Photo by David Oliete - www.davidoliete.com

Structure determination

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http://marciuslab.org
http://3DGenomes.org
http://cnag.crg.eu

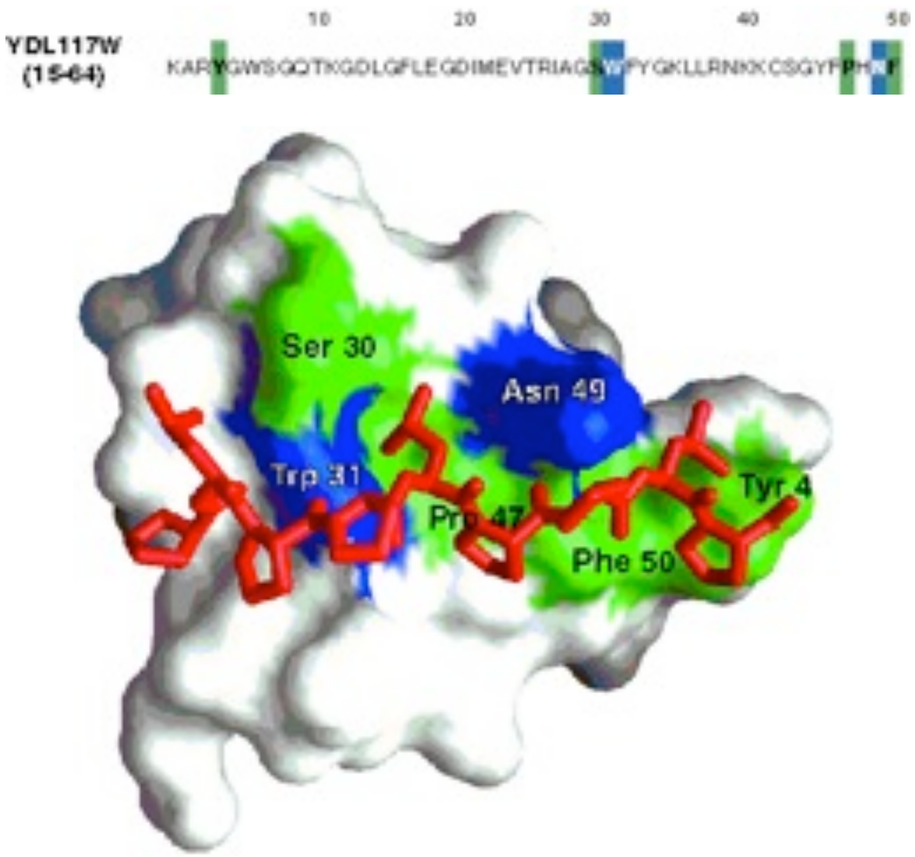
The importance of the 3D structure

The biochemical function of a molecule is defined by its interactions

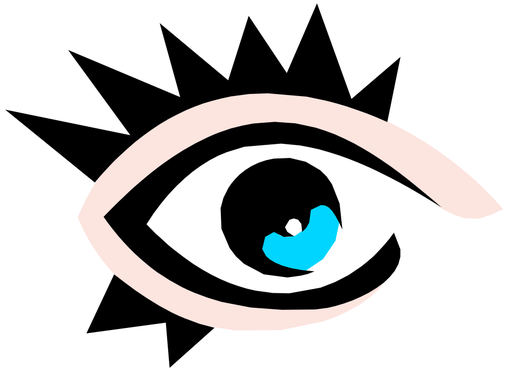
The biological function is in large part a consequence of these interactions

The 3D structure is more informative than sequence alone

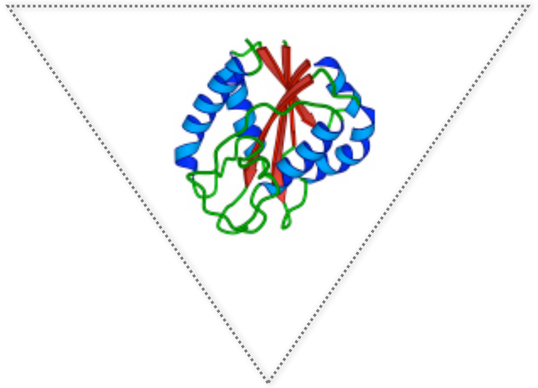
Evolution tends to conserve function and function depends more directly on structure than on sequence

