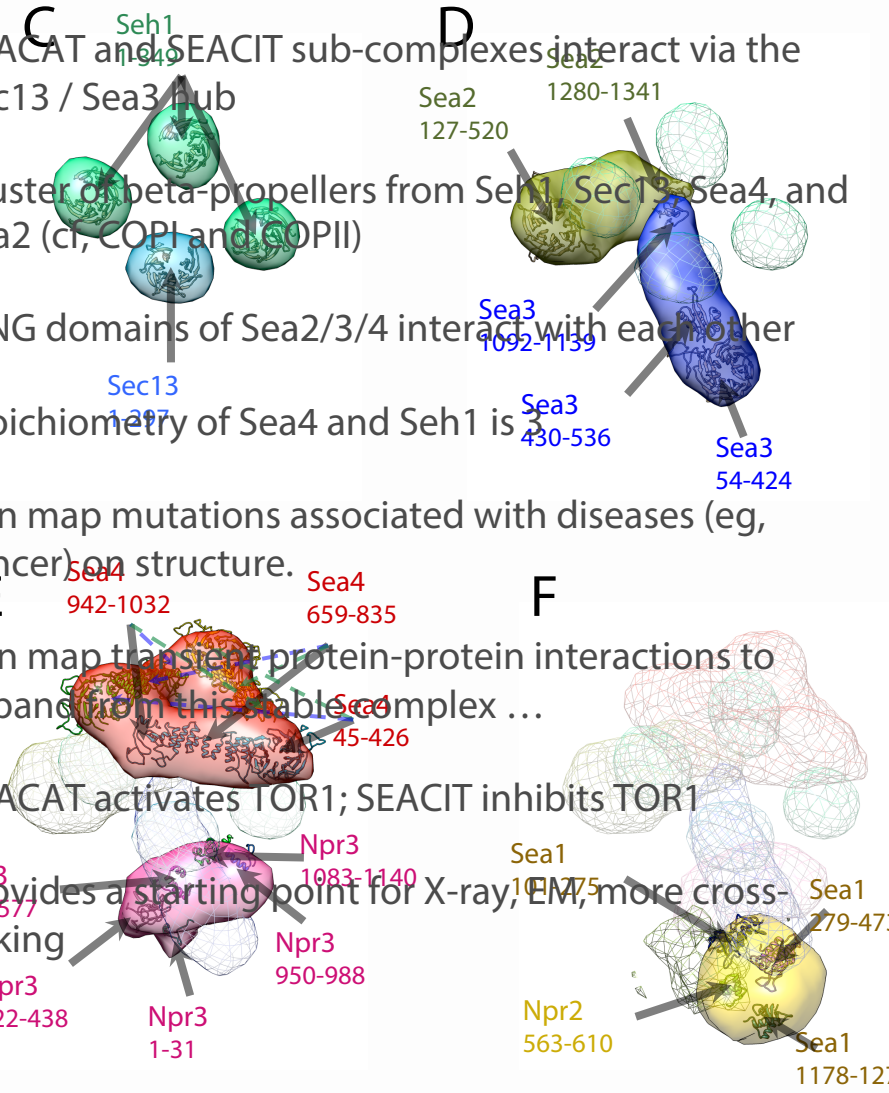


Algret *et al.*, "Molecular Architecture and Function of the SEA Complex, a Modulator of the TORC1 Pathway", MCP, 2014

