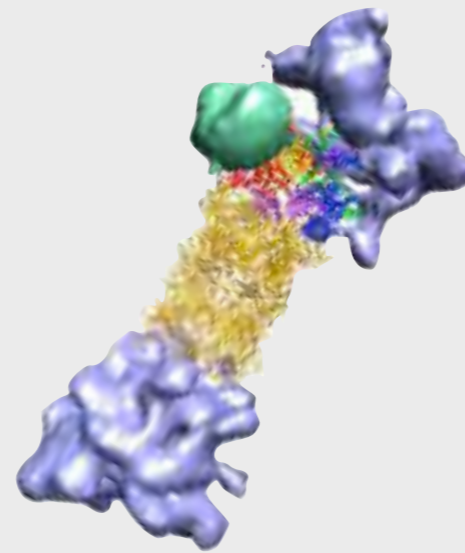
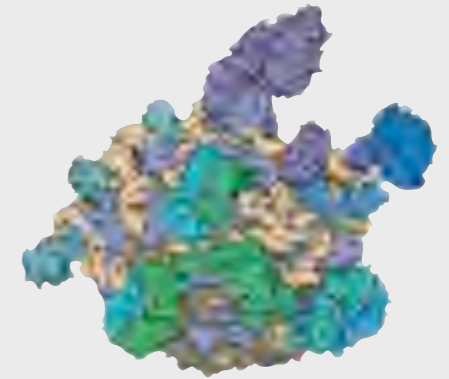
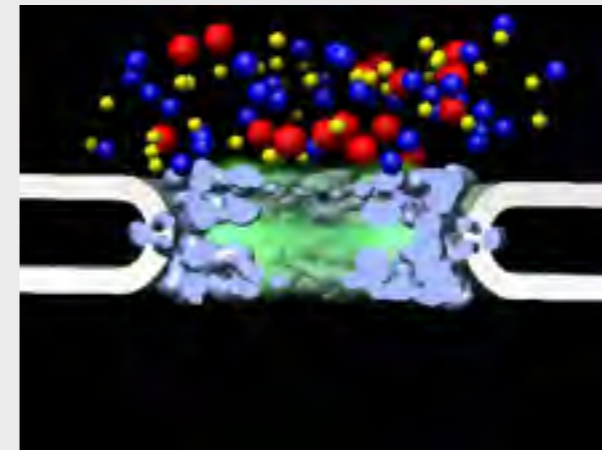
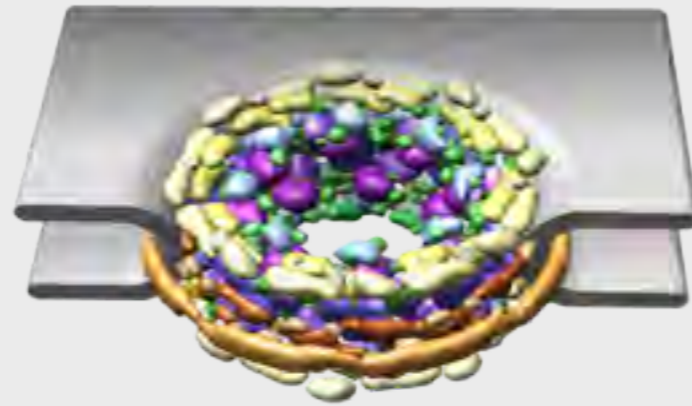
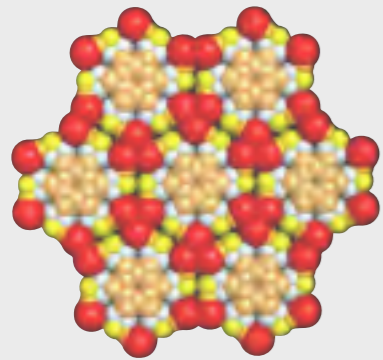


Integrative structure determination of macromolecular assemblies



Andrej Sali
<http://salilab.org/>



Department of Bioengineering and Therapeutic Sciences
Department of Pharmaceutical Chemistry
California Institute for Quantitative Biosciences
University of California, San Francisco

Disseminating structural models

Publishing models in a **printed paper**



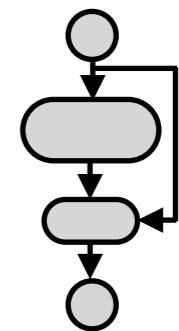
Depositing models in a **computer database**



Depositing **input data** in a computer database



Depositing modeling **protocols** for converting data to models



Enable others to interact with data and models:
test, improve, use data and models





- **Types** of structural models (static and dynamic):
 - **information**: X-ray, NMR, EM, and SAXS structures; “theoretical” models; hybrid models
 - **representation**: atomic, coarse-grained, multi-scale models
- **PDB** is a natural facilitator of establishing conventions, standards, interfaces, assessment criteria, publication criteria, *etc*, thus catalyzing a collaborative community

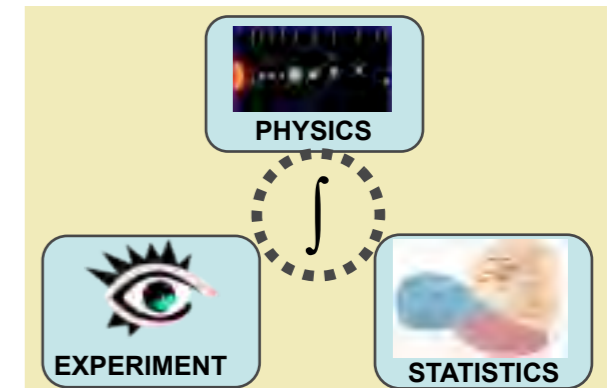
Contents

1. Integrative (hybrid) structure determination
2. Fitting multiple subunits into an EM map subject to restraints from proteomics
3. Structure of the yeast Nup84 complex

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.



X-ray crystallography	NMR spectroscopy	2D & single particle electron microscopy	electron tomography	immuno-electron microscopy	chemical cross-linking	affinity purification mass spectroscopy
subunit structure	subunit structure				subunit structure	
subunit shape	subunit shape	subunit shape	subunit shape			
subunit-subunit contact	subunit-subunit contact	subunit-subunit contact	subunit-subunit contact		subunit-subunit contact	subunit-subunit contact
subunit proximity	subunit proximity	subunit proximity	subunit proximity	subunit proximity	subunit proximity	subunit proximity
subunit stoichiometry	subunit stoichiometry					
assembly symmetry	assembly symmetry	assembly symmetry	assembly symmetry	assembly symmetry		
assembly shape	assembly shape	assembly shape	assembly shape			
assembly structure	assembly structure					

FRET	site-directed mutagenesis	yeast two-hybrid system	gene/protein arrays	protein structure prediction	computational docking	bioinformatics
				subunit structure		
				subunit shape		
subunit-subunit contact	subunit-subunit contact	subunit-subunit contact	subunit-subunit contact		subunit-subunit contact	subunit-subunit contact
subunit proximity		subunit proximity	subunit proximity			

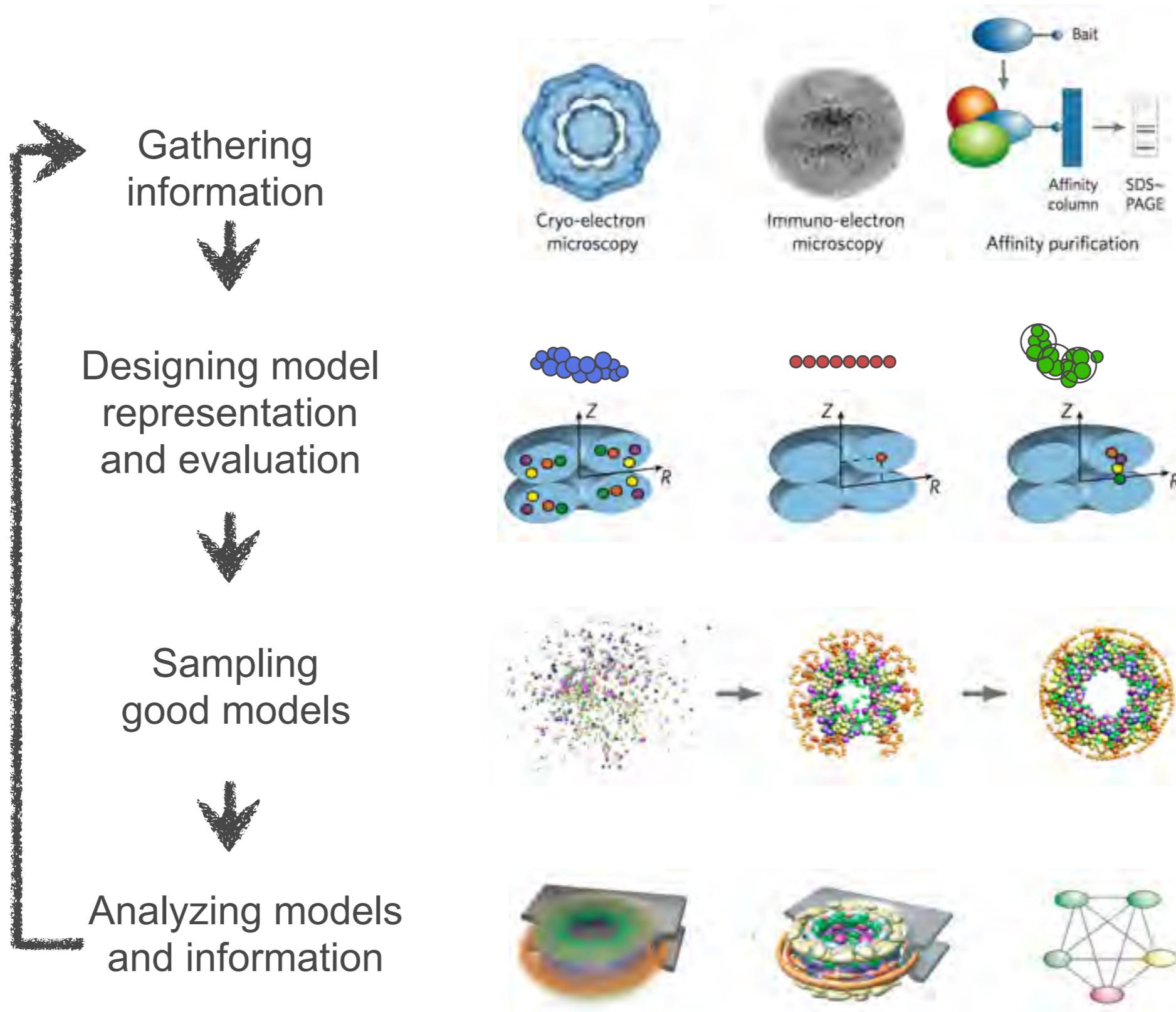
Sali, Earnest, Glaeser, Baumeister. From words to literature in structural proteomics. *Nature* 422, 216-225, 2003.

An approach to integrative structure determination

Alber *et al.* *Nature* **450**, 683-694, 2007.

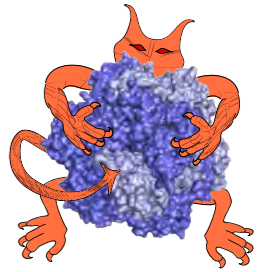
Robinson, Sali, Baumeister. *Nature* **450**, 974-982, 2007.

Alber, Foerster, Korkein, Topf, Sali. *Annual Reviews in Biochemistry* **77**, 11.1–11.35, 2008.