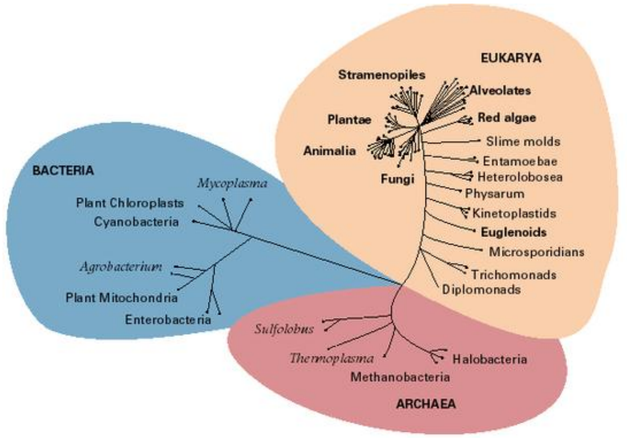


Data groups

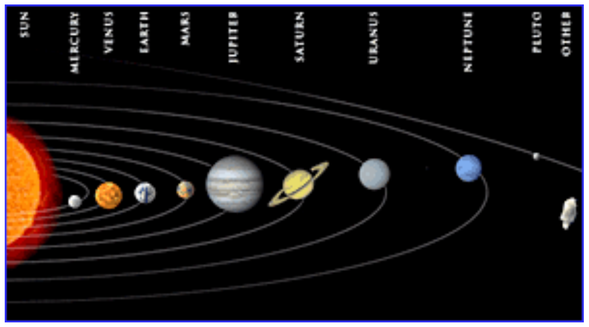


Experimental observations





Statistical rules



Laws of physics

Structure prediction vs determination

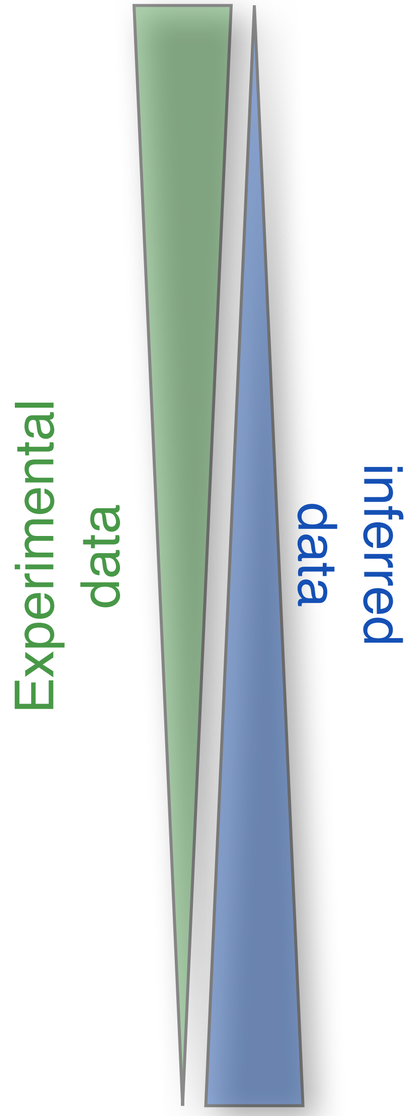
X-Ray

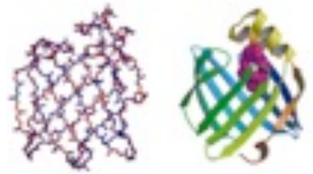
NMR

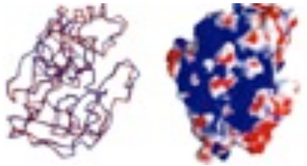
Comparative Modeling

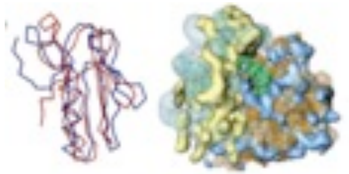
Threading

Ab-initio

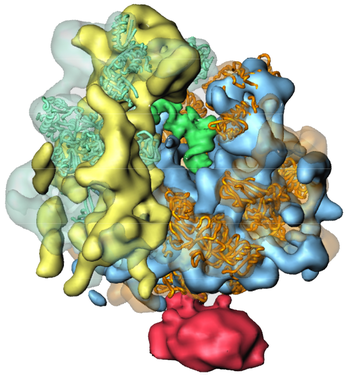


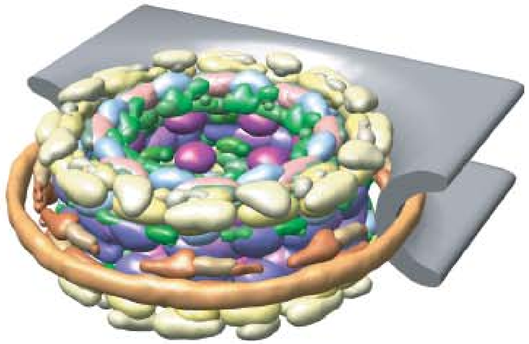


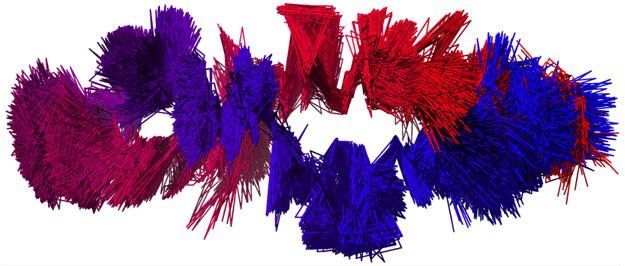


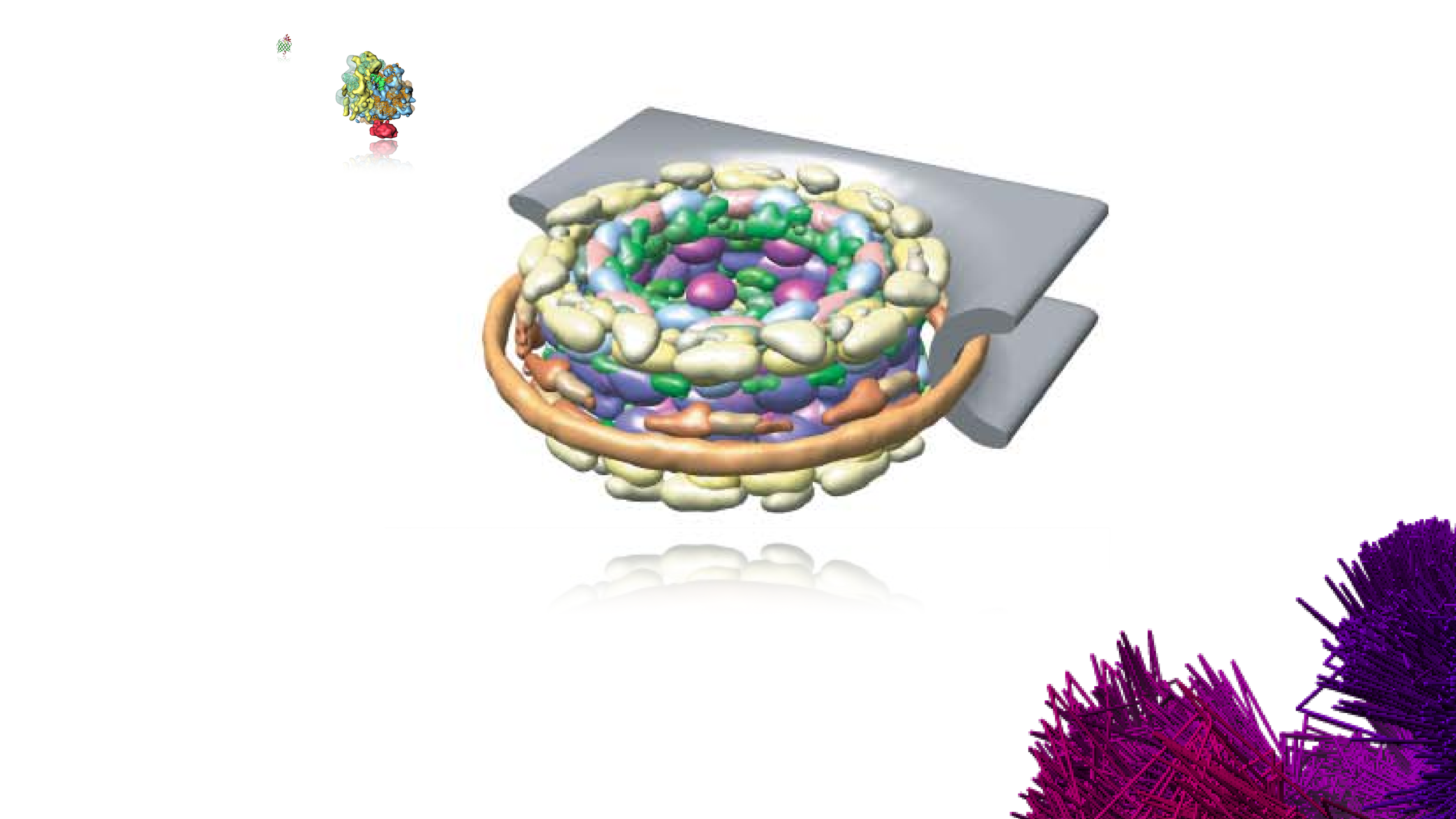


Data integration









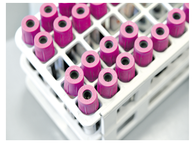
Advantages of integrative modeling

- **It facilitates the use of new information**
- **It maximizes accuracy, precision and completeness of the models**
- **It facilitates assessing the input information and output models**
- **It helps in understanding and assessing experimental accuracy**

Russel, D., Lasker, K., Webb, B., Velázquez-Muriel, J., Tjioe, E., Schneidman-Duhovny, D., Peterson, B., et al. (2012). *PLoS Biology*, *10*(1), e1001244

