

21 января 2016 года

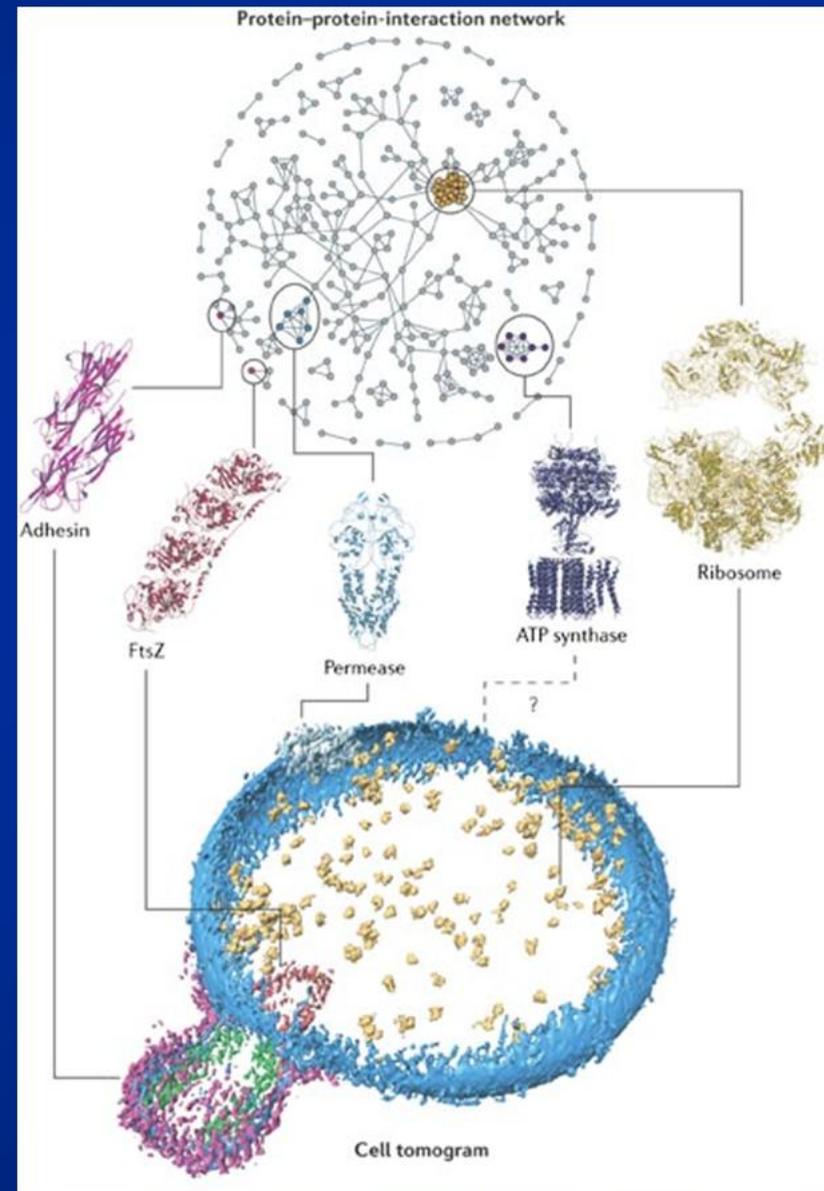
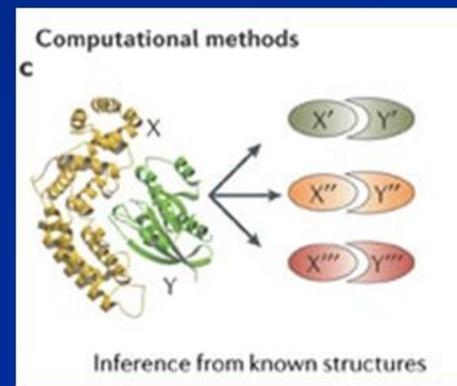
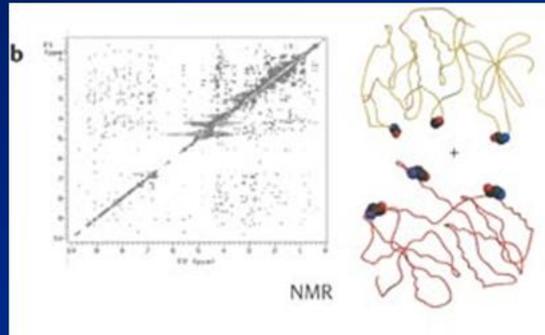
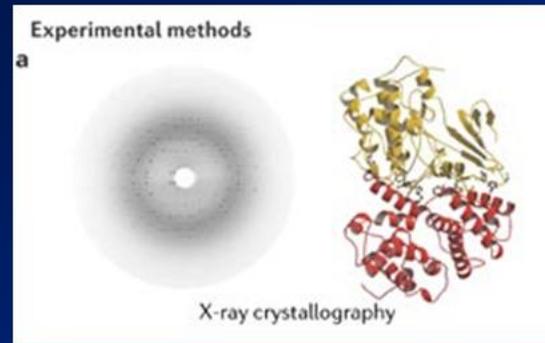
Integrative Approaches for Structure Determination