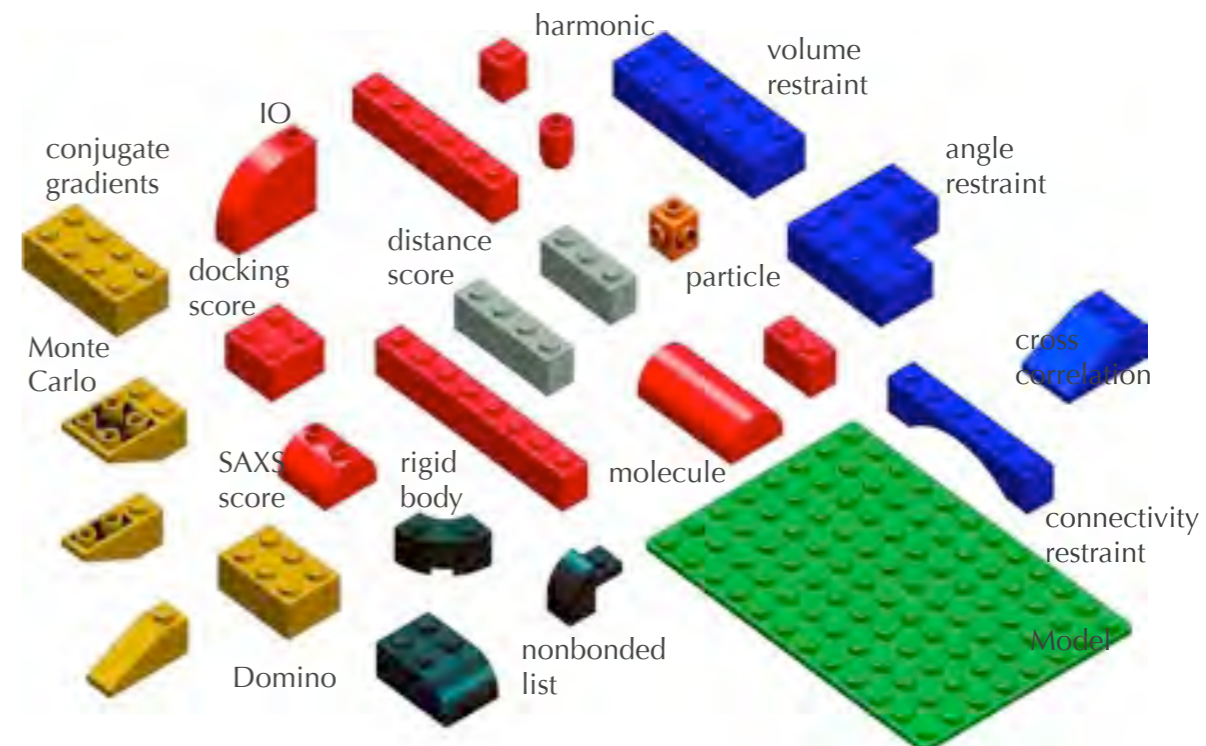
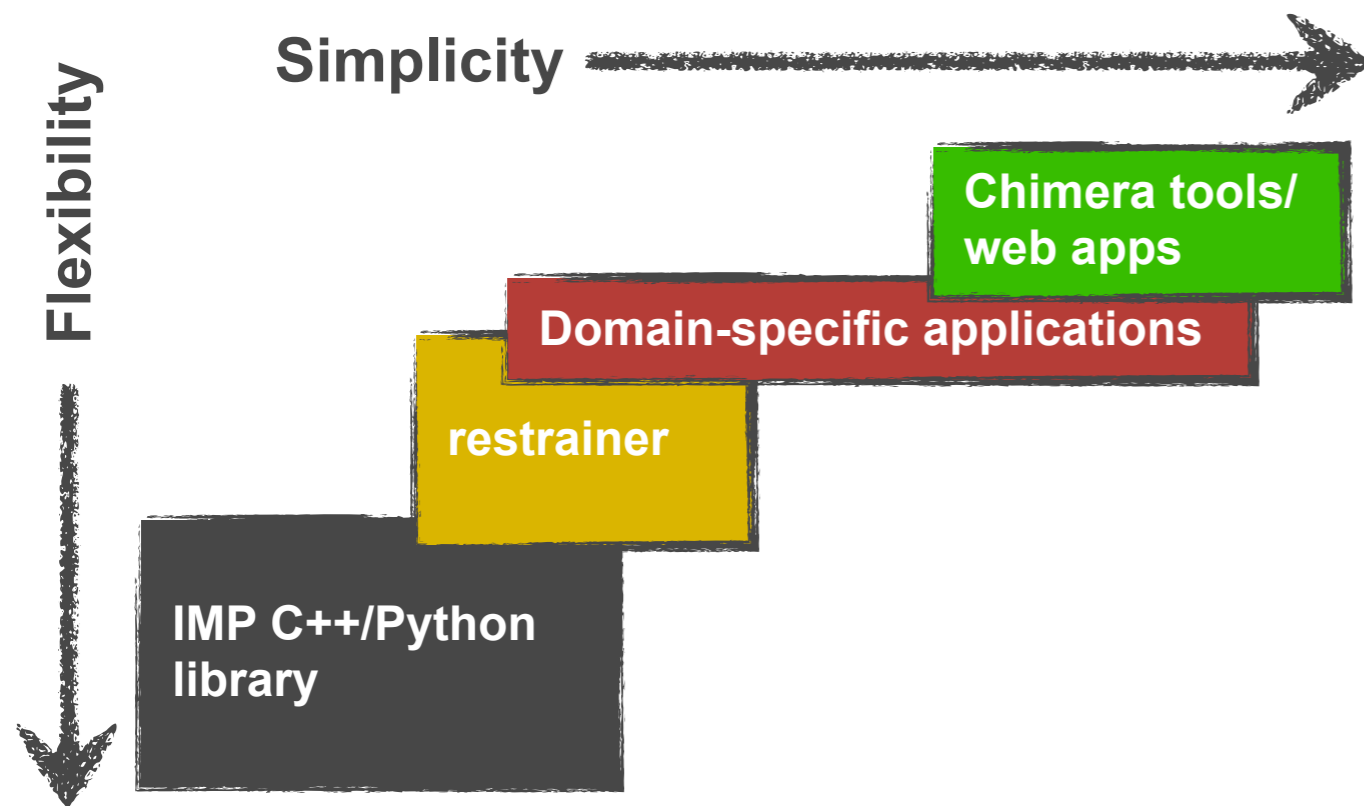


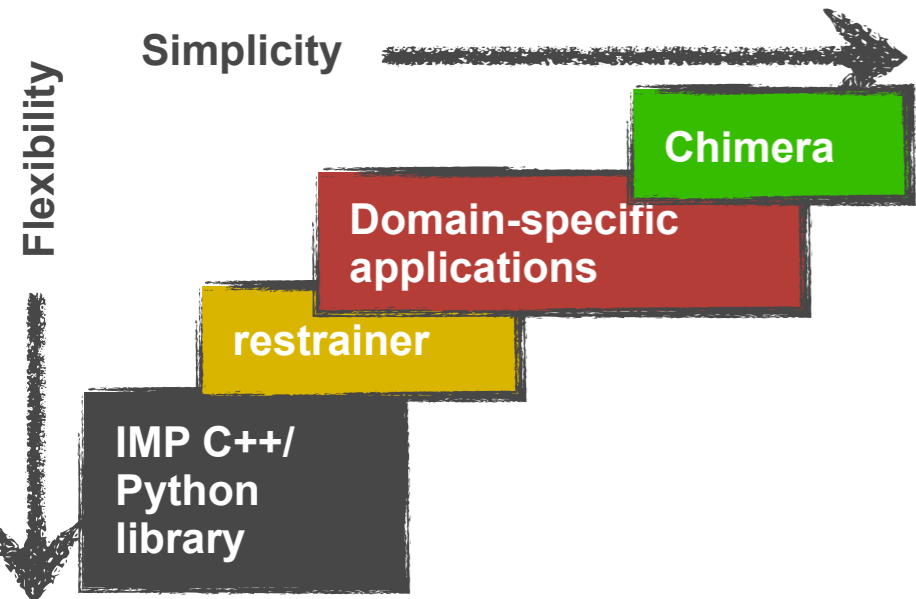
Integrative Modeling Platform (IMP)

D. Russel, K. Lasker, B. Webb, J. Velazquez-Muriel, E. Tijoie, D. Schneidman, F. Alber, B. Peterson, A. Sali, PLoS Biol, 2011.



- IMP-1.0 available at <http://salilab.org/imp/> (3/10/10)
- Open source, SVN, documentation, wiki, examples, mailing lists, unit testing, bug tracking, ...





Modeller interface

Model	GA341	zDOPE	Estimated RMSD	Estimated Overlap (3.5Å)	DOPE
#1.1	1.00	0.19	7.153	0.680	-50178.91
#1.2	1.00	0.27	7.344	0.646	-49518.61
#1.3	1.00	0.25	7.141	0.660	-49662.25
#1.4	1.00	0.20	5.747	0.684	-50079.25
#1.5	1.00	0.23	6.759	0.703	-49857.44
#1.6	1.00	0.30	8.224	0.627	-49275.85
#1.7	1.00	0.21	7.237	0.661	-50021.49
#1.8	1.00	0.30	8.636	0.626	-49274.52
#1.9	1.00	0.19	5.450	0.721	-50168.98
#1.10	1.00	0.24	7.149	0.695	-49801.83

FoXS interface

MultiFit interface

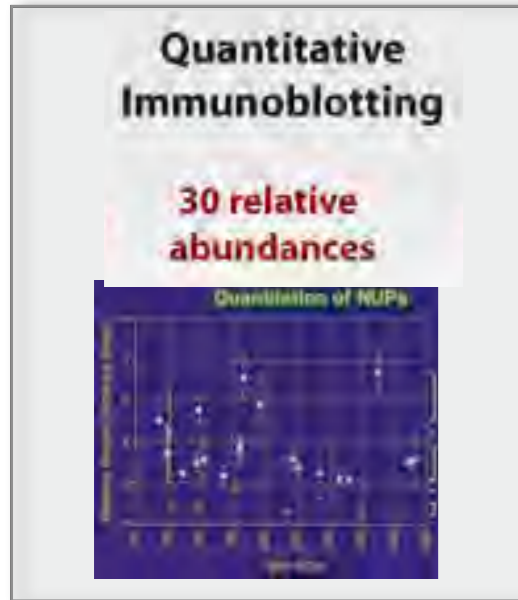
Index	c	Correlation	Name
0		0.95	P0A6F5_EColi.B99990001_fit.pdb_#0
1		0.93	P0A6F5_EColi.B99990001_fit.pdb_#1
2		0.92	P0A6F5_EColi.B99990001_fit.pdb_#2
3		0.91	P0A6F5_EColi.B99990001_fit.pdb_#3
4		0.88	P0A6F5_EColi.B99990001_fit.pdb_#4
5		0.87	P0A6F5_EColi.B99990001_fit.pdb_#5
6		0.84	P0A6F5_EColi.B99990001_fit.pdb_#6
7		0.83	P0A6F5_EColi.B99990001_fit.pdb_#7
8		0.83	P0A6F5_EColi.B99990001_fit.pdb_#8
9		0.83	P0A6F5_EColi.B99990001_fit.pdb_#9
10		0.82	P0A6F5_EColi.B99990001_fit.pdb_#10

Z. Yang, K. Lasker, D. Schneidman-Duhovny, B. Webb, C. Huang, E. Pettersen, T. Goddard, E. Meng, A. Sali, T. Ferrin. UCSF Chimera, MODELLER, and IMP: an integrated modeling system. *J Struct Biol*, in press.

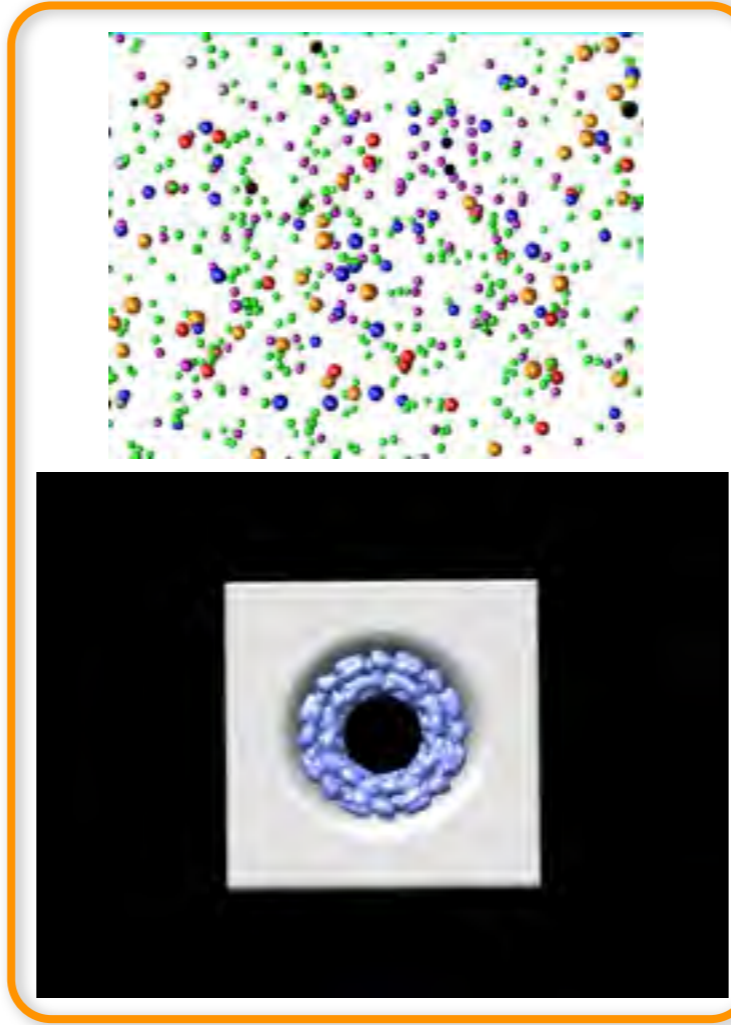
Configuration of 456 proteins in the Nuclear Pore Complex

with M. Rout & B. Chait

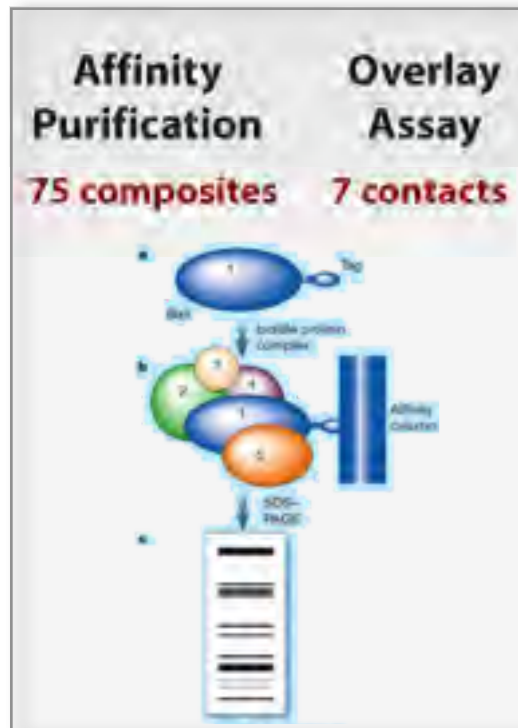
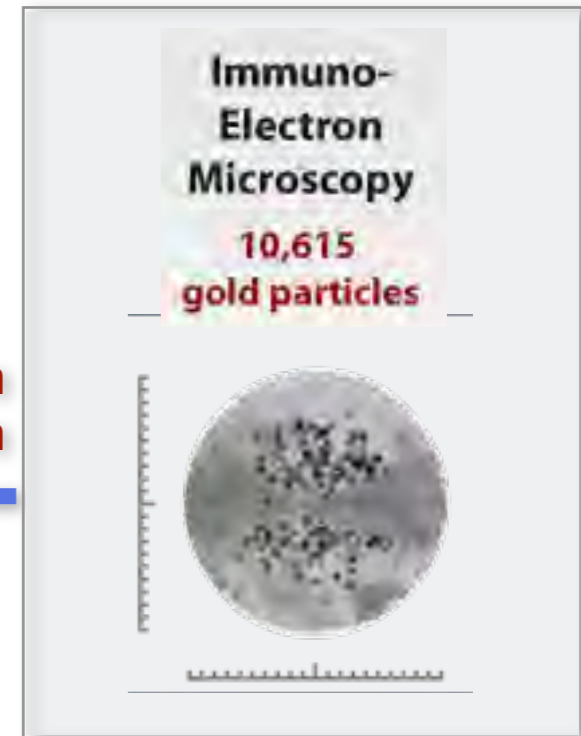
Alber et al. *Nature* 450, 684-694, 2007.
Alber et al. *Nature* 450, 695-702, 2007.