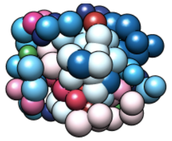
The four stages of integrative modeling

Data collection

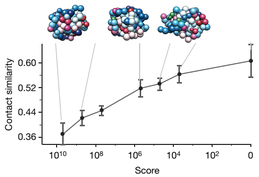


Representation and scoring

Analysis of the results



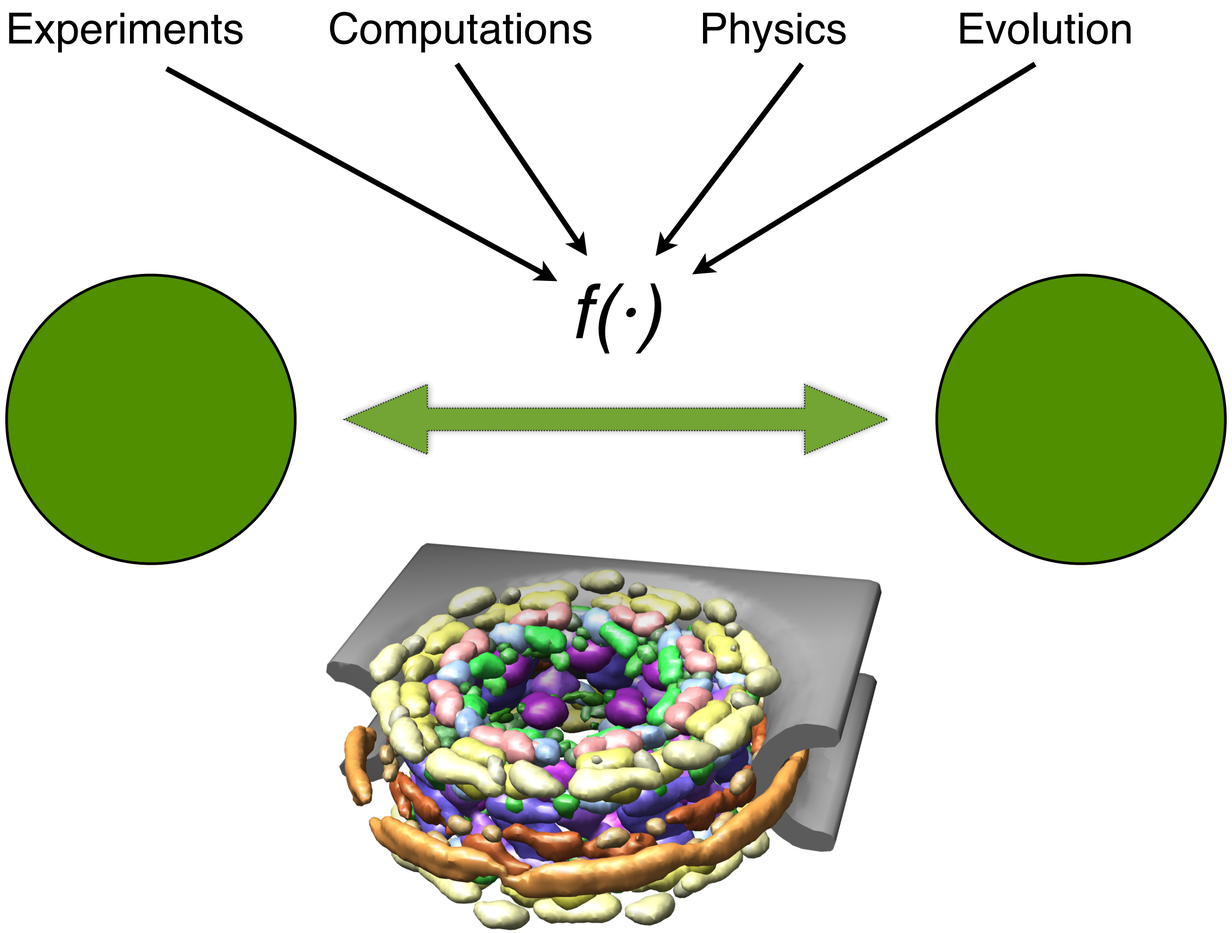
Optimization



UCSF

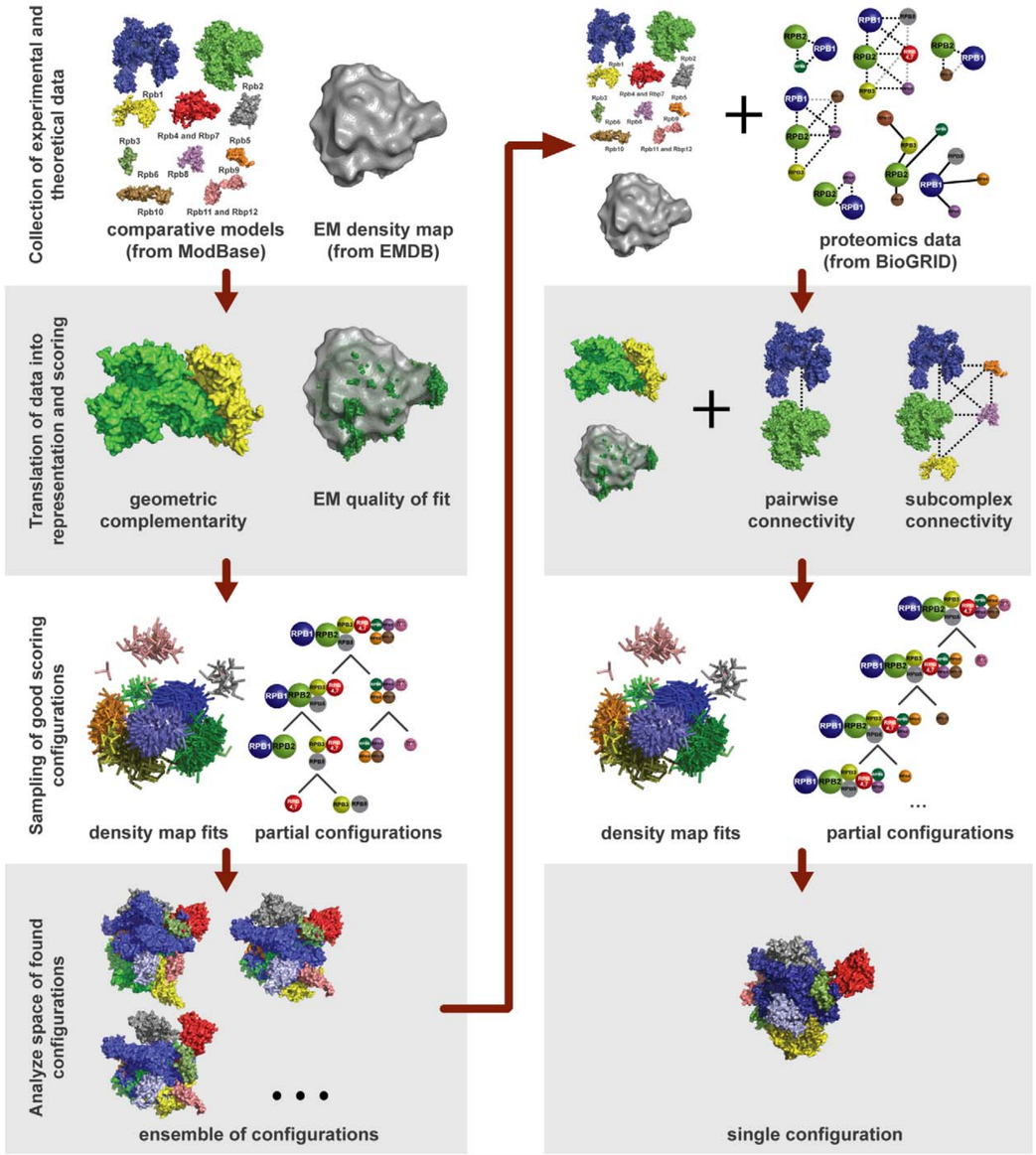
Integrative Modeling Platform

http://www.integrativemodeling.org



From: Russel, D. et al. PLOS Biology 10, e1001244 (2012).

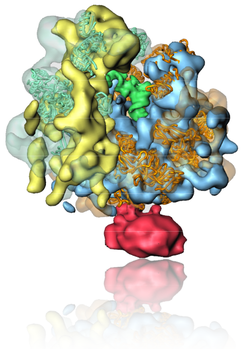
"Toy" example…

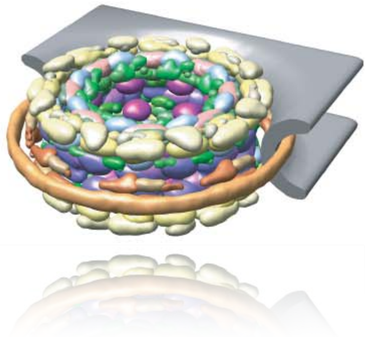


Russel, D., Lasker, K., Webb, B., Velázquez-Muriel, J., Tjioe, E., Schneidman-Duhovny, D., Peterson, B., et al. (2012). PLoS Biology, 10(1), e1001244