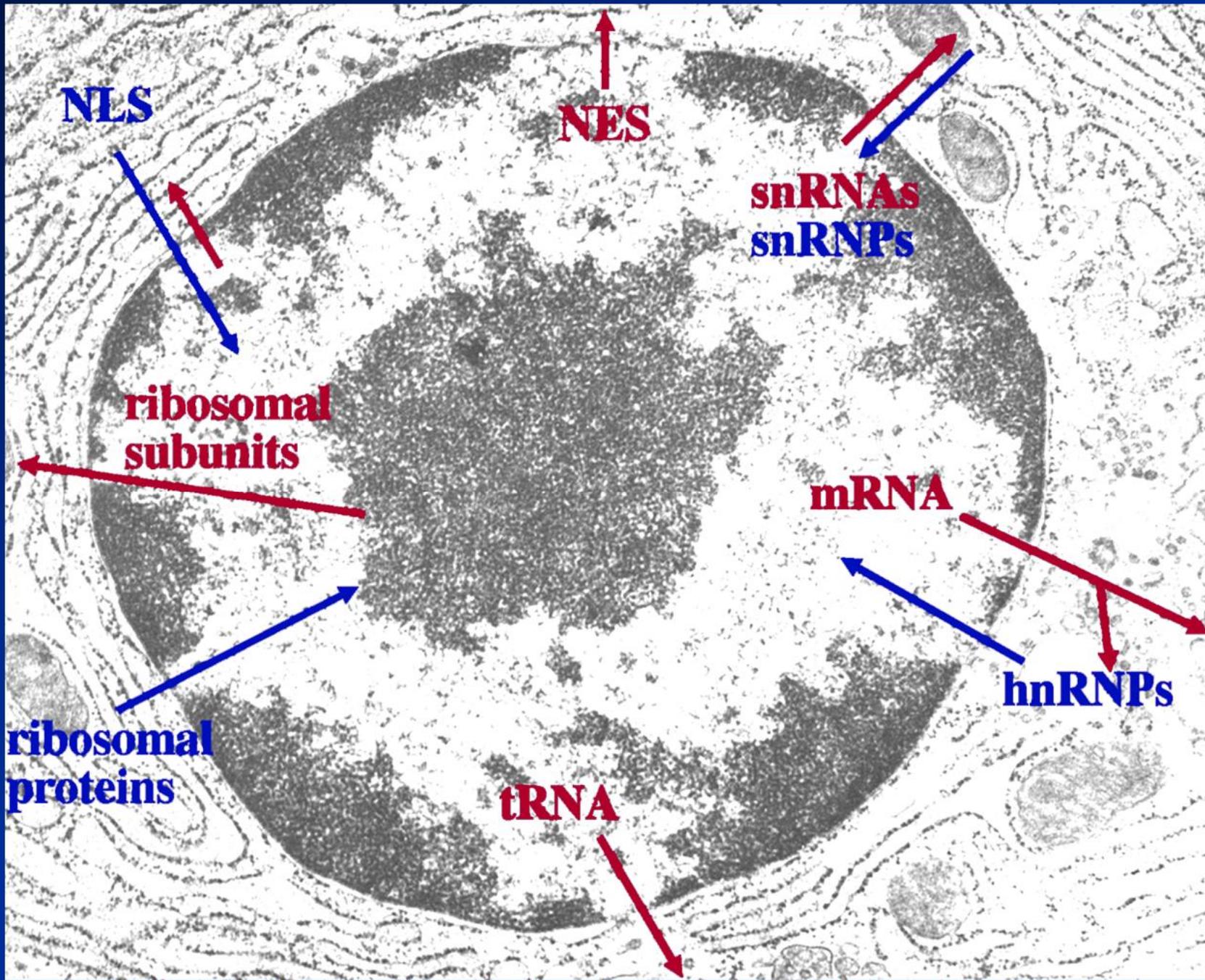
Интегративные подходы к решению
трехмерных структур
макромолекулярных комплексов