Protein Stoichiometry



Protein Localization



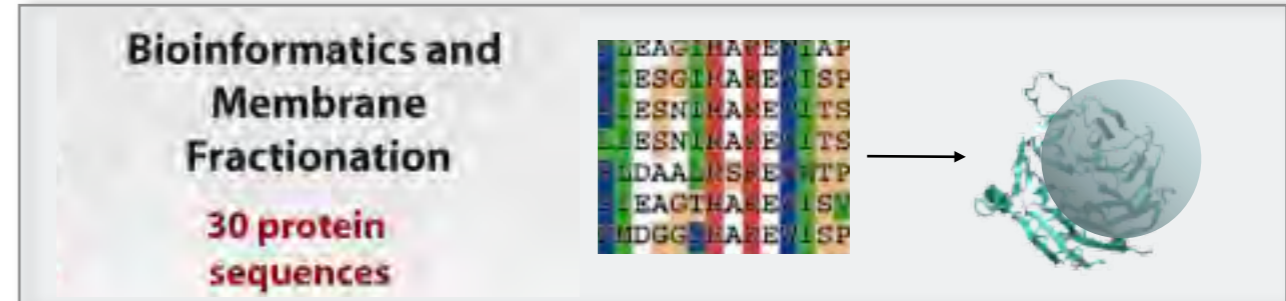
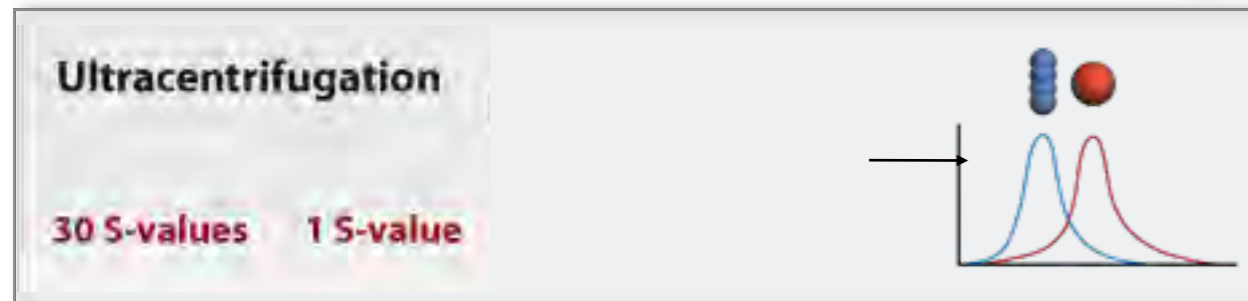
Protein-protein Proximities



Symmetry

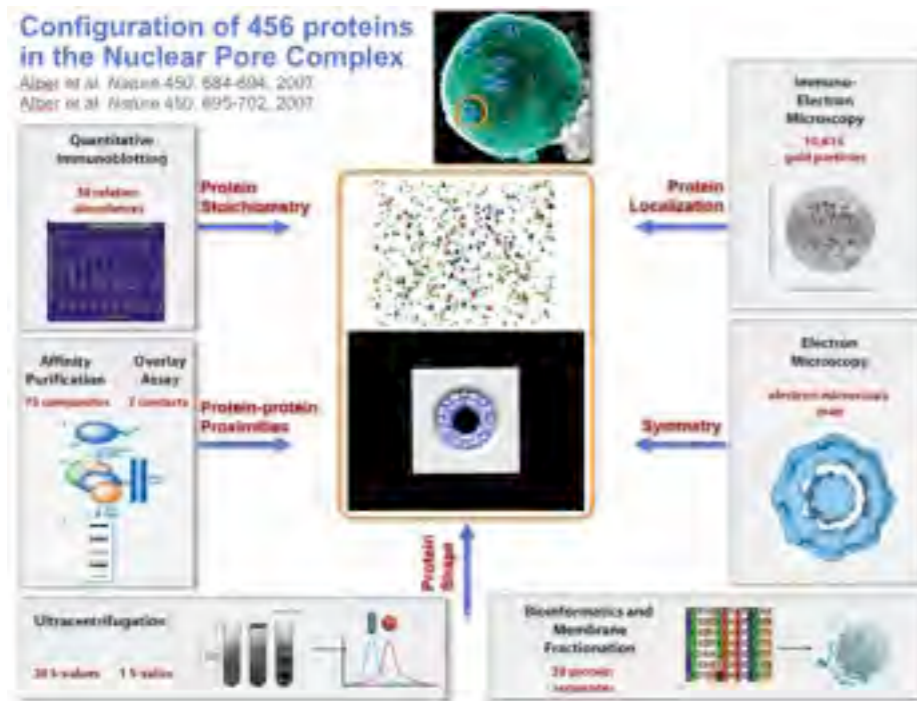


Protein Shape

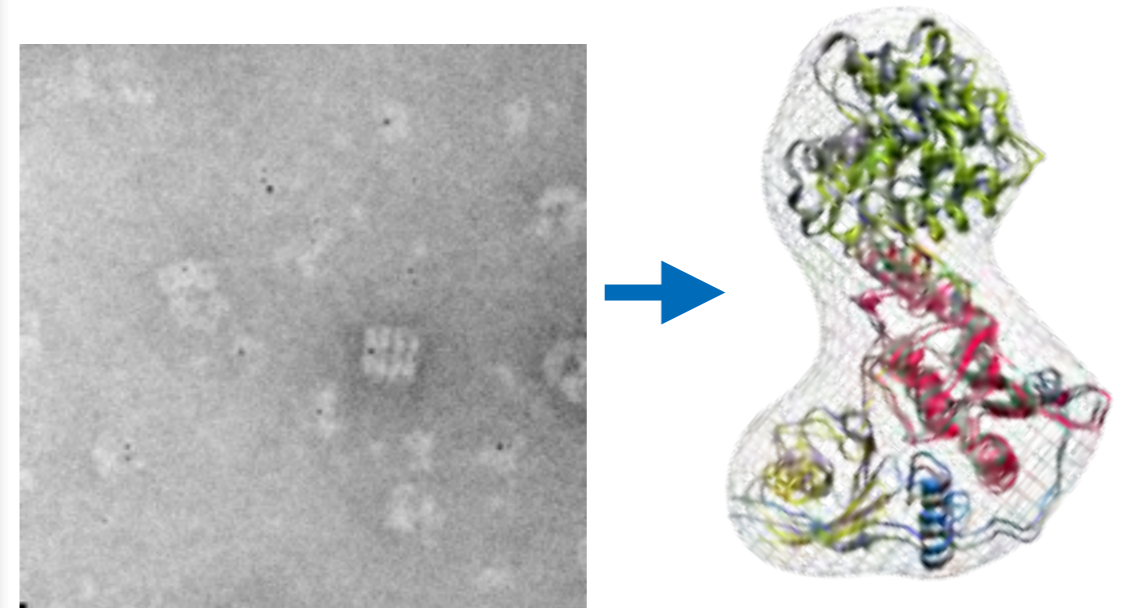


Determination by experiment *versus* prediction by modeling

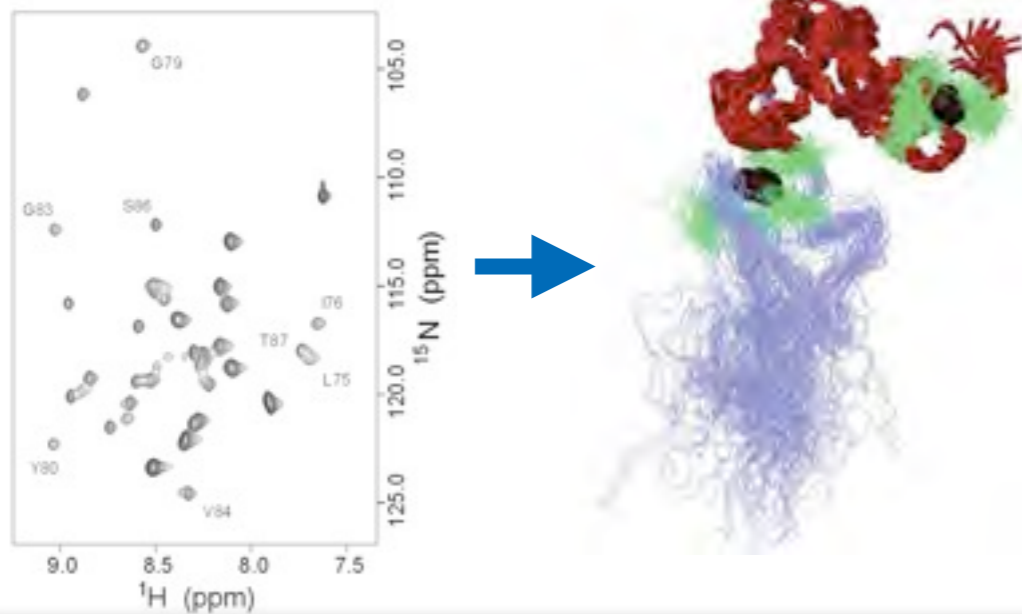
Integrative structure determination



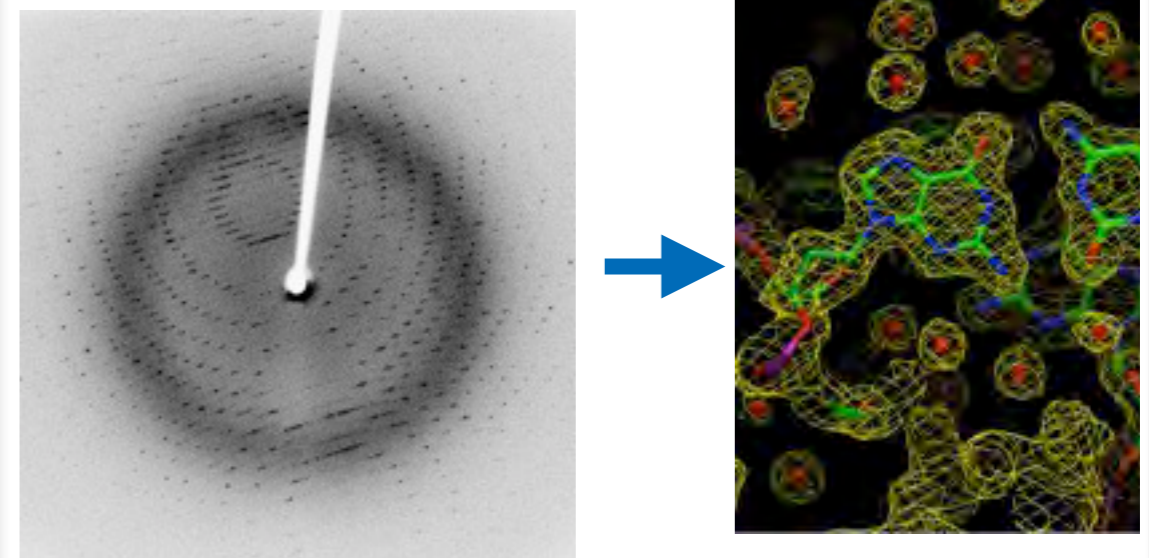
EM microscopy



NMR spectroscopy



X-ray crystallography

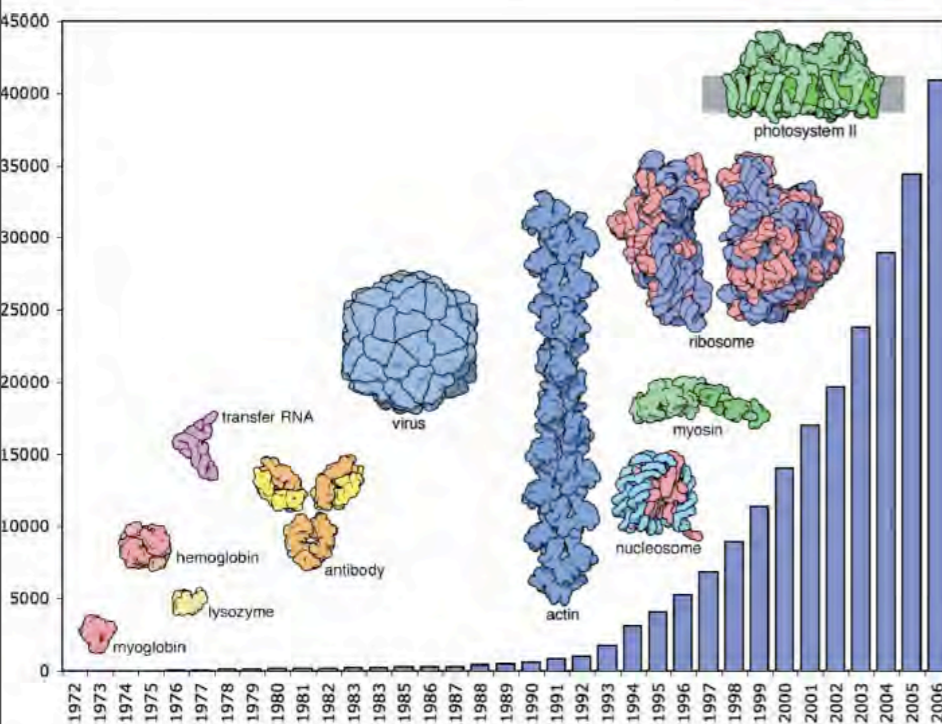


Contents

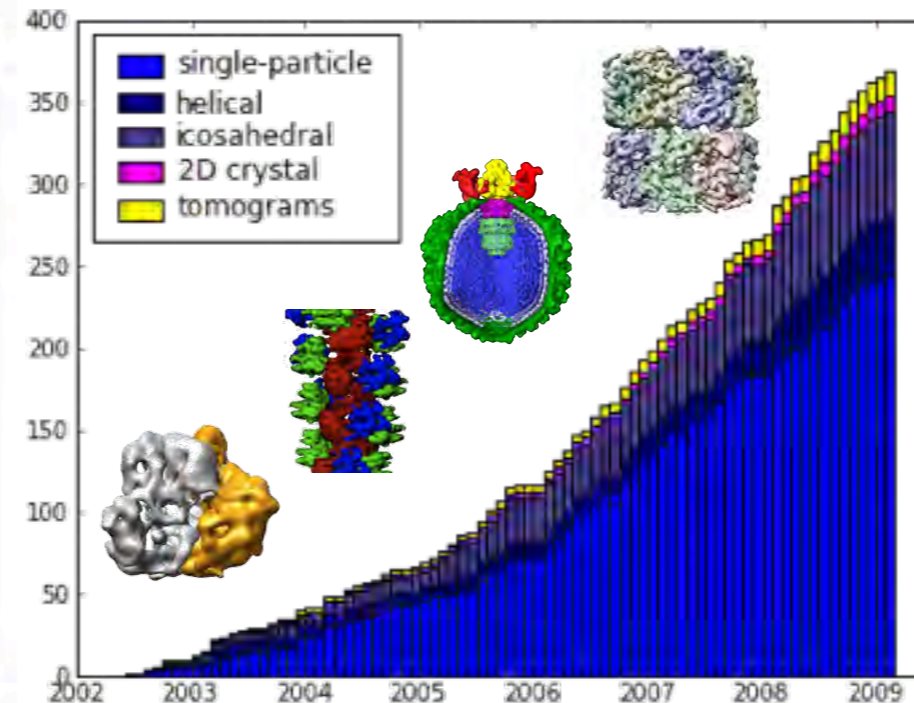
1. Integrative (hybrid) structure determination
2. Fitting multiple subunits into an EM map
subject to restraints from proteomics
3. Structure of the yeast Nup84 complex