"Real" examples

PROTEINS



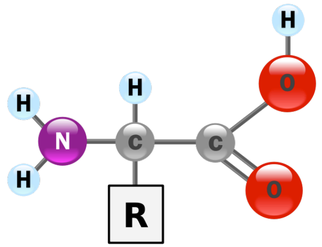


COMPLEXES

GENOMES

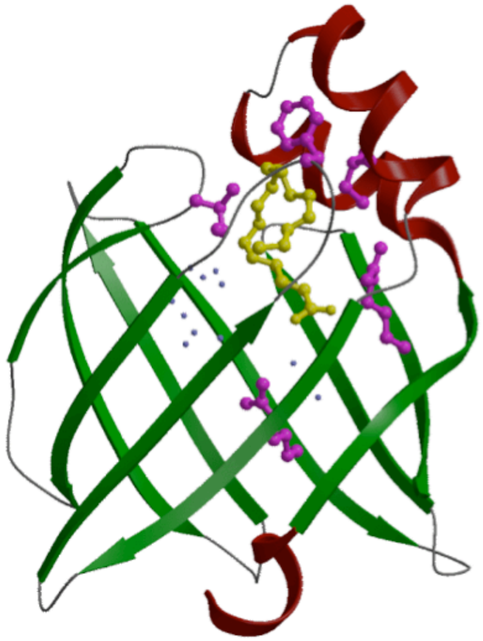
Proteins

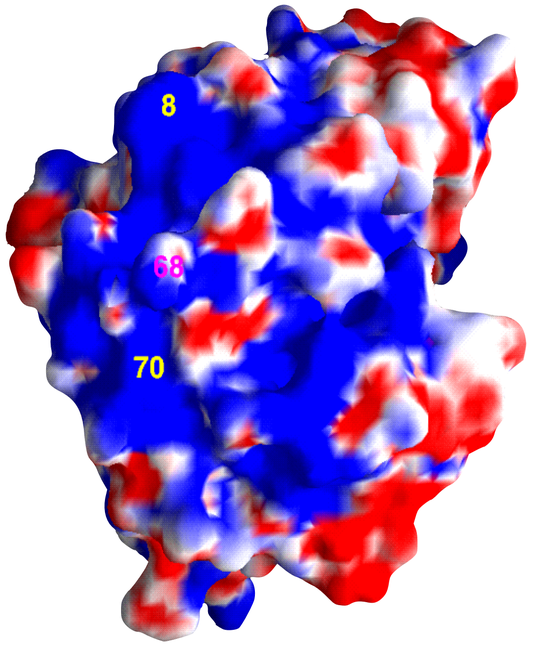
Single data type



Amino Acids

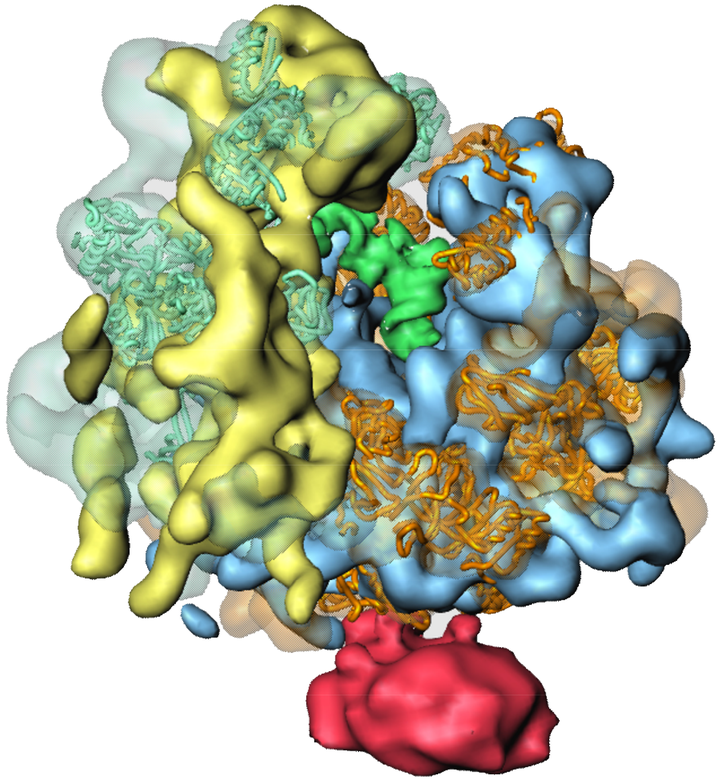
X-Ray;
NMR;
Modeling

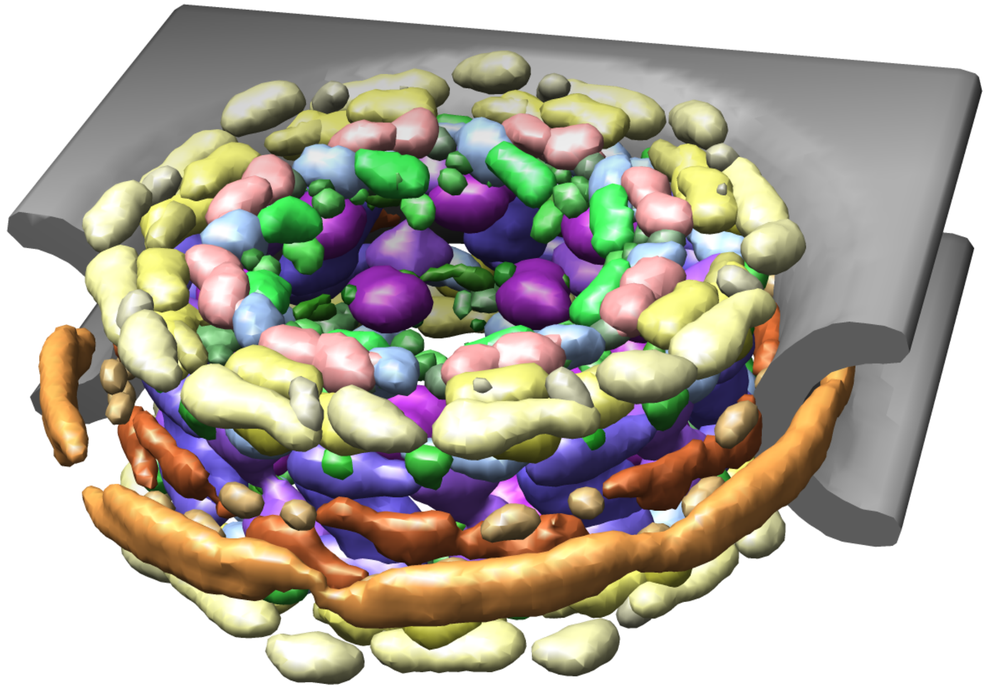




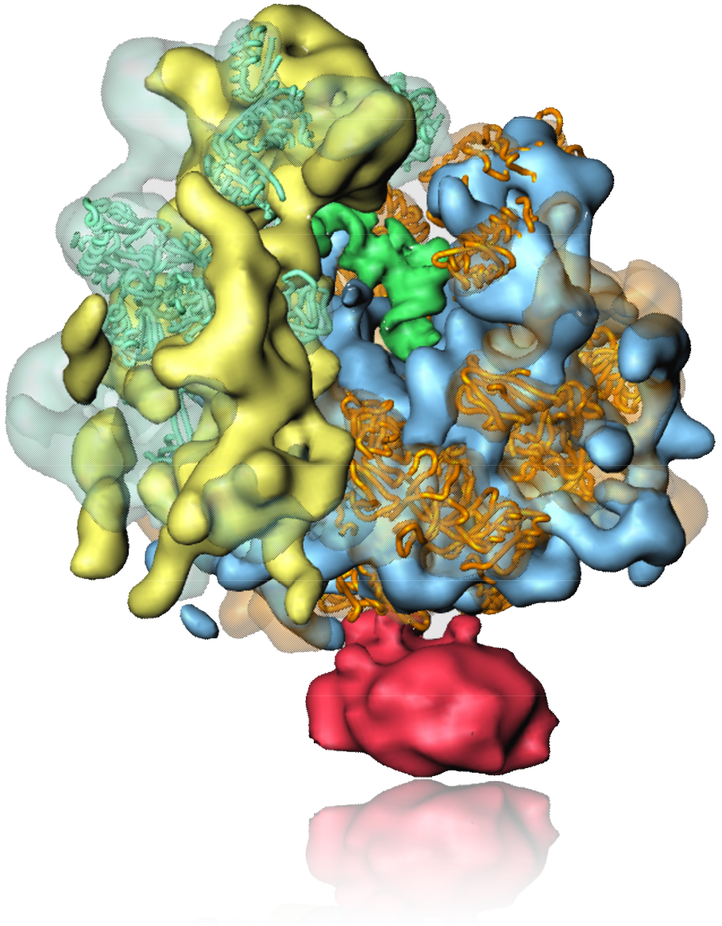
Complexes

Multiple data types





S. cerevisiae ribosome



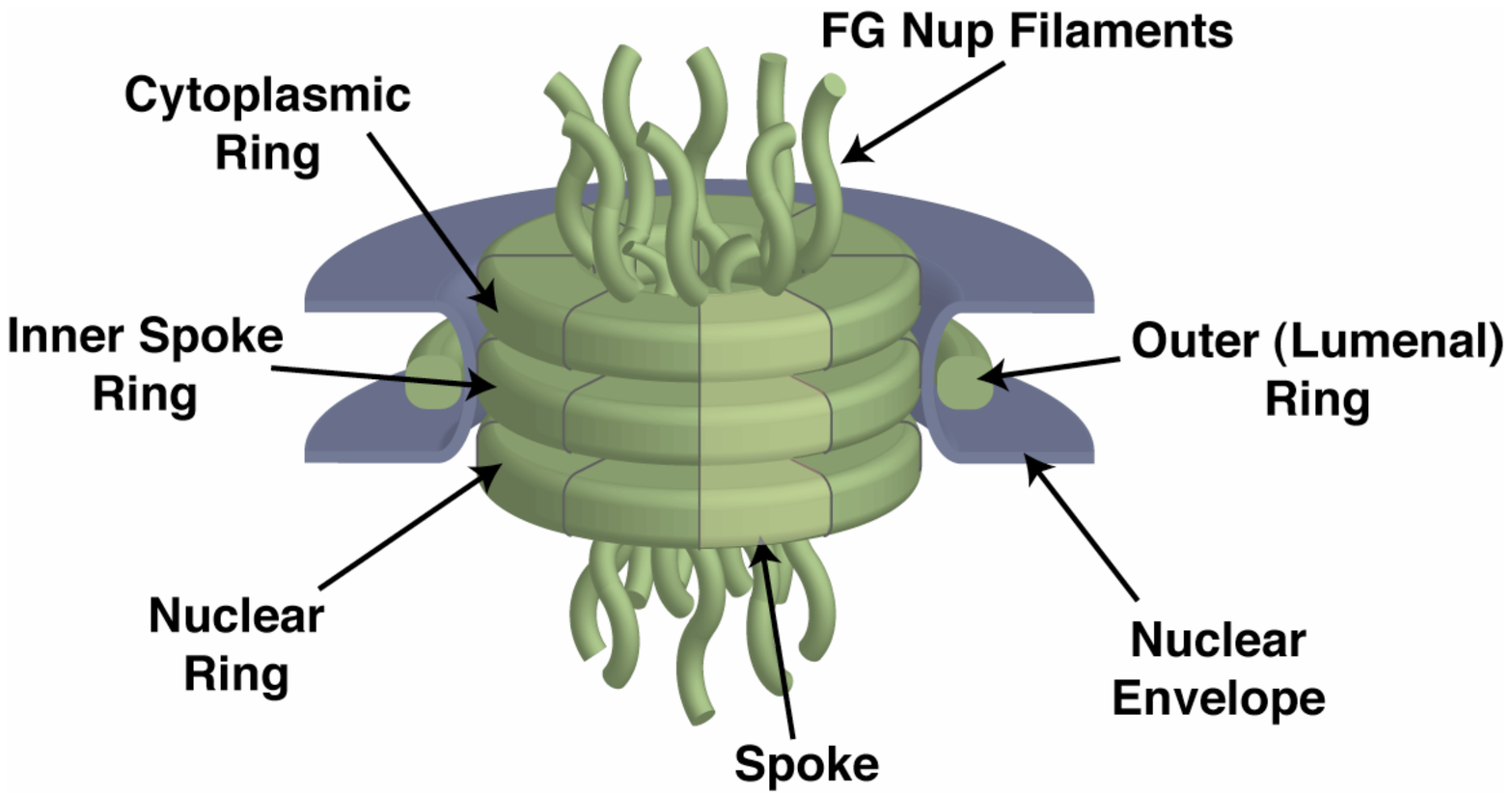
Fitting of comparative models into 15Å cryo-electron density map.

43 proteins could be modeled on 20-56% seq.id. to a known structure.