Svetlana Dokudovskaya,
С.С. Докудовская

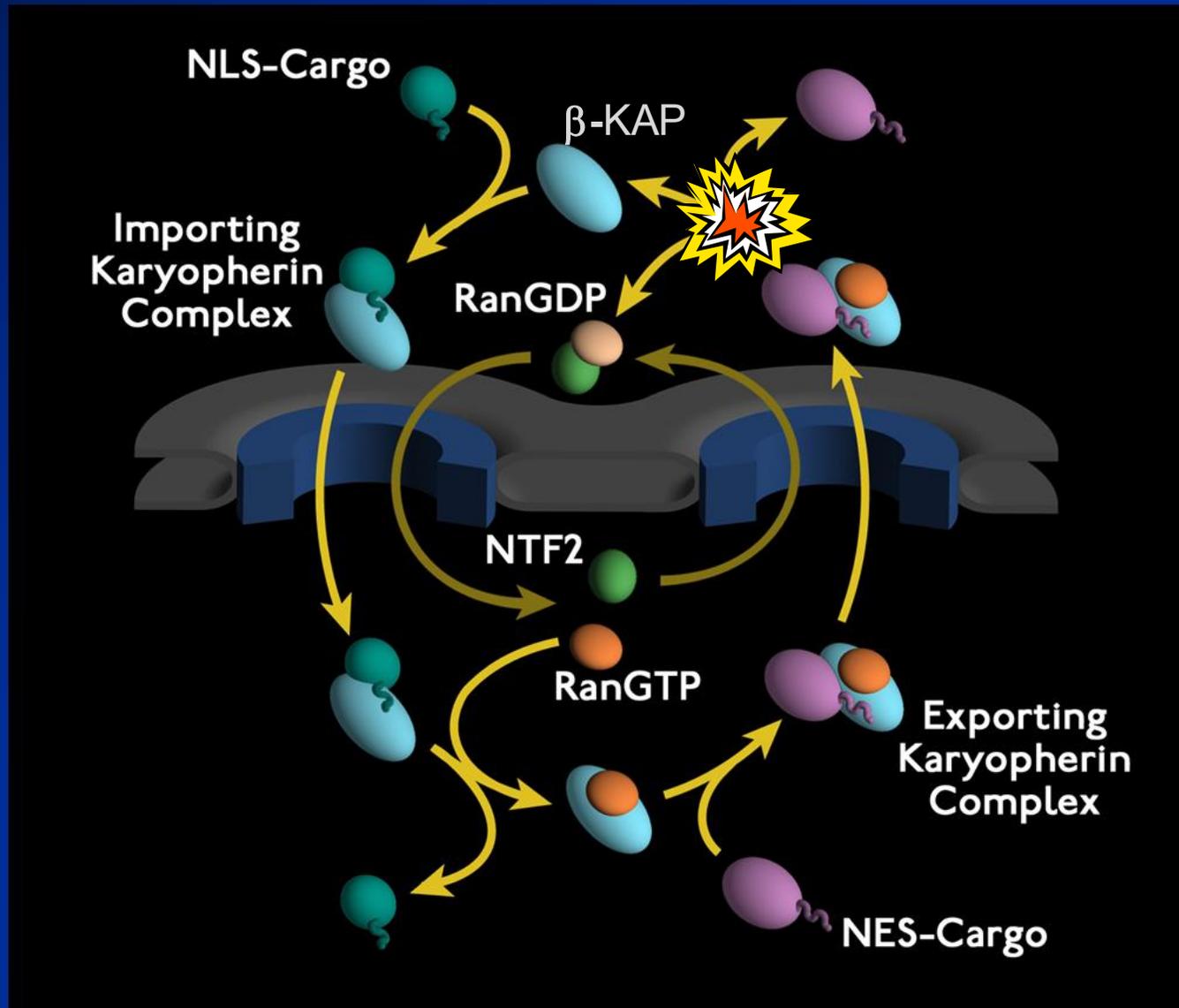
UMR8126 CNRS, Institut Gustave Roussy,
Villejuif, FRANCE