Assembly architecture from atomic structures of subunits, EM density map of assembly, and proteomics

Protein Data Bank



EM Data Bank

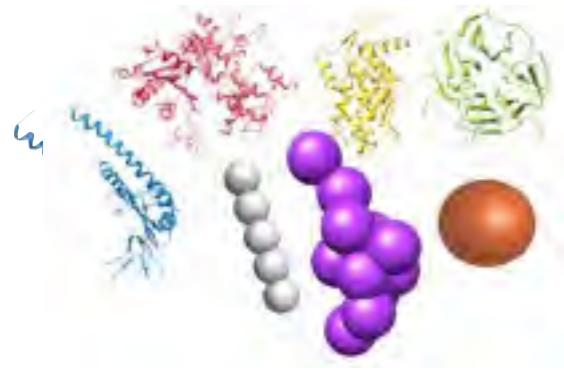


BioGrid, ...



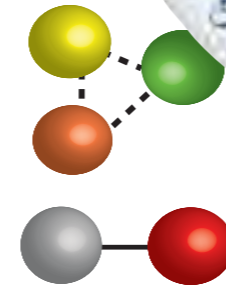
Fitting multiple subunits into a density map: Scoring

Input:



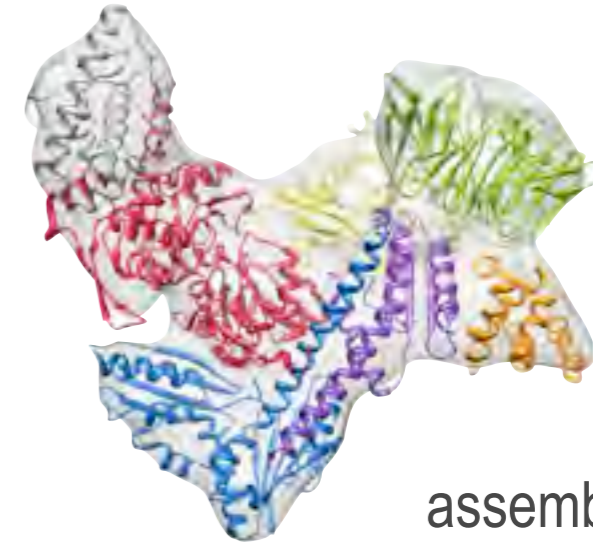
atomic, coarse
components

low resolution density
map of the assembly



proteomics
data

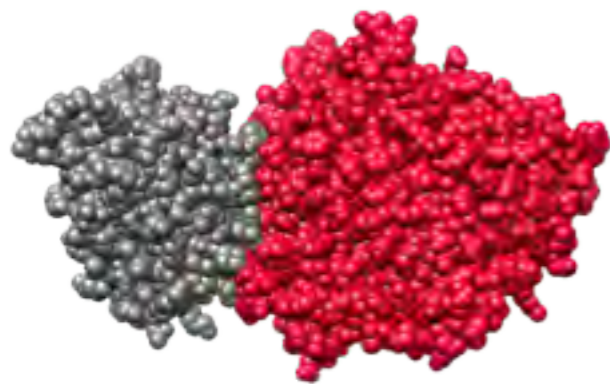
Output:



assembly
configuration

Find assembly configurations that satisfy:

Shape complementarity



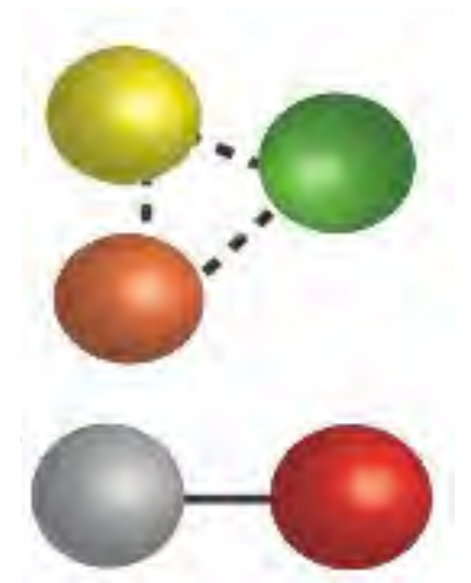
Quality-of-fit



Envelope protrusion

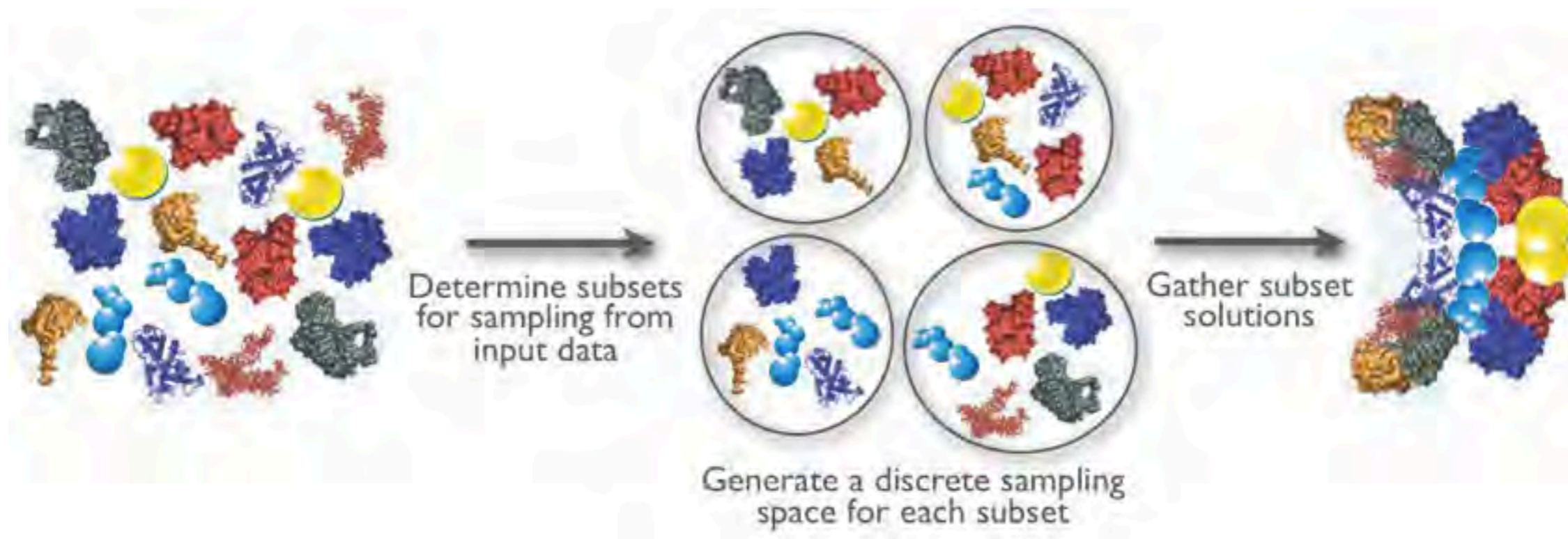


Connectivity



K. Lasker, M. Topf, A. Sali, H. Wolfson, *J. Mol. Biol.* 388, 180-194, 2009.
K. Lasker et al, *Proteins*, 2010.
K. Lasker et al, *Mol Cel Prot*, 2010.

Optimization / sampling

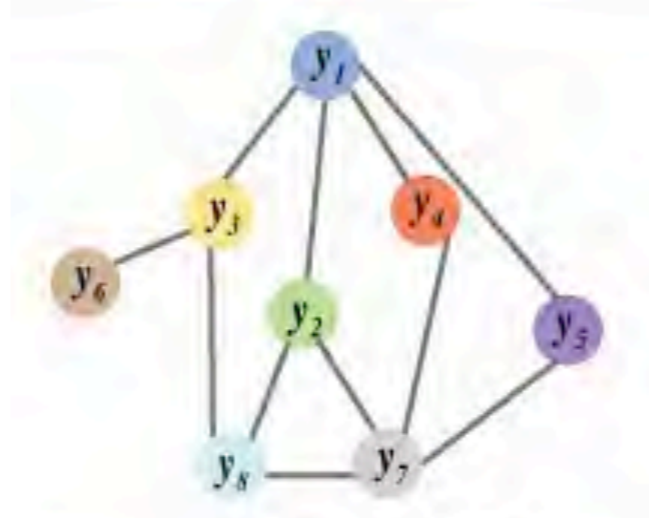




Divide-and-Conquer (DOMINO)

1. **Represent** the scoring function as a graph.

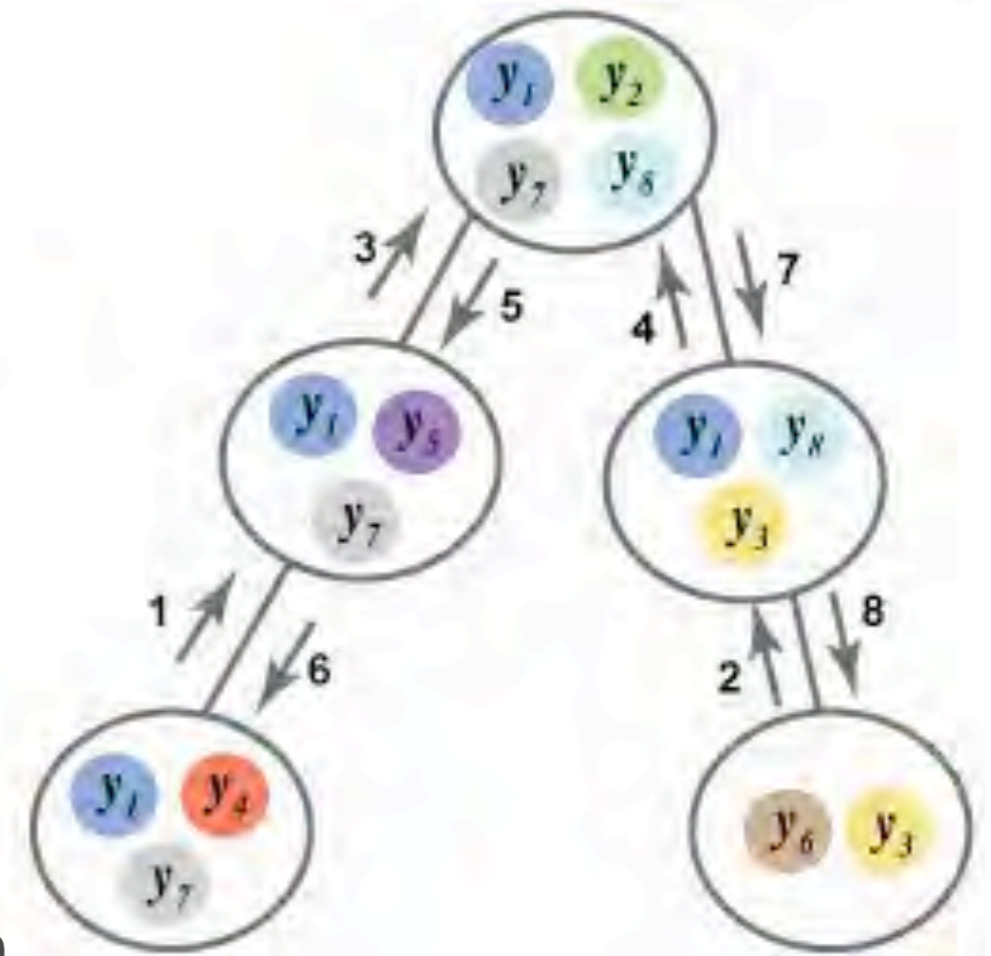
$$F(y_1, \dots, y_8) = \alpha_2(y_2) + \alpha_6(y_6) + \alpha_7(y_7) \\ + \beta_{1,2}(y_1, y_2) + \beta_{1,3}(y_1, y_3) + \beta_{1,4}(y_1, y_4) + \beta_{1,5}(y_1, y_5) \\ + \beta_{2,7}(y_2, y_7) + \beta_{2,8}(y_2, y_8) + \beta_{3,6}(y_3, y_6) + \beta_{3,8}(y_3, y_8) \\ + \beta_{4,7}(y_4, y_7) + \beta_{5,7}(y_5, y_7) + \beta_{7,8}(y_7, y_8)$$



2. **Decompose** the set of variables into relatively decoupled subsets (a junction tree algorithm).

3. **Optimize** each subset independently by a traditional optimizer, to get the optimal and a number of suboptimal solutions.