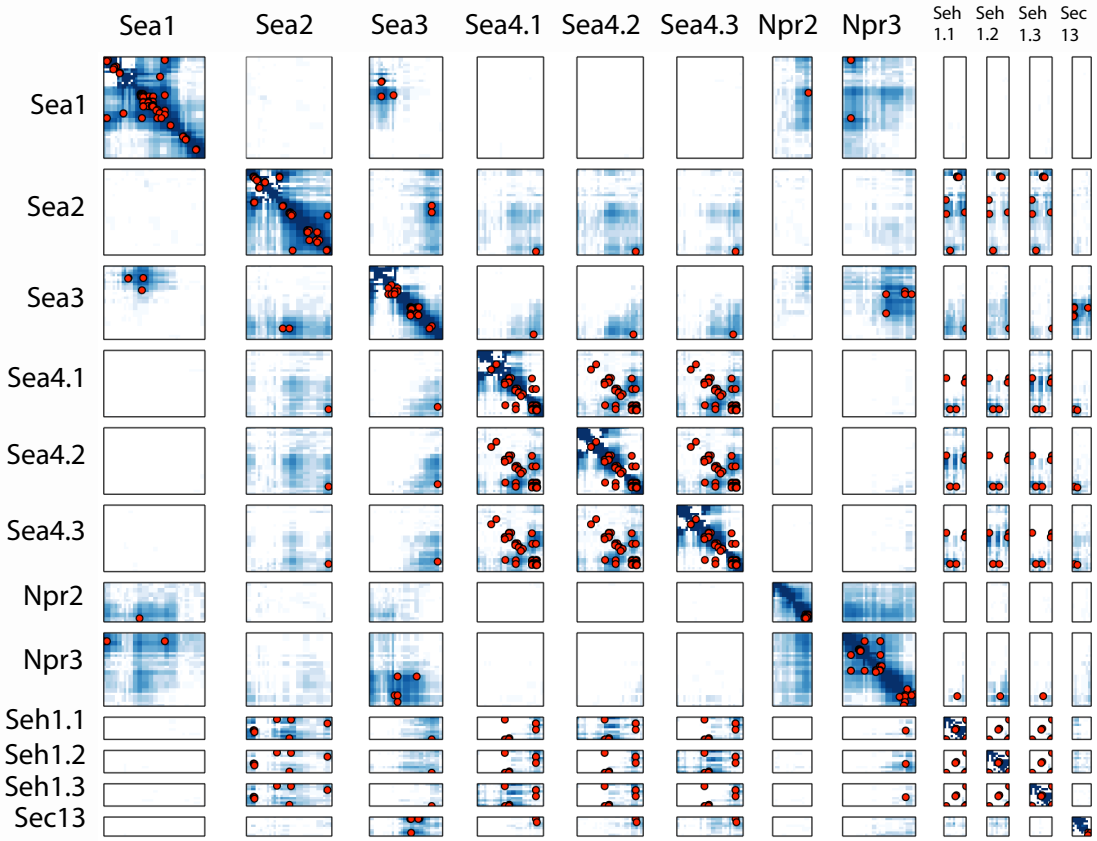
Molecular architecture of the SEA complex



- 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 cross-links

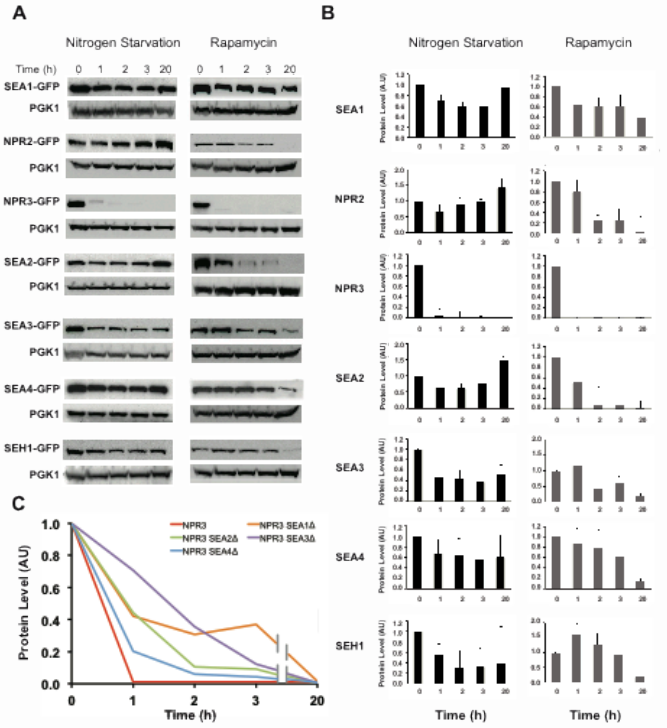


The proximities of any two residues in the topological map were measured by their relative "contact frequency". A contact between a pair of residues is defined when their corresponding bead surfaces are less than 20 Å from each other.