How to study cellular functional modules

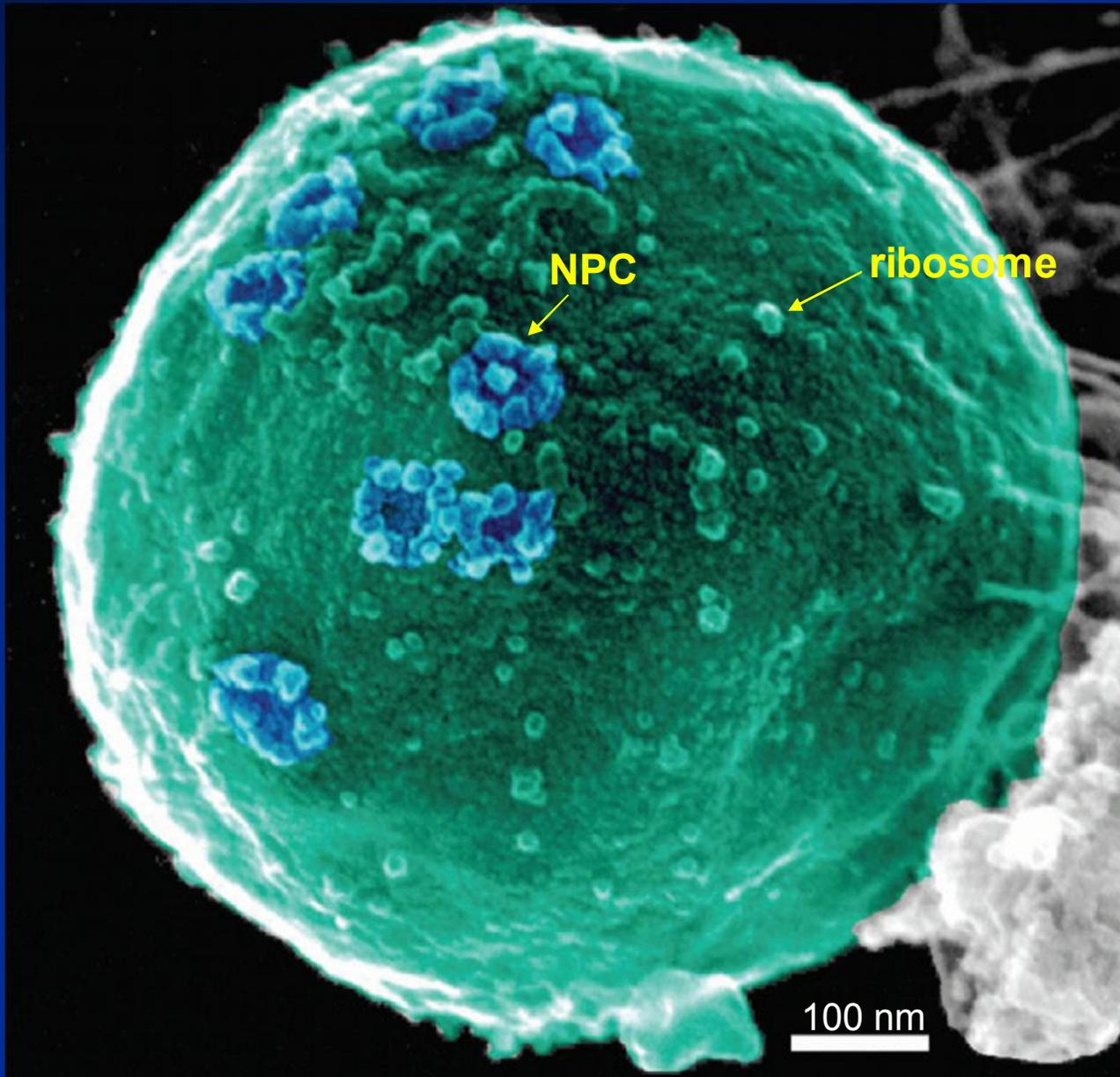




Karyopherin Mediated Transport



The Nuclear Pore Complex (NPC)

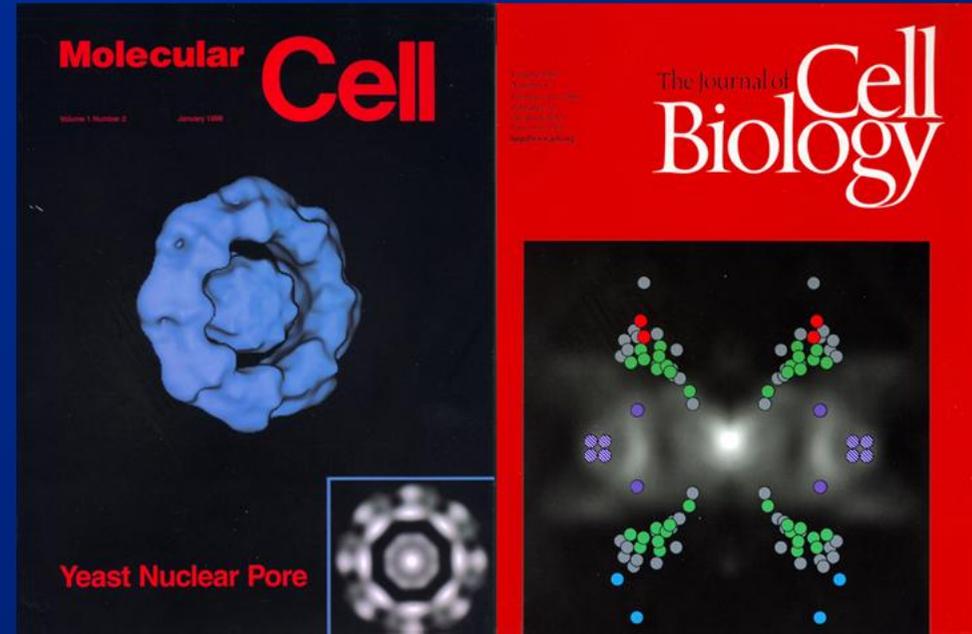


NPC:
-50 MDa
-480 proteins
of ~30 different types

Ribosome:
-2 MDa
-80 proteins
-3 RNAs

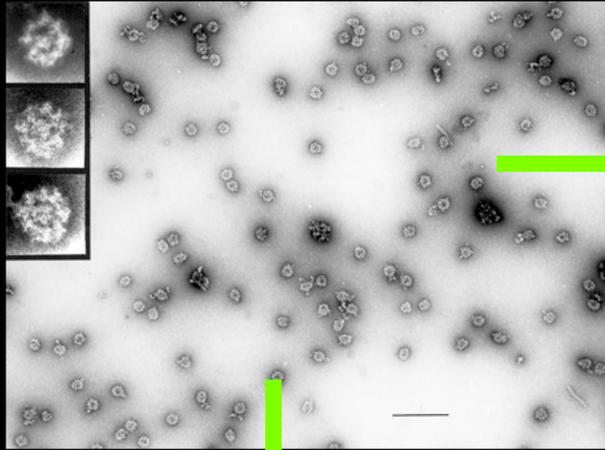
Kiseleva, *Nat. Cell. Biol.* 6, 497, 2004.

Working Out the Structure of the NPC - We Need:

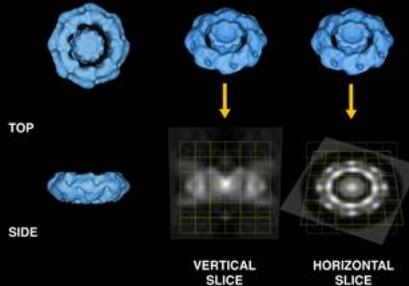


- x What the Whole NPC Looks Like X
- x An NPC Component List X
- x Amount of Each Component in the NPC X
- x Where the Components Go in the NPC X
- x What Each Component Looks Like X
- x How the Components Fit Together X