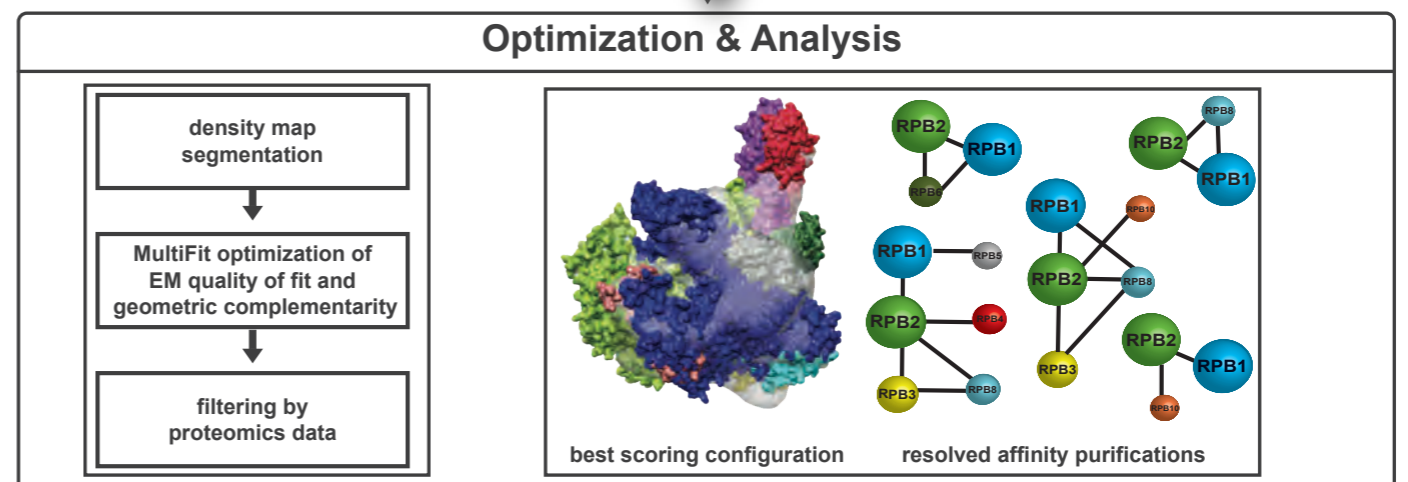
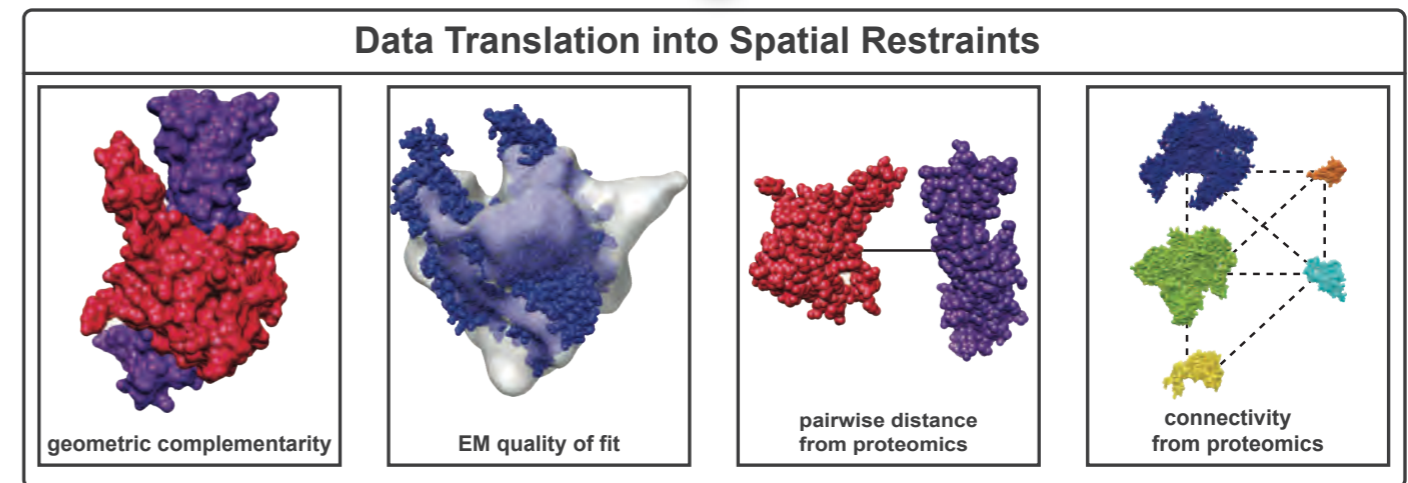
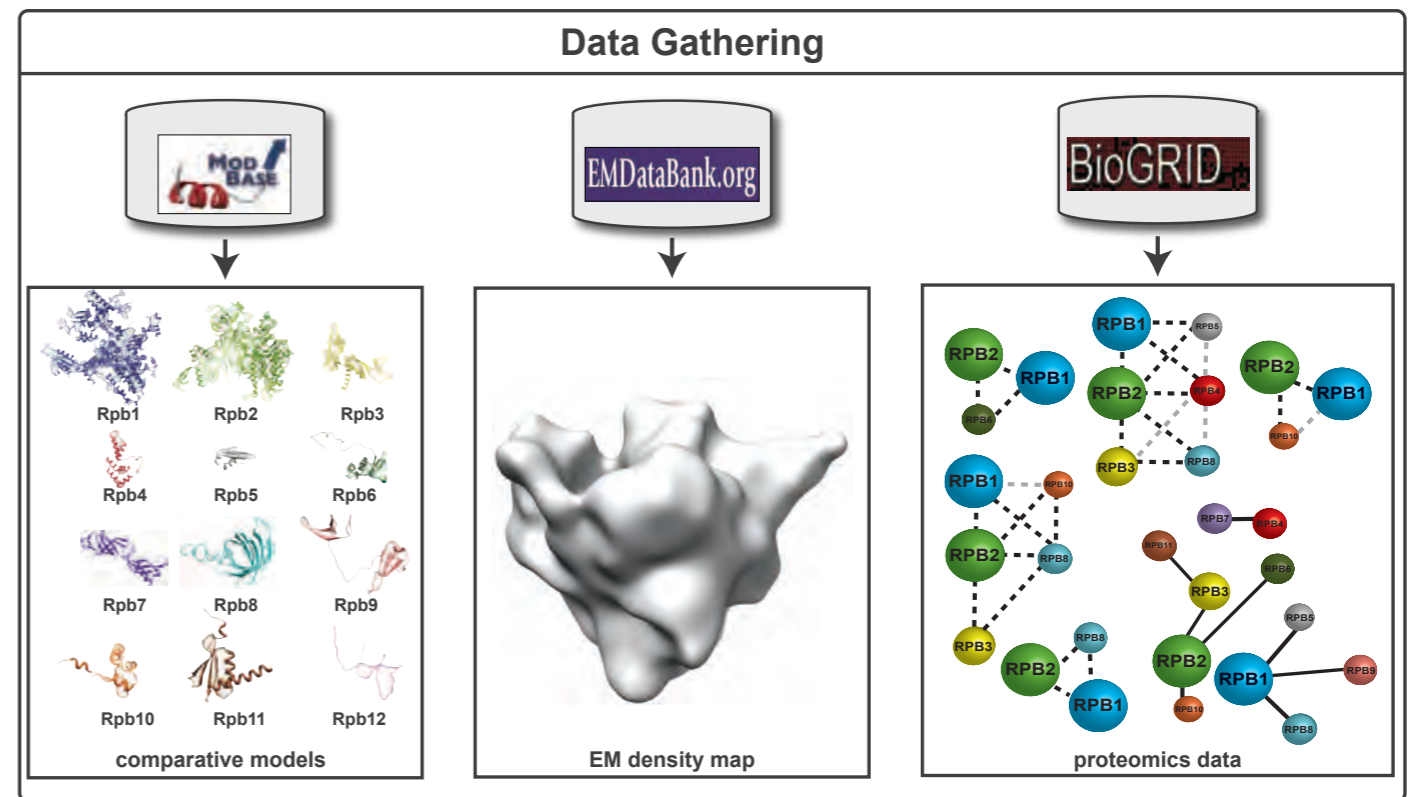
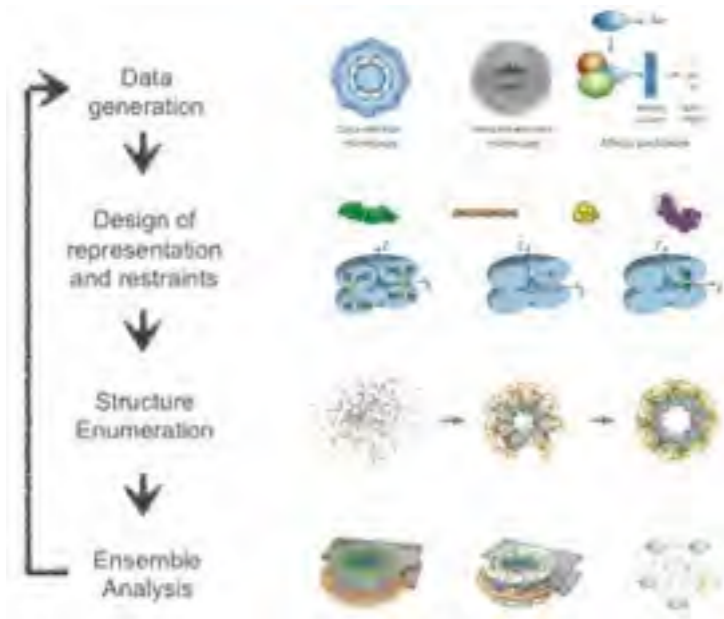
4. **Gather** subset solutions into the best possible global solutions (message passing algorithms; eg, belief-propagation).



K. Lasker, M. Topf, A. Sali, H. Wolfson, J. Mol. Biol. 388, 180-194, 2009.
M.I. Jordan, Graphical models. *Stat. Sci.* 19, 140–155, 2004.