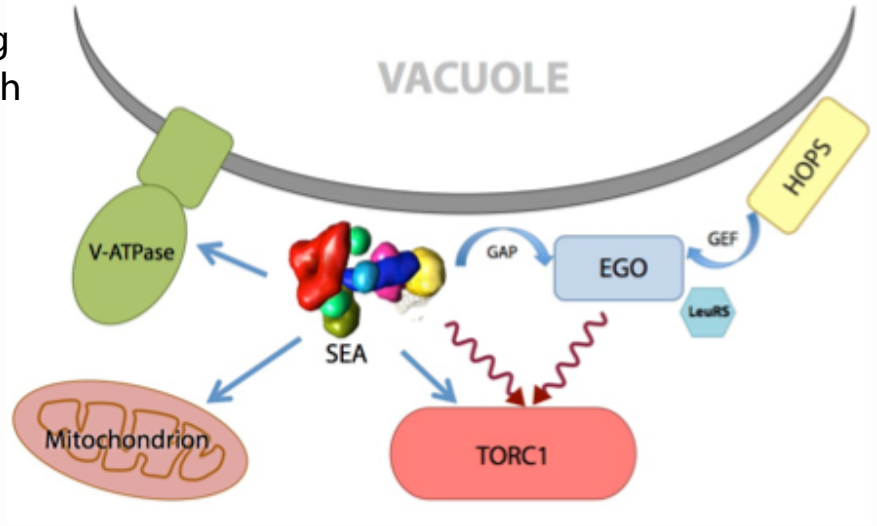
Crosslinks were plotted as the red dots, and the residue contact frequency is indicated by a color ranging from white (0) to dark blue (1). Each box contains the contact frequency between the corresponding pair of the SEA complex proteins.