Yeast NPC Preparation

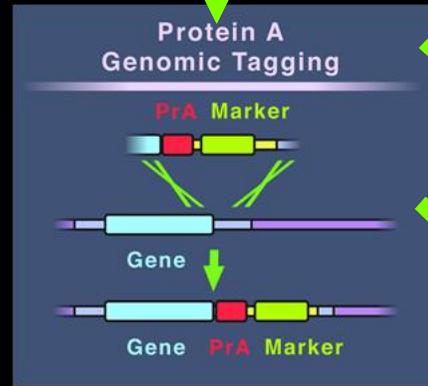
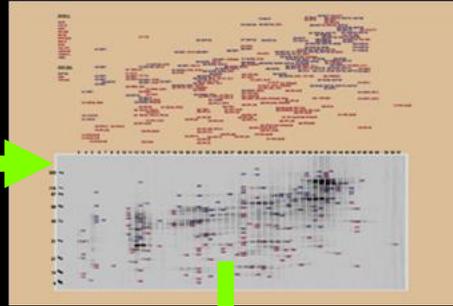


Electron Cryomicroscopy



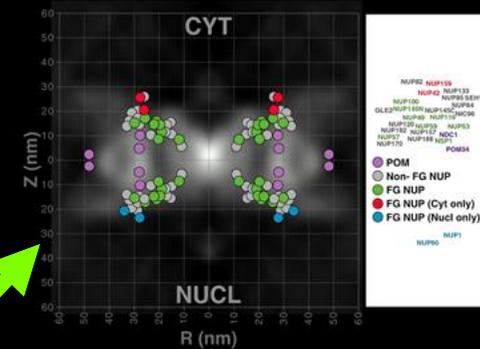
What the Whole NPC Looks Like

Protein Separation, Mass Spectrometric Identification & Genomic Tagging



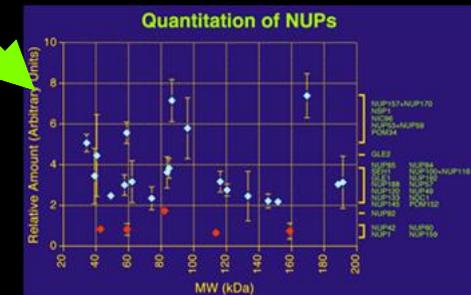
NPC Component List

Immunolectron Microscopy



Where the Components Go in the NPC

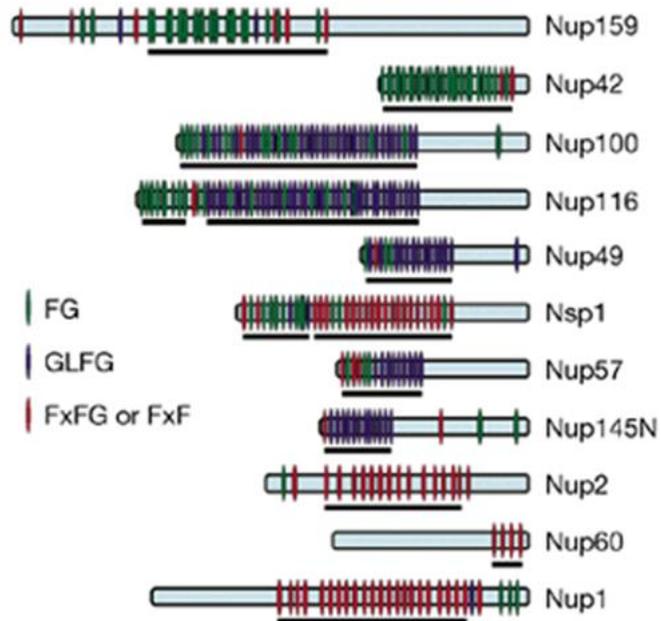
Quantitative Western Blotting



Amount of Each Component in the NPC

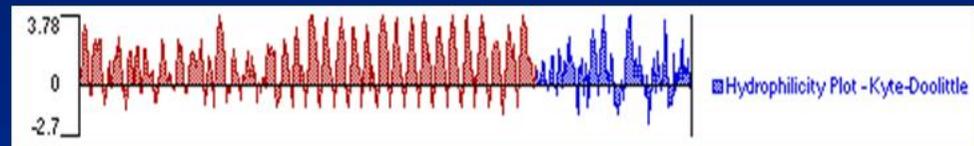
Yeast FG Nucleoporins (FG NUPs)

FG Repeat Nups in Yeast



Consensus sequence of FG repeat region of Nsp1p:

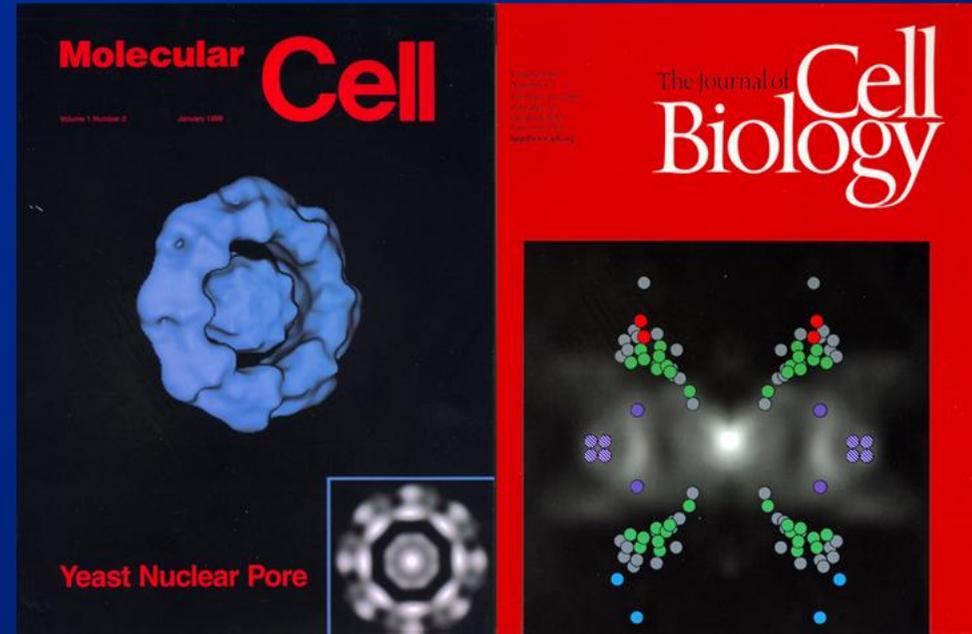
```
. . . PSFSFGAKSDENKAGATSK  
PAFSFGAKPEEKDDNSSK  
PAFSFGAKSNEDKQDGTAK  
PAFSFGAKPAEKNNNETSK . . .
```