The modeled fraction of the proteins ranges from 34-99%.

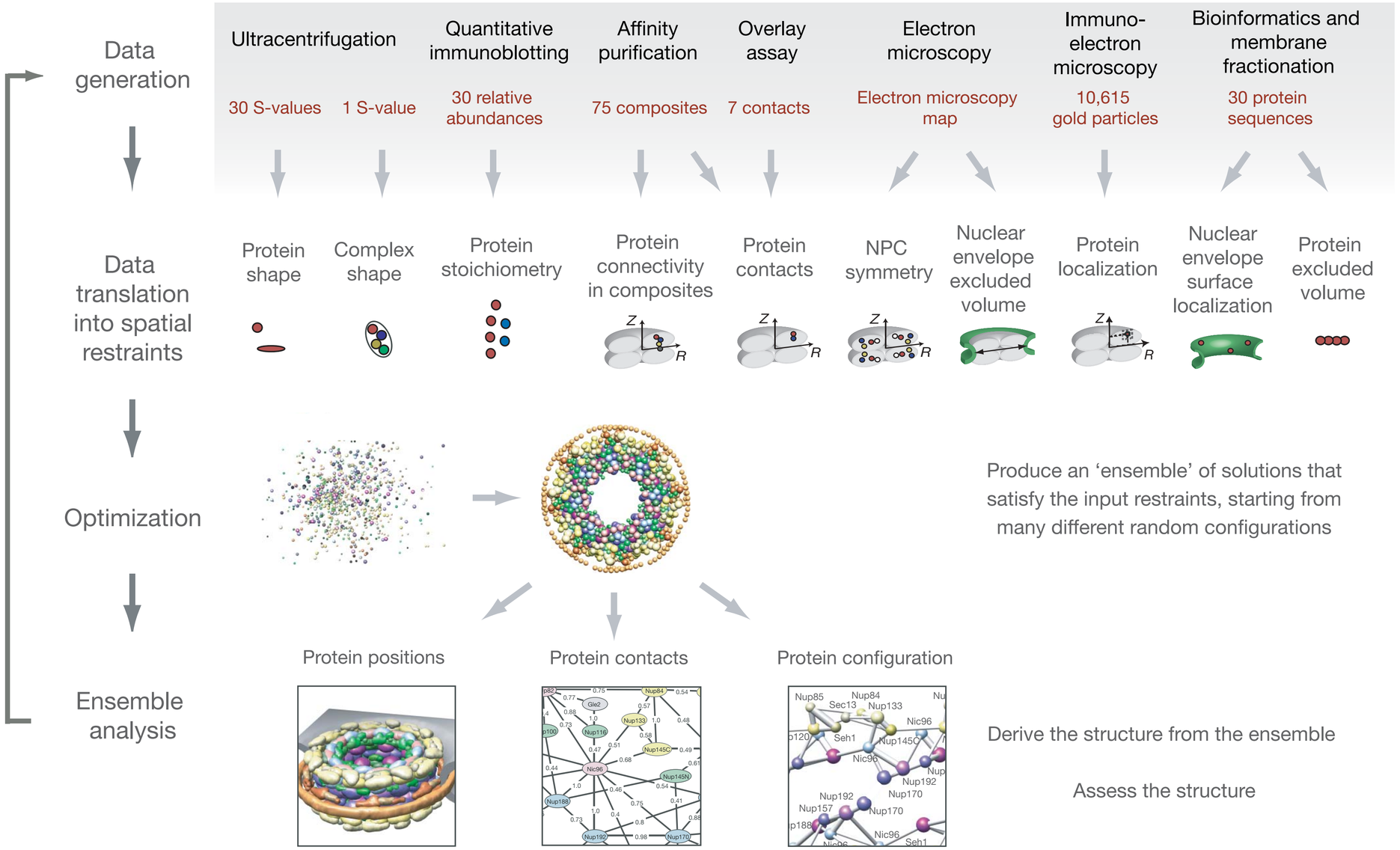
C. Spahn, R. Beckmann, N. Eswar, P. Penczek, A. Sali, G. Blobel, J. Frank. Cell 107, 361-372, 2001.

The nuclear pore complex



Alber, F., Dokudovskaya, S., Veenhoff, L. M., Zhang, W., Kipper, J., Devos, D., Suprapto, A., et al. (2007). *Nature*, *450*(7170), 695–701

Integrative Modeling of the NPC



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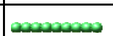
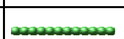
Representation

436 proteins!