Proof-of-principle: Integrative structure determination of human RNAPII

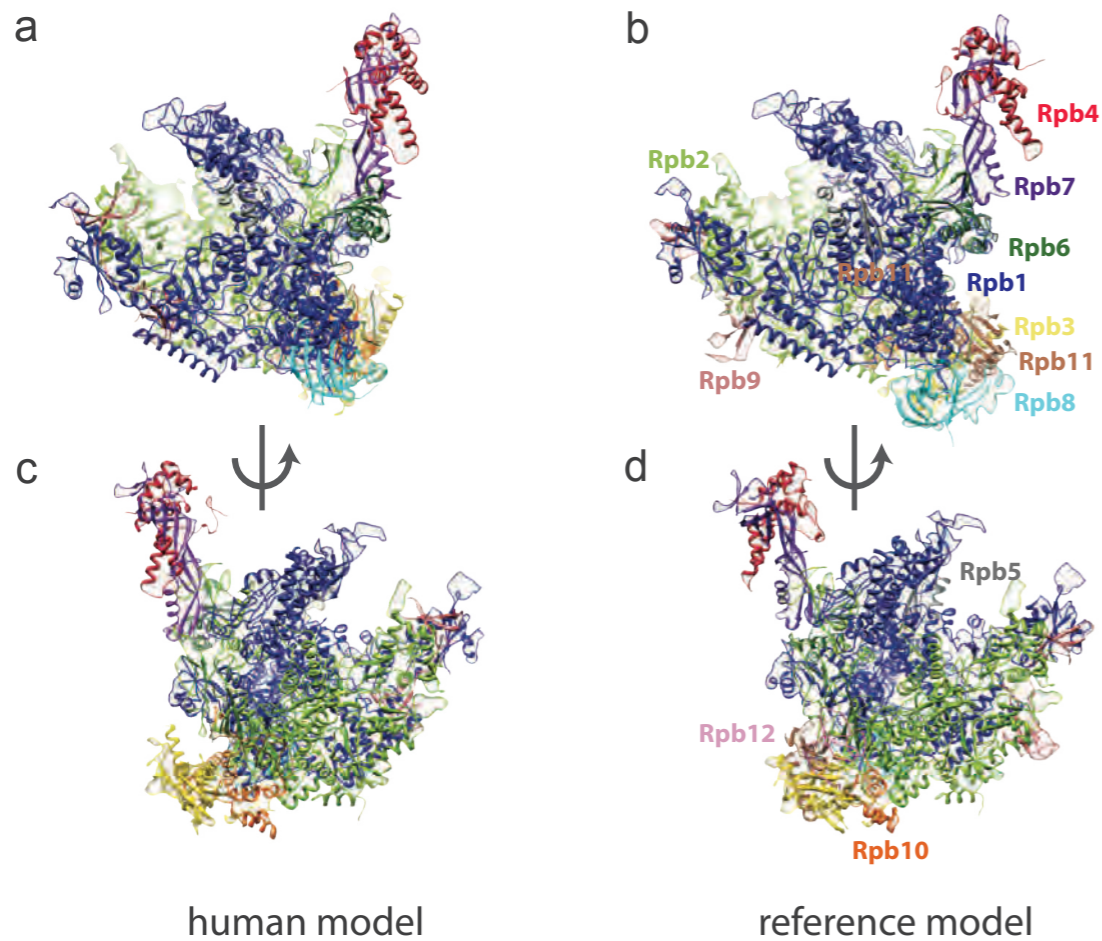
Lasker *et al*, MCP 2010



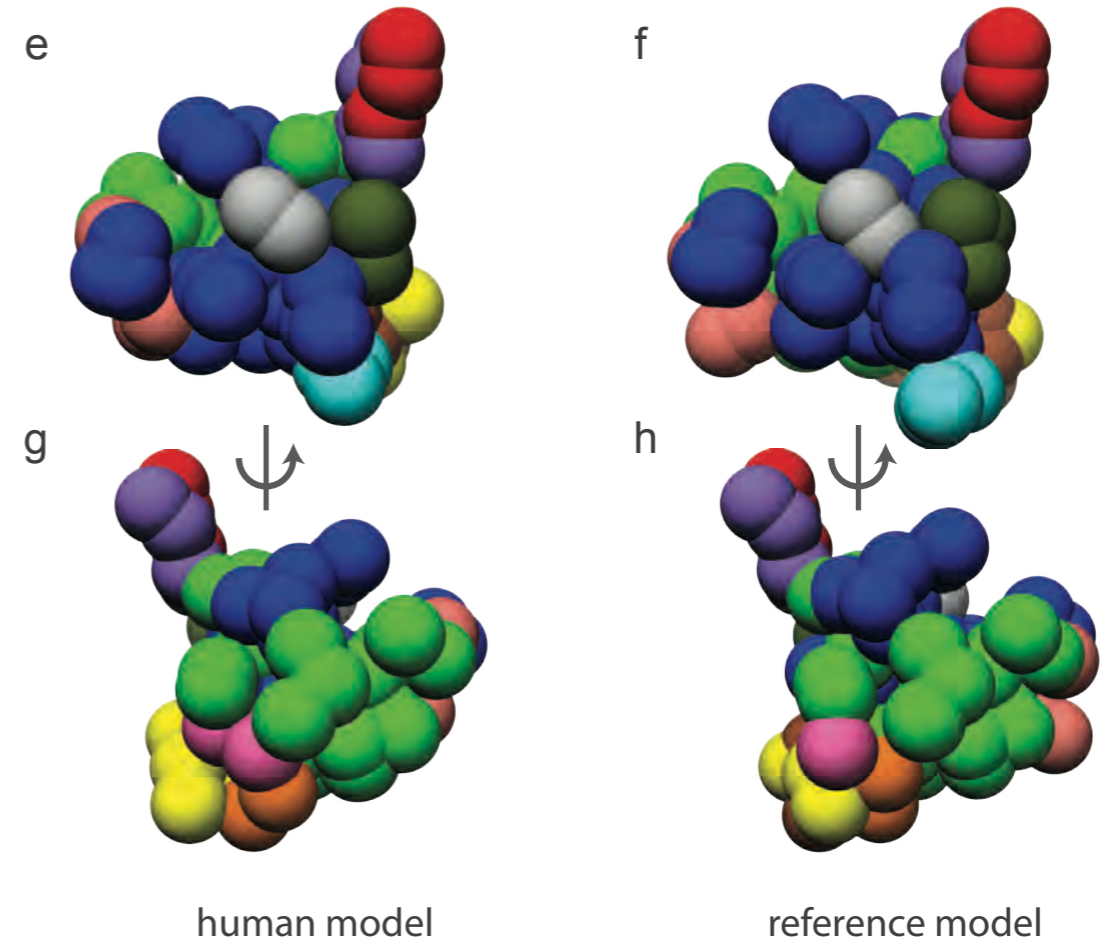
Cramer *et al*, *Science*, 2000 (X-ray)
 Kostek *et al*, *Structure*, 2006 (EM)
 Gavin *et al*, *Nature* 2006 (proteomics)
 Krogan *et al*, *Nature*, 2006 (proteomics)

Assessment of an integrative model of human RNAPII

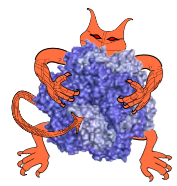
I. atomic representation



II. coarse-grained representation



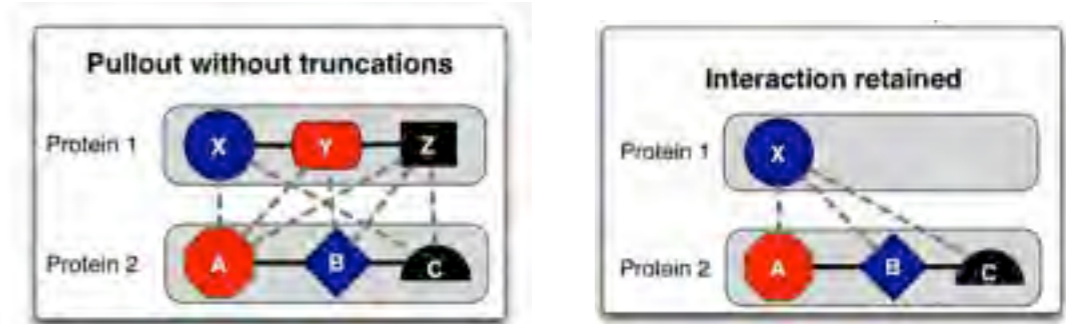
reference model - human subunit models fit on the corresponding subunits in the crystallographic yeast RNAPII structure



Additional configurational restraints

1. Affinity purification with domain deletion constructs

Orienting subunits by identification of interacting domains
J. Phillips; with J. Fernandez, M. Rout:



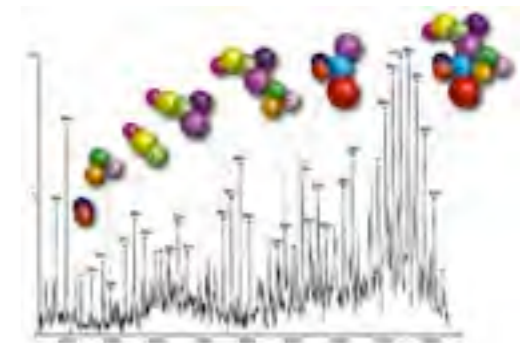
2. 2D EM class averages

Filtering models by matching their optimal projections to images
J. Velazquez, D. Schneidman



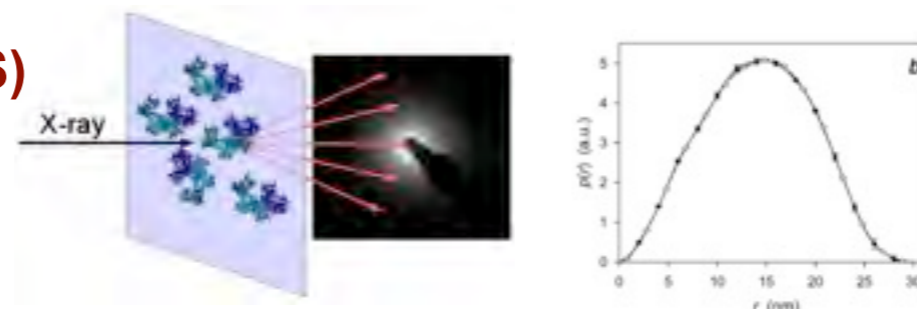
3. Assembly subcomplex stoichiometry by native mass spectrometry

Ambiguous network of protein proximities
D. Russel, J. Phillips; with A. Politis, C. Robinson:



4. Small Angle X-ray Scattering (SAXS)

Filtering models by their shape
D. Schneidman, S.-J. Kim



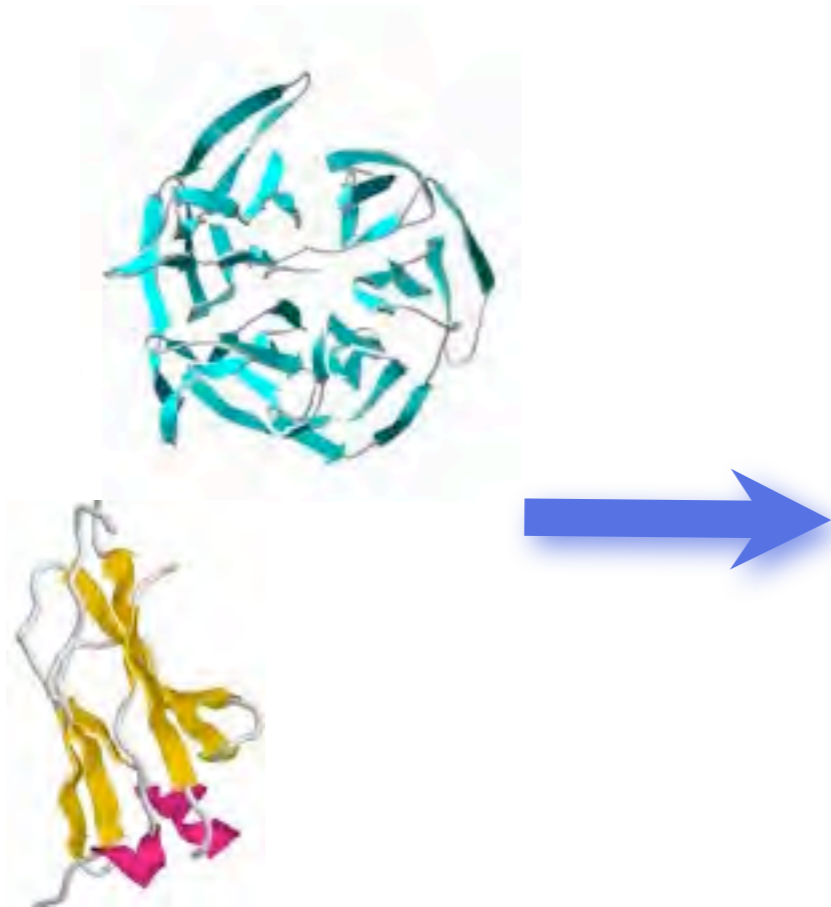
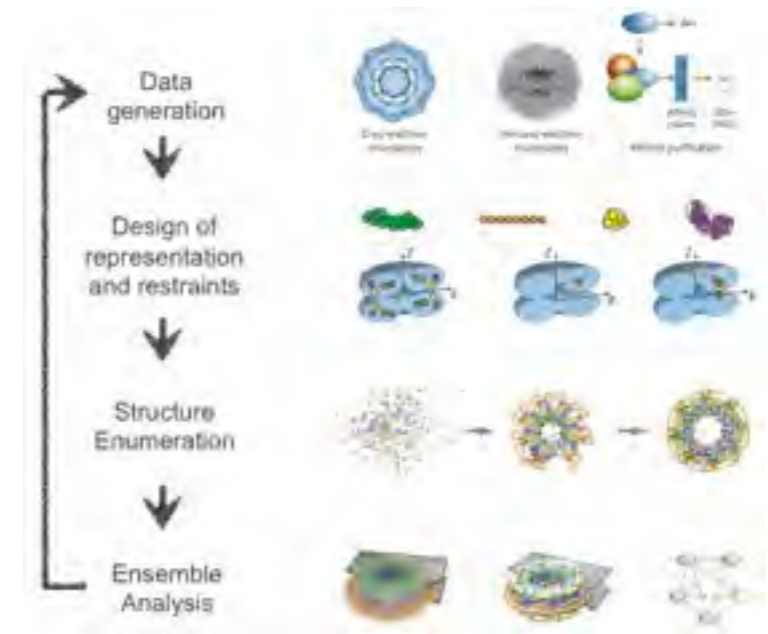
$$\chi^2 = \frac{1}{Q} \sum_{k=1}^Q \frac{1}{\sigma_{\text{exp}}^2(q_k)} \cdot (I_{\text{exp}}(q_k) - c \cdot I_m(q_k))^2$$

Contents

1. Integrative (hybrid) structure determination
2. Fitting multiple subunits into an EM map subject to restraints from proteomics
3. **Structure of the yeast Nup84 complex**

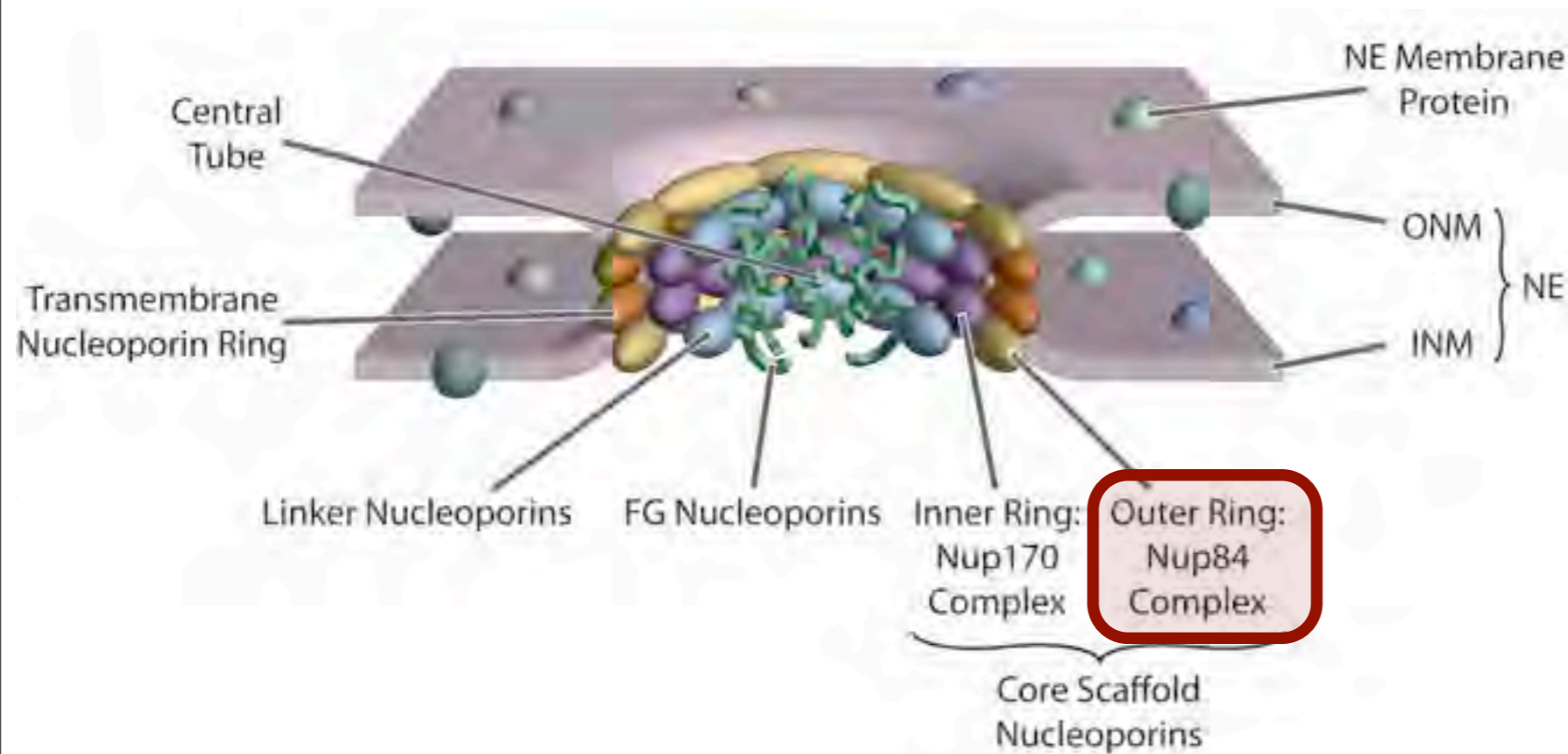
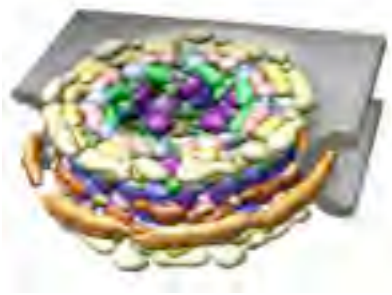
Towards a higher resolution structure of the NPC

Characterize structures of the individual subunits, then fit them into the current low-resolution structure, aided by additional experimental information.