FG NUP Characteristics:

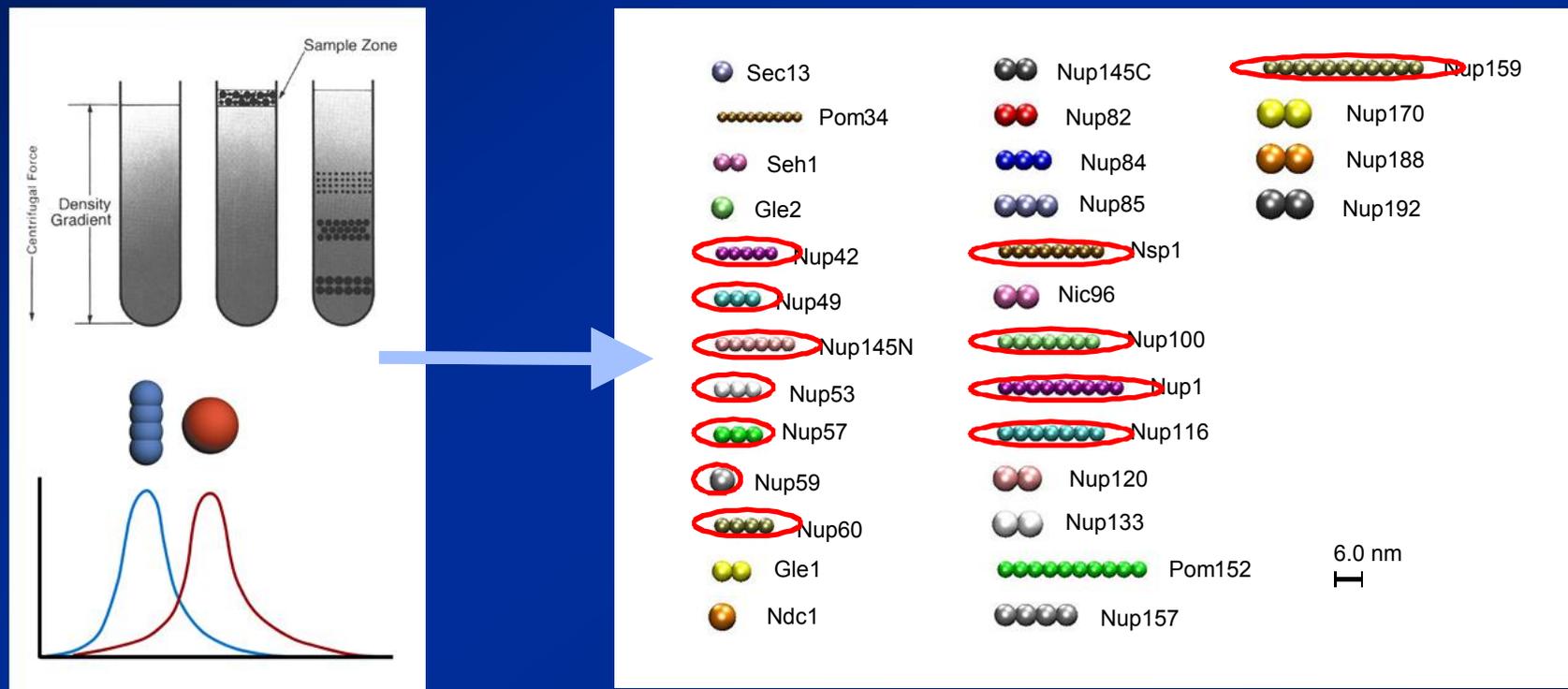
- ⇒ Disordered Filaments
- ⇒ Found at NPC Filaments
- ⇒ Karyopherin Docking Sites

Working Out the Structure of the NPC - We Need:



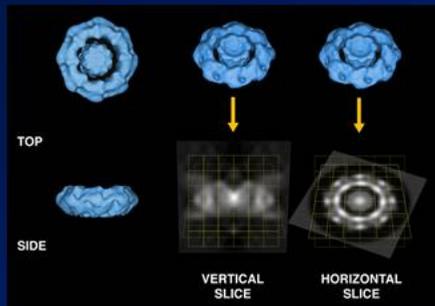
- X What the Whole NPC Looks Like X
- X An NPC Component List X
- X Amount of Each Component in the NPC X
- X Where the Components Go in the NPC X
- X What Each Component Looks Like X
- X How the Components Fit Together X