τ	N_τ^1	N_τ^2	κ	$\{B_j^\kappa\}$	n_κ	r	τ	N_τ^1	N_τ^2	κ	$\{B_j^\kappa\}$	n_κ	r
Nup192	1	1	1,2,5		2	3.0	**Nup1**	0	1	1,5		9	1.5
			3	-	1	-				2		2	1.5
Nup188	1	1	1,2,5		2	3.0				3	-	1	-
			3	-	1	-				4		7	1.5
Nup170	1	1	1,2,5		2	2.9	**Nsp1**	2	2	1,5		12	1.3
			3	-	1	-				2		3	1.3
Nup157	1	1	1,2,5		3	2.5				3	-	1	-
			3	-	1	-				4		9	1.3
Nup133	1	1	1,2,5		2	2.7	**Gle1**	1	0	1,2,5		2	2.1
			3	-	1	-				3	-	1	-
Nup120	1	1	1,2,5		2	2.6	**Nup60**	0	1	1,5		4	1.6
			3	-	1	-				2,3		1	1.6
Nup85	1	1	1,2,5		3	2.0				4		3	1.6
			3	-	1	-	**Nup59**	1	1	1,5		4	1.6
Nup84	1	1	1,2,5		3	2.0				2		2	1.6
			3	-	1	-				3	-	1	-
Nup145C	1	1	1,2,5		2	2.3				4		2	1.6
			3	-	1	-	**Nup57**	1	1	1,5		3	1.8
Seh1	1	1	1,2,3,5		1	2.2				2,3		1	1.8
Sec13	1	1	1,2,3,5		1	2.1				4		2	1.8
Gle2	1	1	1,2,3,5		1	2.3	**Nup53**	1	1	1,5		3	1.7
Nic96	2	2	1,2,5		2	2.4				2,3		1	1.7
			3	-	1	-				4		2	1.7
Nup82	1	1	1,2,5		2	2.3	**Nup145N**	0	2	1,5		6	1.5
			3	-	1	-				2,3		1	1.5

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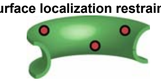
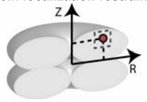
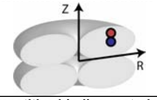
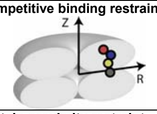
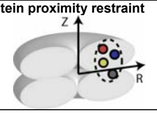
Scoring

Data generation		Data interpretation				
Method	**Experiments**	**Restraint**	R_C	R_O	R_A	**Functional form of activated feature restraint**
Bioinformatics and Membrane fractionation	30 nup sequences	Protein excluded volume restraint	-	-	1,864 *1,863/2	**Protein-protein**: Violated for $f < f_o$. f is the distance between two beads, f_o is the sum of the bead radii, and σ is 0.01 nm. Applied to all pairs of particles in representation κ=1: $B^{ms} = \{B_j^{\kappa=1}(\theta,s,\tau,i)\}$
	30 nup sequences	Surface localization restraint 	-	-	48	**Membrane-surface location:** Violated if $f \neq f_o$. f is the distance between a protein particle and the closest point on the NE surface (half-torus), f_o = 0 nm, and σ is 0.2 nm. Applied to particles: $B^{ms} = \{B_j^{\kappa=1}(\theta,s,\tau,i) \mid \tau \in (\text{Ndc1,Pom152,Pom34})\}$
	30 Nup sequences and immuno-EM (see below)		-	-	64	**Pore-side volume location:** Violated if $f < f_o$. f is the distance between a protein particle and the closest point on the NE surface (half-torus), f_o = 0 nm, and σ is 0.2 nm. Applied to particles: $B^{ms} = \{B_j^{\kappa=1}(\theta,s,\tau,i) \mid \tau \in (\text{Ndc1,Pom152,Pom34})\}$
			-	-	80	**Perinuclear volume location:** Violated if $f > f_o$,, f is the distance between a protein particle and the closest point on the NE surface (half-torus), f_o = 0 nm, and σ is 0.2 nm. Applied to particles: $B^{ms} = \{B_j^{\kappa=1}(\theta,s,\tau,i)\, \tau \in (\text{Pom152})\}$
Hydrodynamics experiments	1 S-value	Complex shape restraint	1	164	1	**Complex diameter** Violated if $f < f_o$. f is the distance between two protein particles representing the largest diameter of the largest complex, f_o is the complex maximal diameter $D=19.2-R$, where R is the sum of both particle radii, and σ is 0.01 nm. Applied to particles of proteins in composite C_{45}: $B^{ms} = \{B_j^{\kappa=1}(\theta,s,\tau,i) \mid \tau \in C_{33}\}$
	30 S-values	Protein chain restraint	-	-	1,680	**Protein chain** Violated if $f \neq f_o$. f is the distance between two consecutive particles in a protein, f_o is the sum of the particle radii, and σ is 0.01 nm. Applied to particles: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \kappa = 1\}$
Immuno-Electron microscopy	10,940 gold particles	Protein localization restraint 	-	-	456	**Z-axial position** Violated for $f < f_o$. f is the absolute Cartesian Z-coordinate of a protein particle, f_o is the lower bound defined for protein type τ, and σ is 0.1 nm. Applied to particles: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \kappa = 1, j = 1\}$
					456	Violated for $f > f_o$. f is the absolute Cartesian Z-coordinate of a protein particle, f_o is the upper bound defined for protein type τ, and σ is 0.1 nm. Applied to particles: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \kappa = 1, j = 1\}$
			-	-	456	**Radial position** Violated for $f < f_o$. f is the radial distance between a protein particle and the Z-axis in a plane parallel to the X and Y axes, f_o is its lower bound defined for protein type τ, and σ is 0.1 nm. Applied to particles: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \kappa = 1, j = 1\}$
					456	Violated for $f > f_o$. f is the radial distance between a protein particle and the Z-axis in a plane parallel to the X and Y axes, f_o is its upper bound defined for protein type τ, and σ is 0.1 nm. Applied to particles: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \kappa = 1, j = 1\}$
Overlay assays	13 contacts	Protein interaction restraint 	20	112	20	**Protein contact** Violated for $f > f_o$. f is the distance between two protein particles, f_o is the sum of the particle radii multiplied by a tolerance factor of 1.3, and σ is 0.01 nm. Applied to particle: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \kappa \in (2,4,9), \theta \in (1,2,3)\}$
Affinity purification	4 complexes	Competitive binding restraint 	1	132	4	**Protein contact** Violated for $f > f_o$. f is the distance between two protein particles, f_o is the sum of the particle radii multiplied by a tolerance factor of 1.3, and σ is 0.01 nm. Applied to : $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \theta \in (1,2,3), \kappa \in (2,4,6), \tau = (Nup82, Nic96, Nup49, Nup57)\}$
	64 complexes	Protein proximity restraint 	692	25,348	692	**Protein proximity** Violated for $f > f_o$. f is the distance between two protein particles, f_o is the maximal diameter of a composite complex, and σ is 0.01 nm. Applied to particles: $B = \{B_j^{\kappa}(\theta,s,\tau,i) \mid \theta \in (1,2,3), \kappa \in (2,4,9)\}$