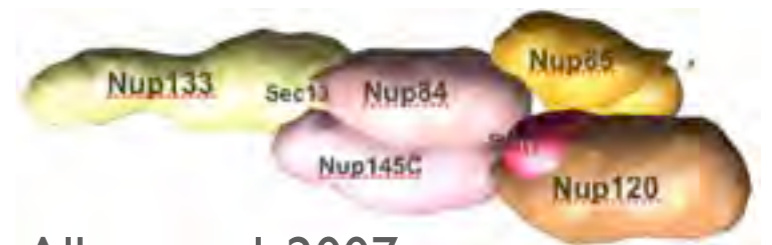


Alber *et al.* *Nature* 450, 684-694, 2007.
Alber *et al.* *Nature* 450, 695-702, 2007.

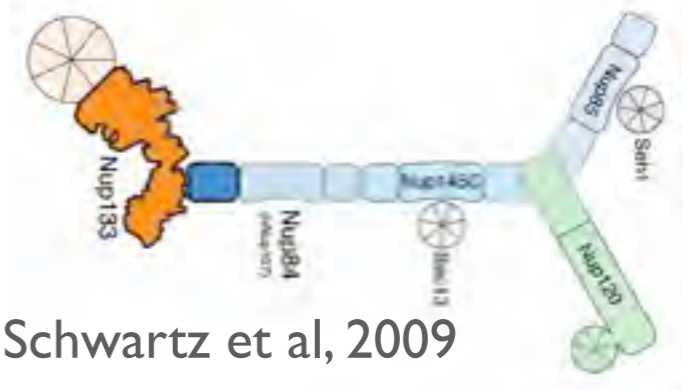
The Nup84 complex in the NPC



Lutzmann et al, 2002



Alber et al, 2007



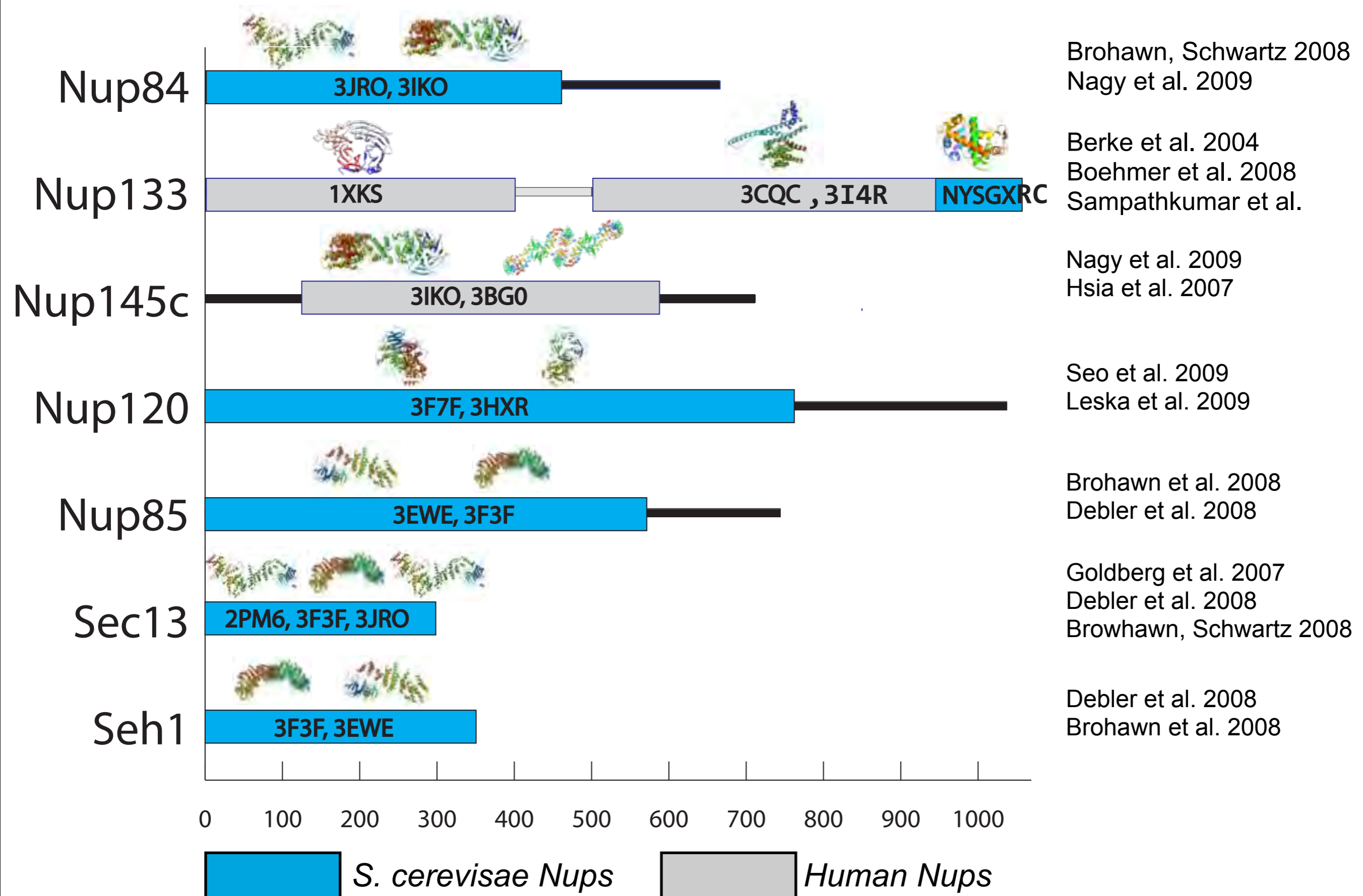
Schwartz et al, 2009



Kampmann et al, 2009

- 7-protein complex
- Forms the two outer rings of the NPC
- Present in 16 copies in the NPC
- Proteins share a common ancestor with vesicle coating complexes

Nup84 complex: Representation



Nup84 complex: Data

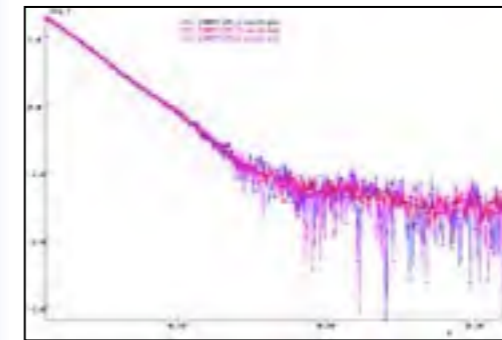
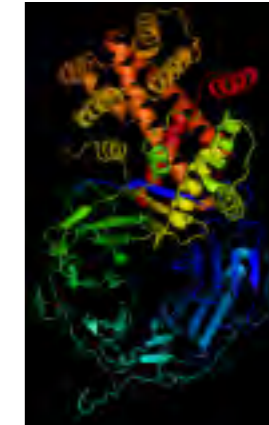
Subunit positions & orientations

Affinity purifications with domain truncations
J. Fernandez, J. Franke, B. Chait, M. Rout

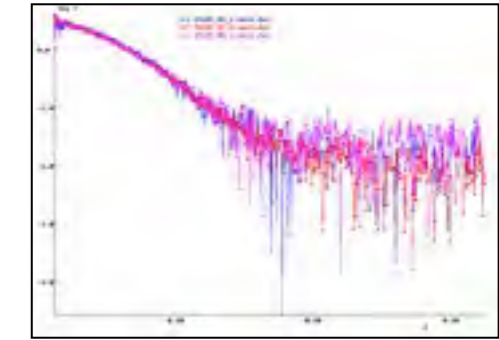


Subunit conformations

Small angle X-ray scattering
S.J. Kim, A. Martel, H. Tsuruta, NYSGXRC, J. Tainer



Nup133



Nup120

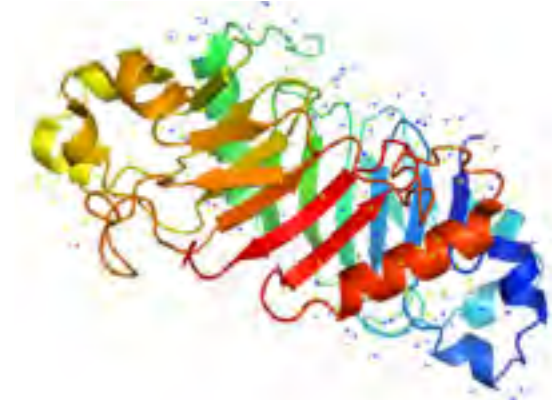
Negative stain EM particle averages at ~3nm resolution
R. Diaz, D. Stokes, J. Velazquez