Determining Shape of the Nups: Sedimentation Analysis

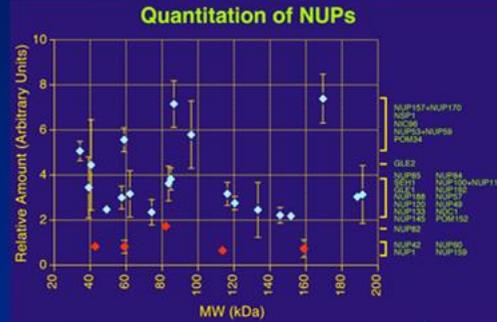


Gives us a Rough Shape for Each Nup

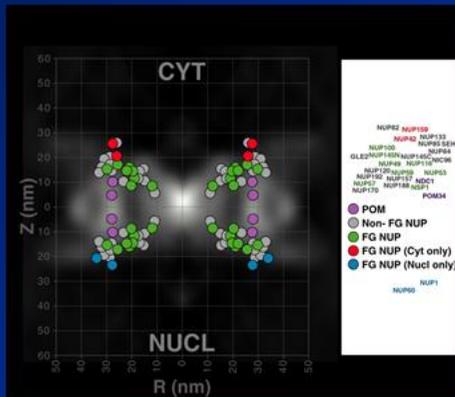
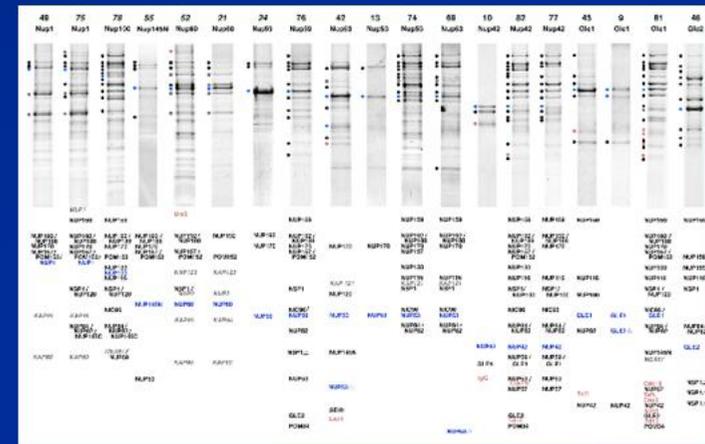
Experimental Data - Spatial Restraints



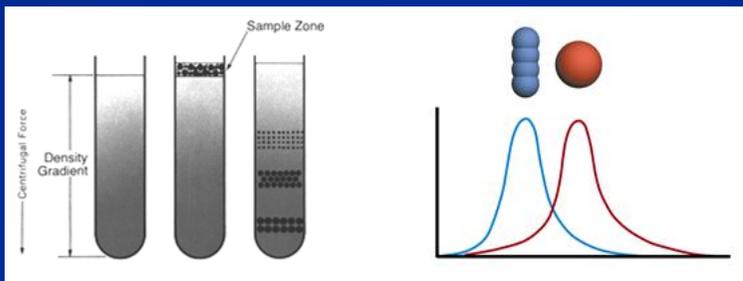
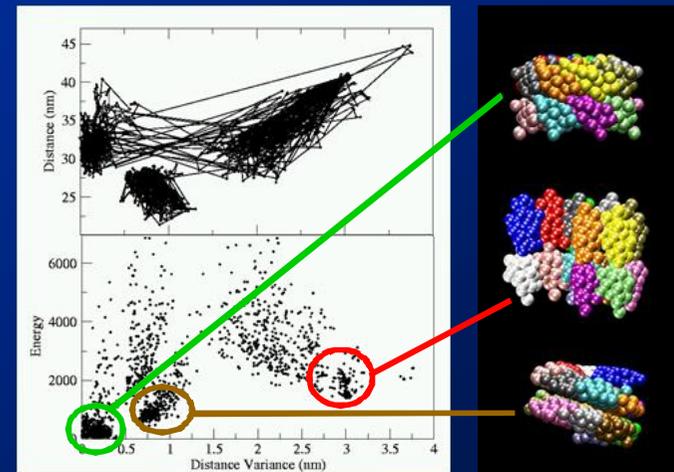
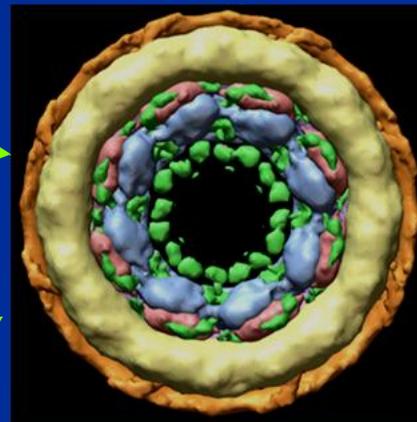
Overall NPC shape



NUP Stoichiometry



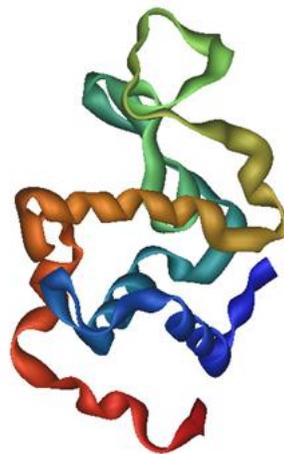
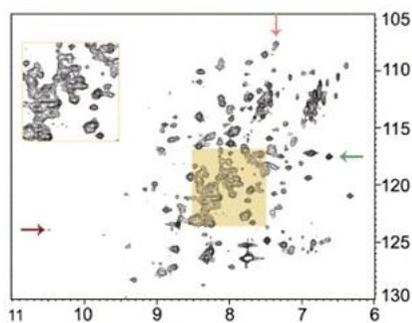
NUP Localization