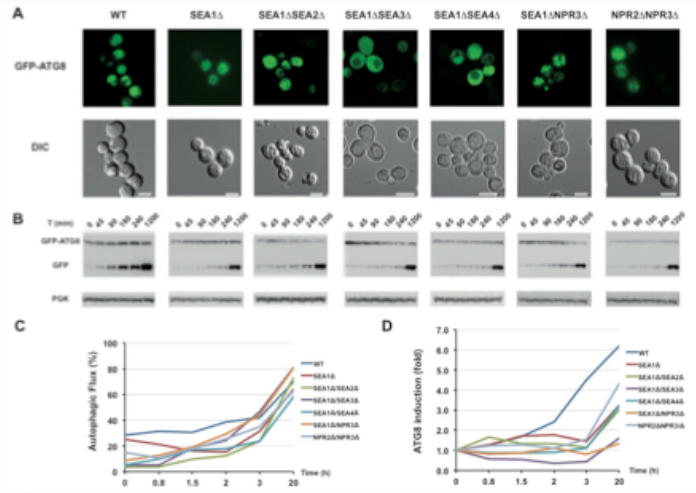
Functional implications

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

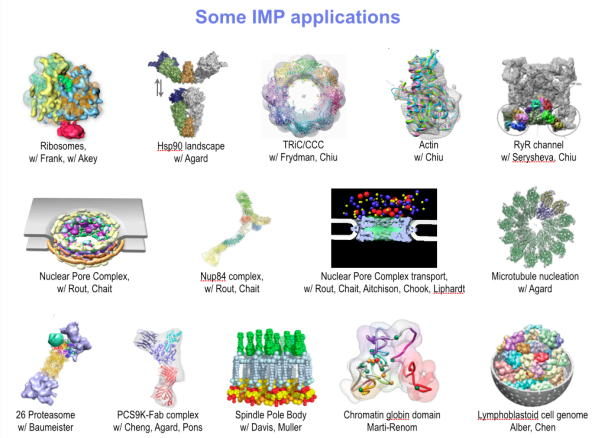


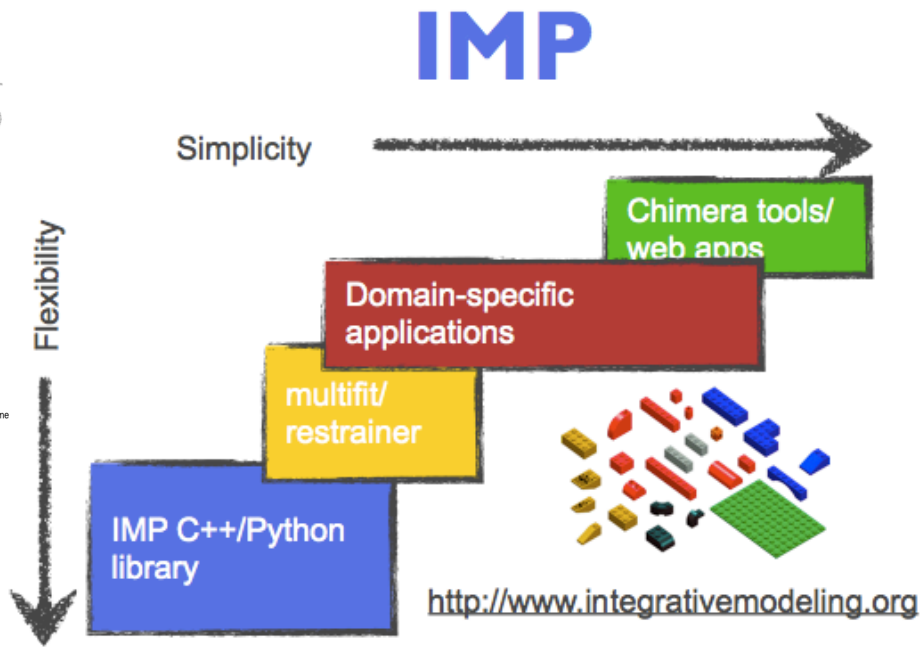


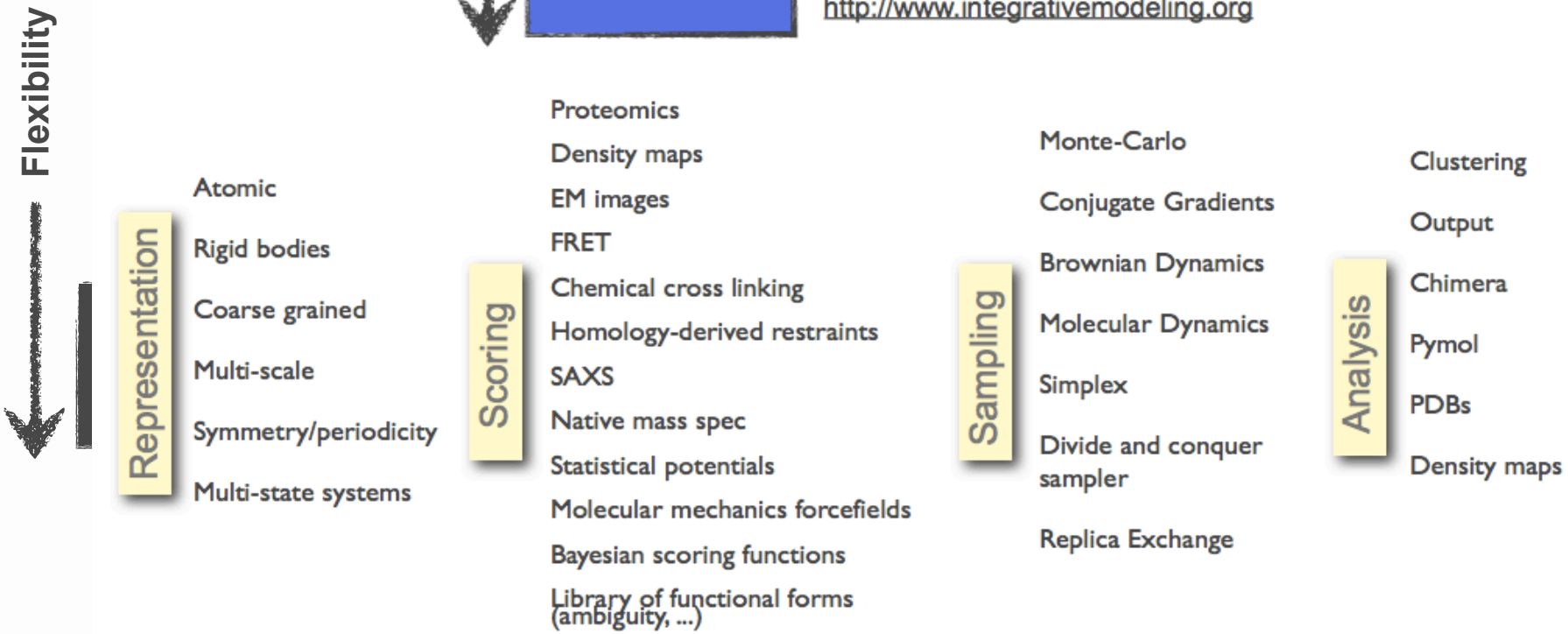
Sea1 is involved in the regulation of general autophagy

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.

D. Russel *et al.*, "Putting the pieces together: integrative modeling platform software for structure determination of macromolecular assemblies.," PLoS Biol., vol. 10, no. 1, p. e1001244, Jan. 2012.







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San Francisco

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  Benjamin Timney
  Zhanna Hakhverdyan
  Natalia Ketaren
  Michael Rout

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  William Rice

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OF YESHIVA UNIVERSITY

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  Lester Carter
  Thomas Weiss
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SSRL
SLAC NATIONAL ACCELERATOR LABORATORY
BL4-2 Biological Small Angle Scattering/Diffraction

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  John Tainer

The SIBYLS Beamline

- Stokes Lab (NYU)
  Paula Upla

NEW YORK UNIVERSITY

Please download the tutorial from github

git clone https://github.com/salilab/Workshop_SEA.git