High-throughput crystallography
NYSGXRC, P. Sampathkumar, M. Sauder, S. Burley

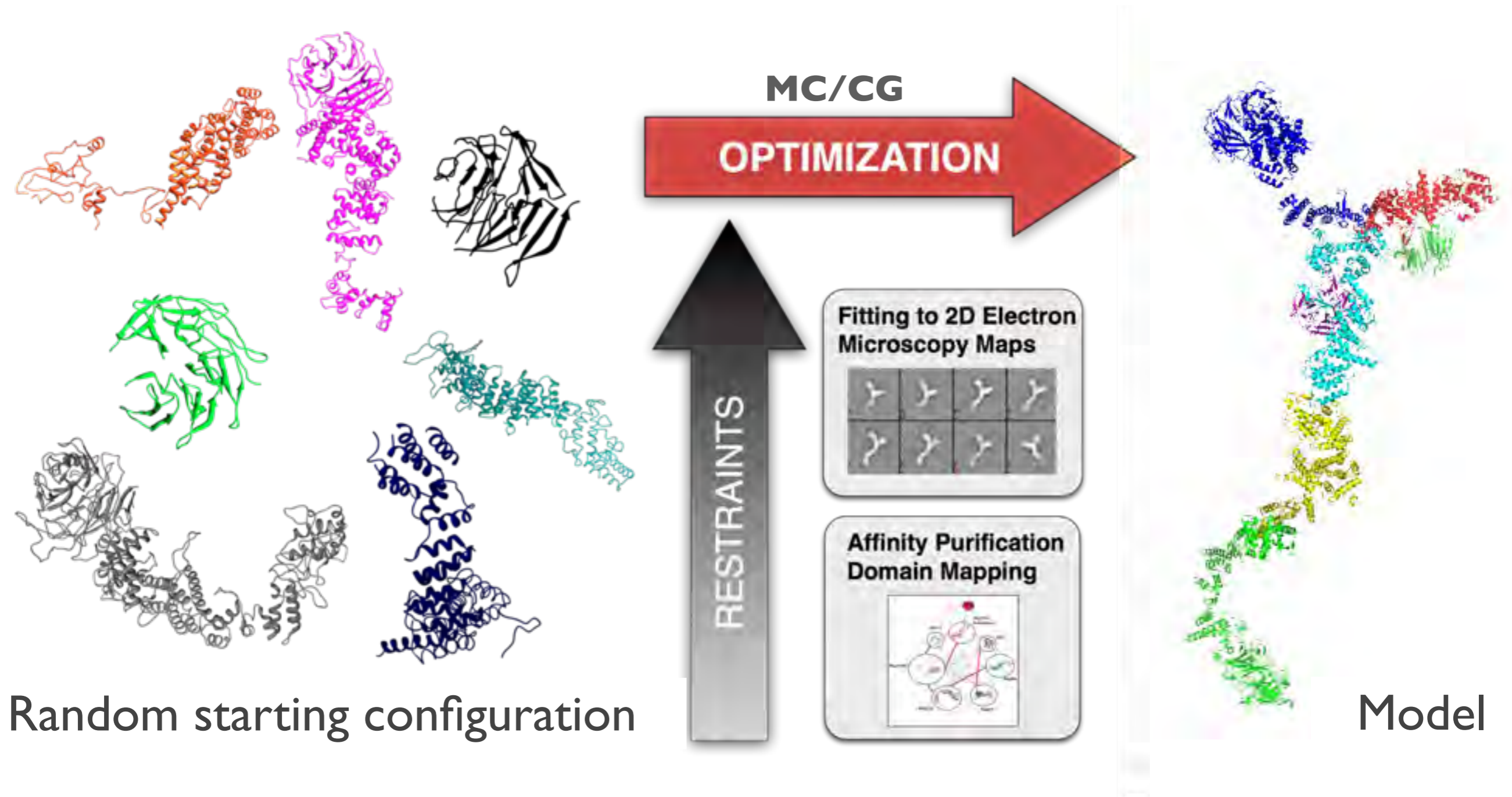


Yeast Nup133

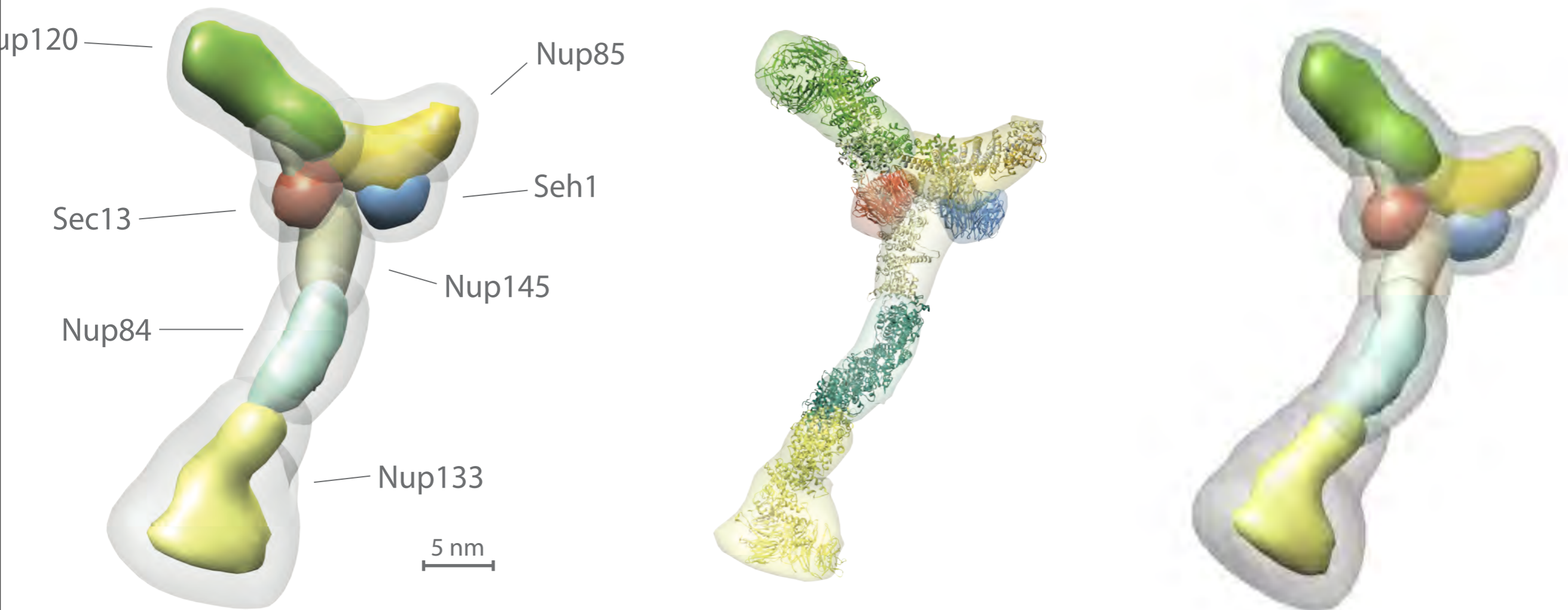


Yeast Nup145

Nup84 complex: Optimization



Nup84 complex: Ensemble of good scoring solutions



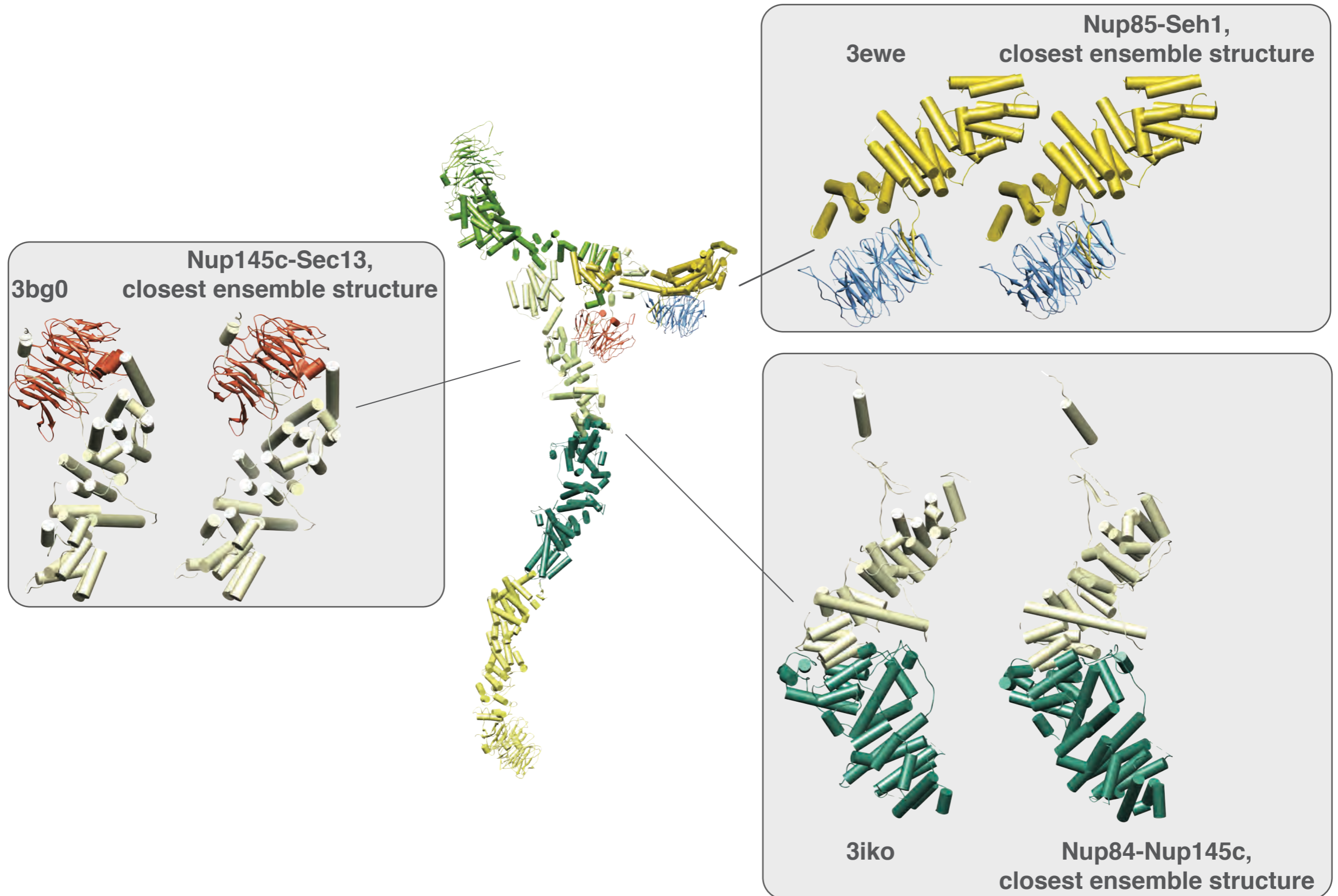
- 10,000 good scoring structures
- All restraints are satisfied (2D-EM, domain deletion, ...)
- Domain-domain orientations are resolved uniquely.
- Full ensemble precision is ~1 nm

Assessing the well-scoring models

1. Existence of a good-scoring model.
2. Precision of the ensemble of good-scoring models.
3. Check model against unused data (cross-validation).
4. Known precision / accuracy for “similar” cases.
5. Non-random patterns in the model.

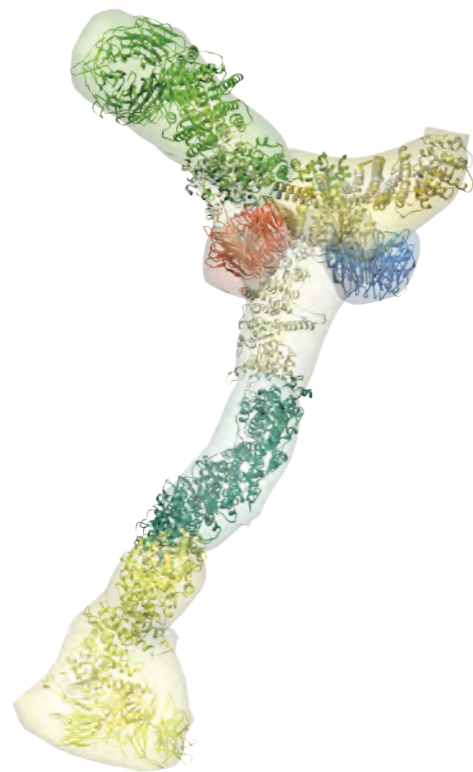
Modeling facilitates assessing the **data as well as models in terms of precision and accuracy.**

Assessment: Agreement with heterodimeric crystallographic structures



Towards a near-atomic structure of the NPC

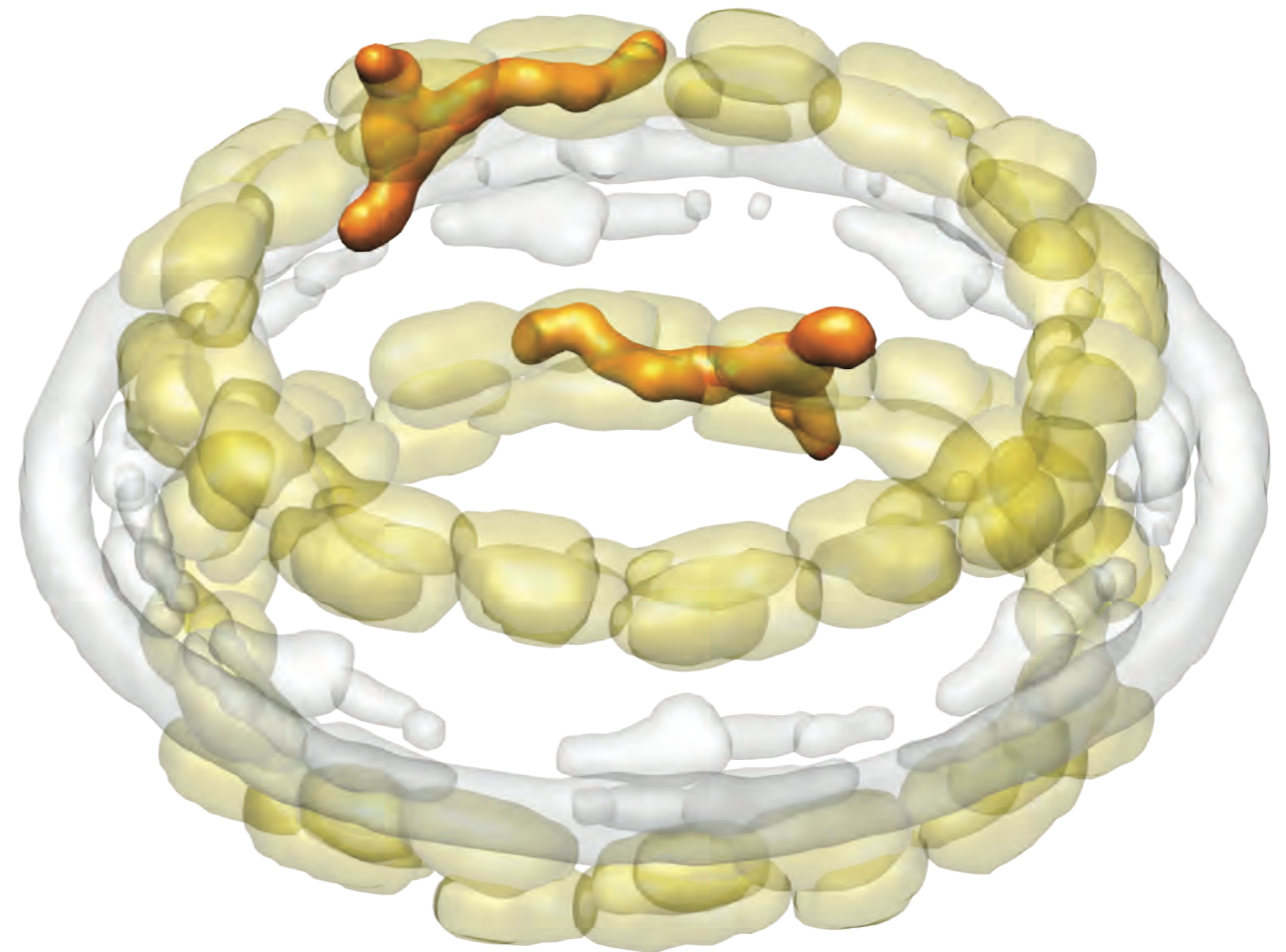
Nup84 complex



16 x

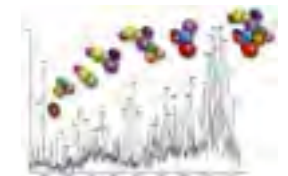
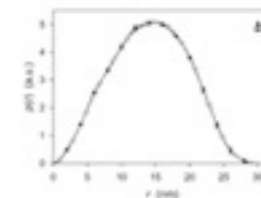
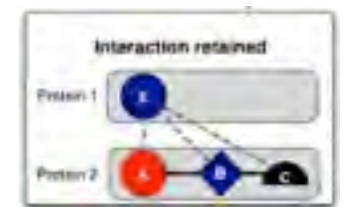
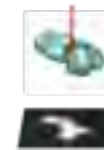
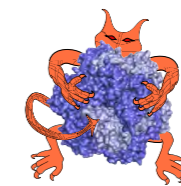
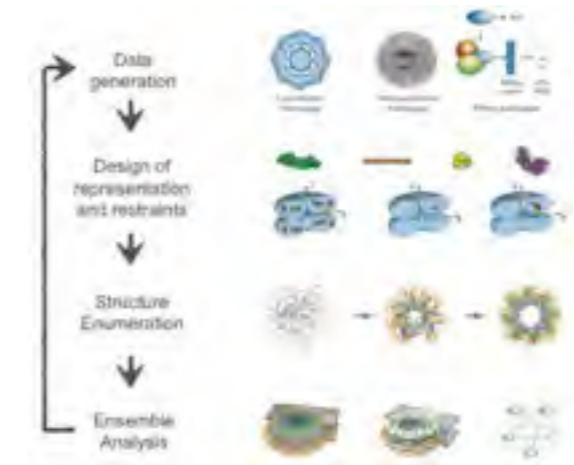


NPC



Conclusions

1. Assembly structure determination benefits greatly from the inclusion of all available information, including heterogeneous data sources.
2. Open source *Integrative Modeling Platform* (IMP). Developers and users of IMP are most welcome.
3. General and efficient assembly of subunit models based on domain deletion pullouts, 2D EM projections, 3D EM maps, SAXS profiles, and native MS.
4. Near atomic model of the Nup84 complex.



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