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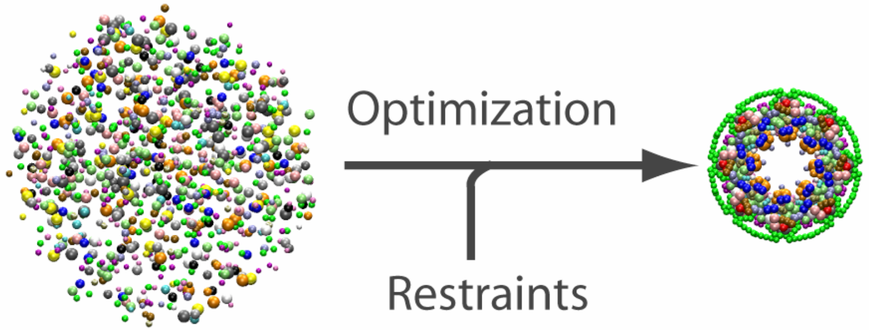
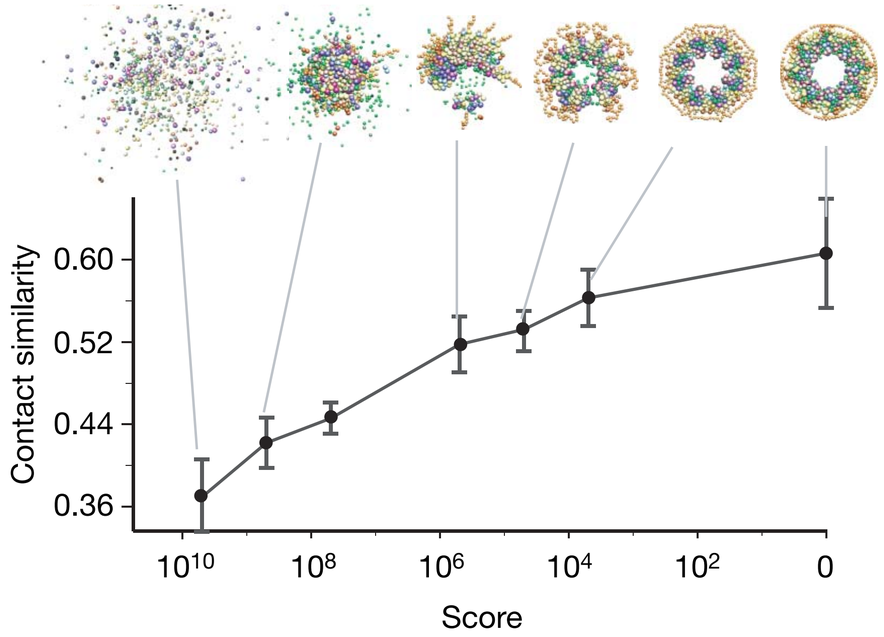
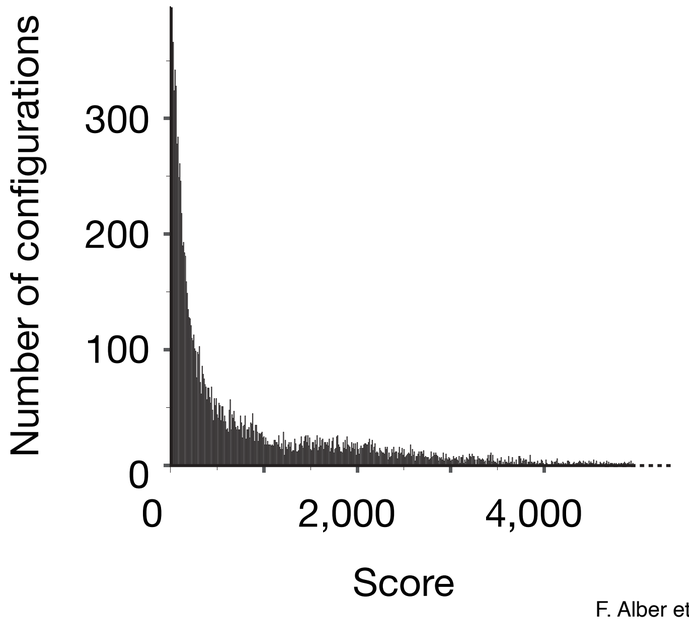
Optimization







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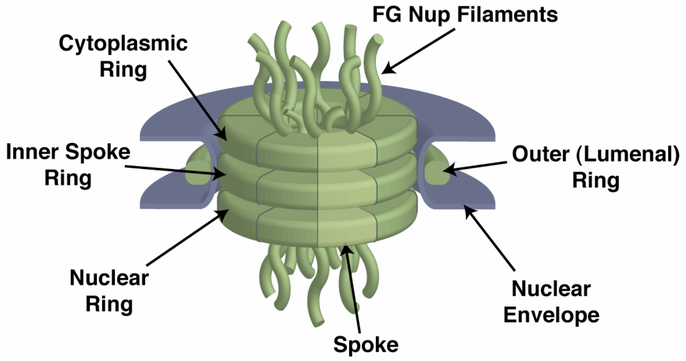
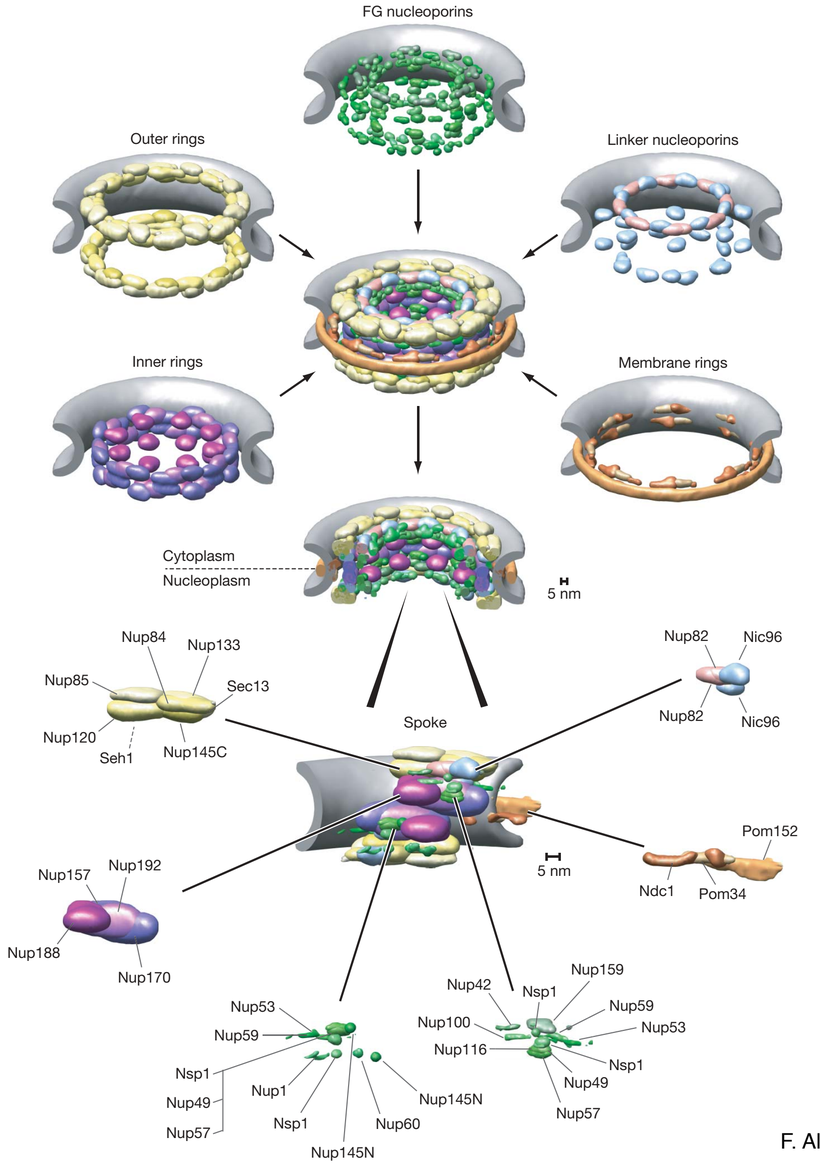
The structure of the nuclear pore complex





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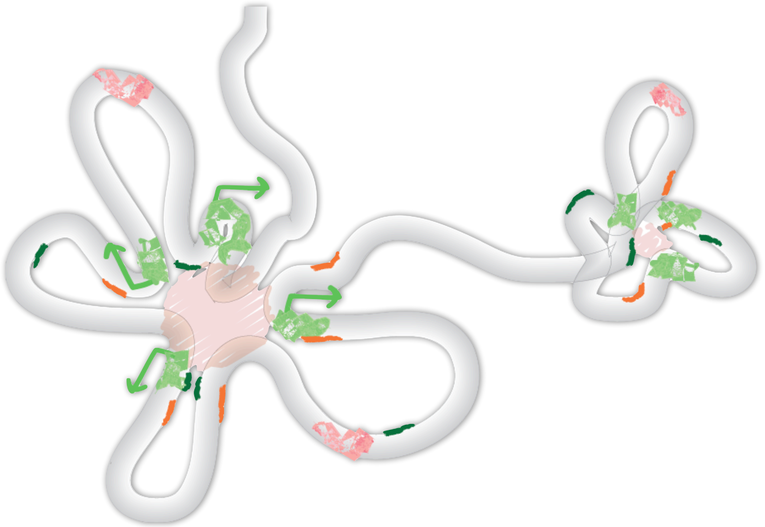
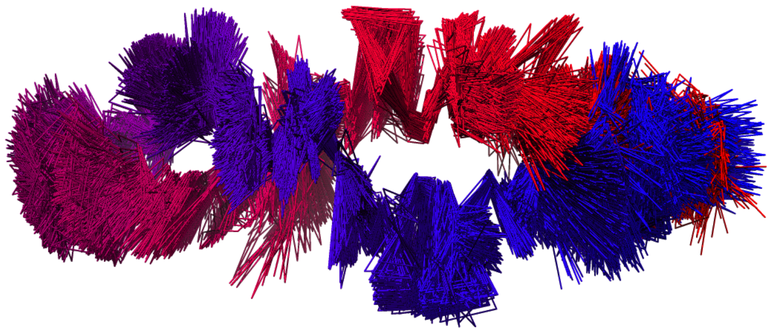
Genomes

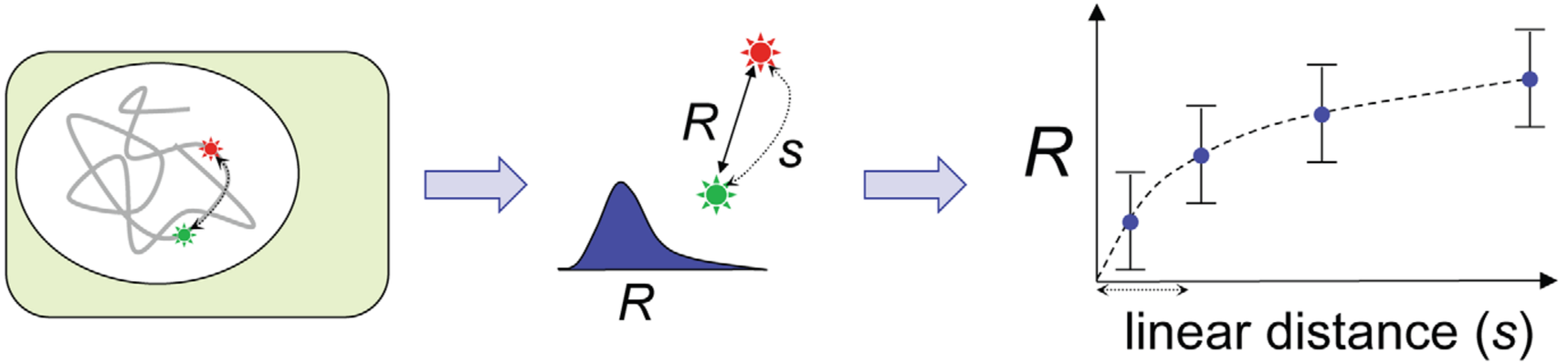
Limited data types



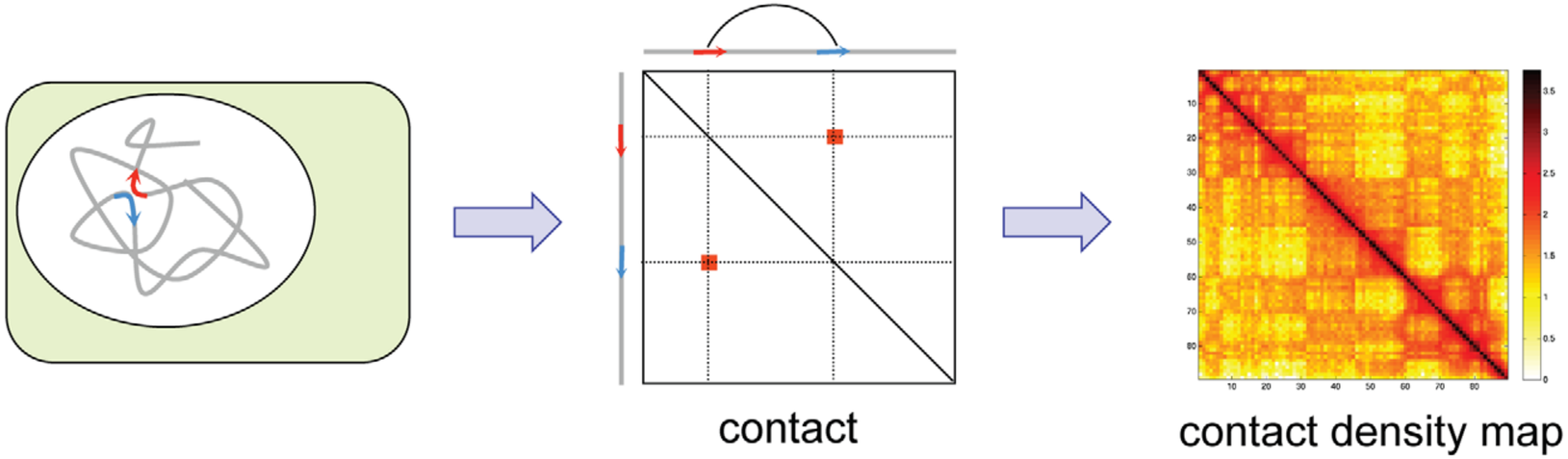
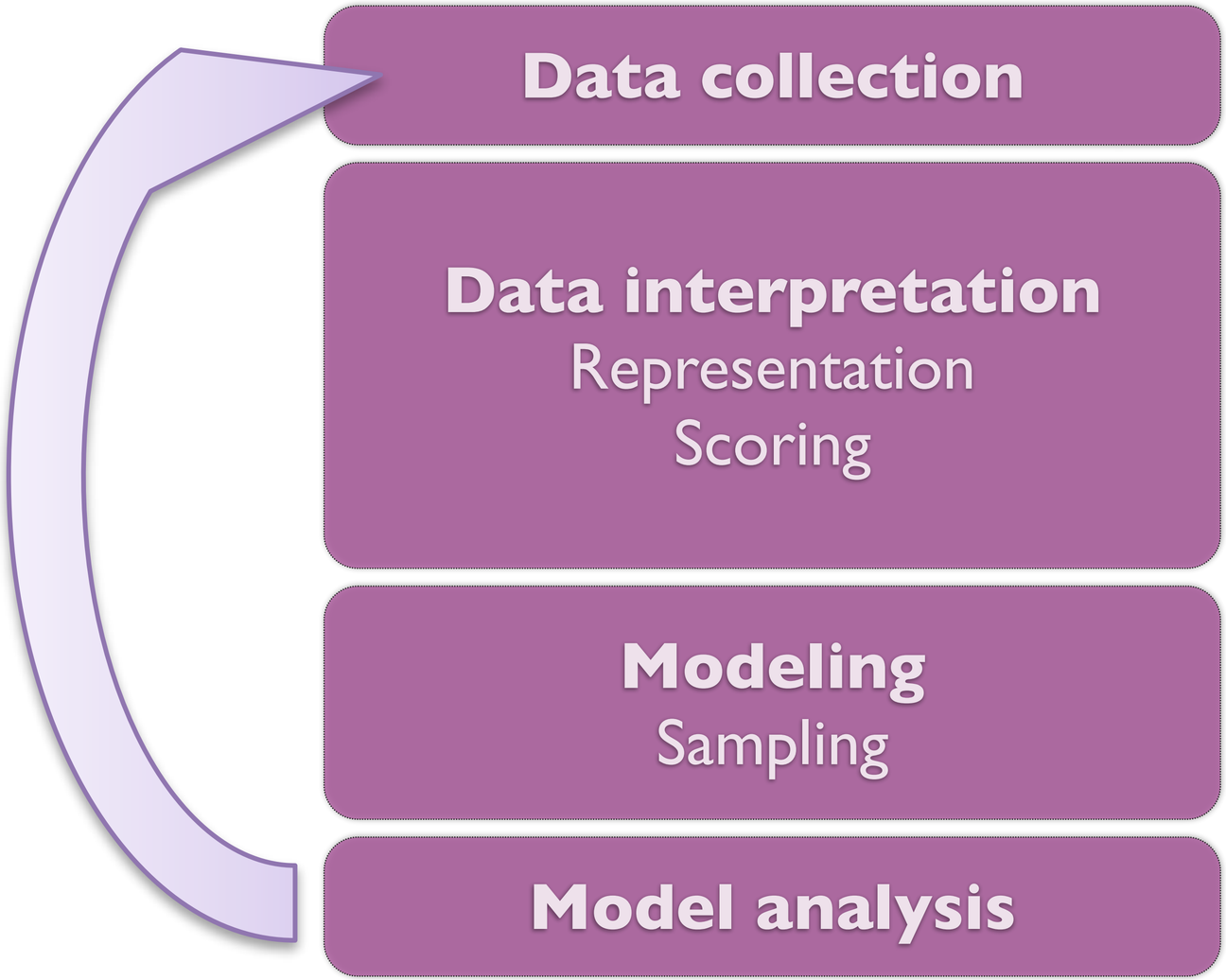


Main approaches

Light microscopy (FISH)



Cell/molecular biology (3C-based methods)



Take home message