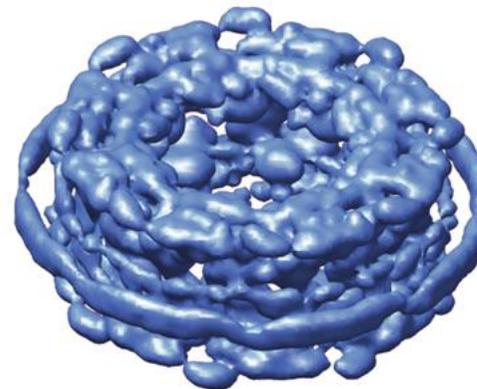
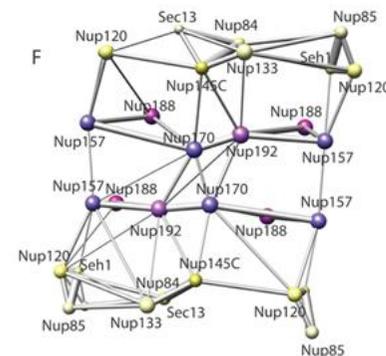
NUP Shape

Structure Determination by Satisfaction of Spatial Restraints - the NMR Concept

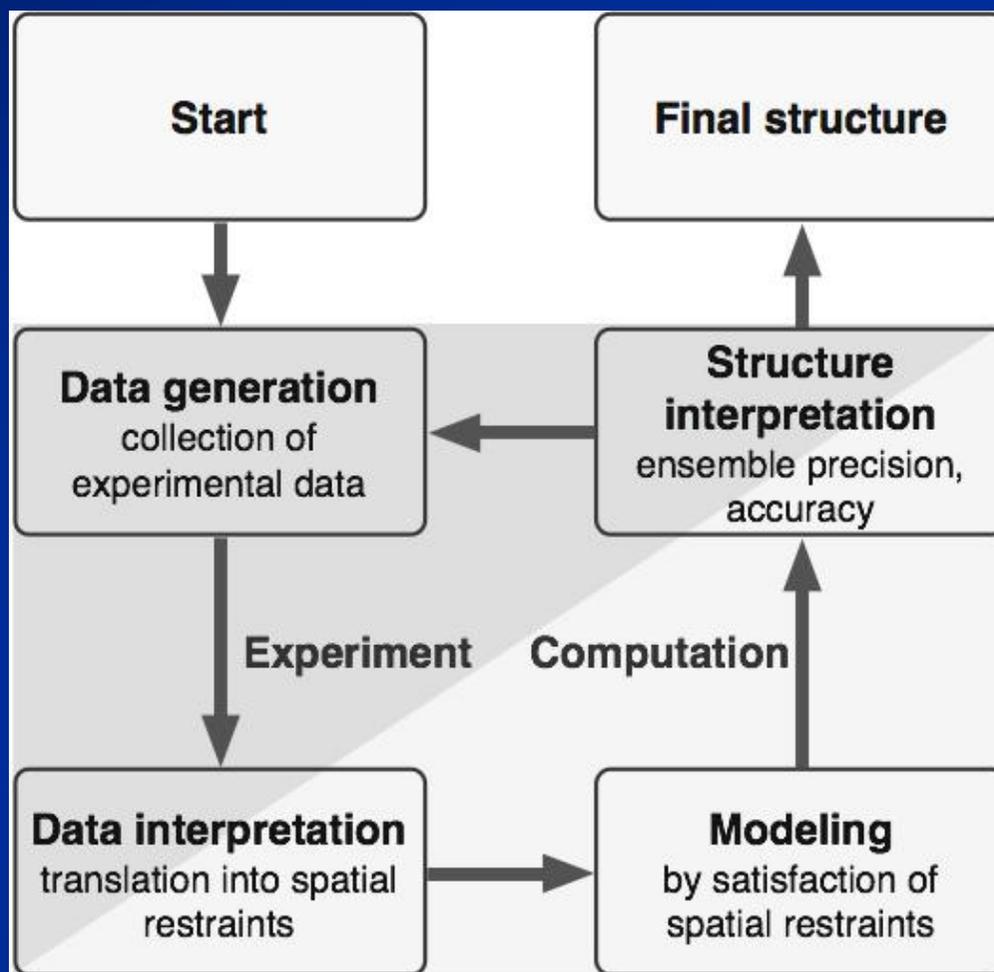
NMR



Our Method



General Strategy of Structure Determination



Determining the 3D Structure of the NPC using the MODELLER Program

(Frank Alber, Andrej Sali)

* Represent proteins as spheres

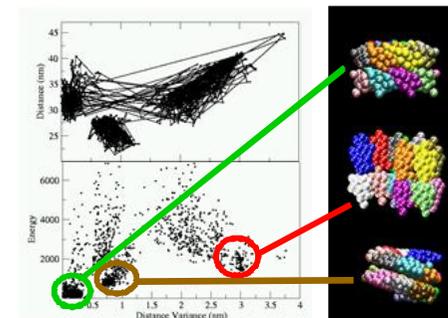
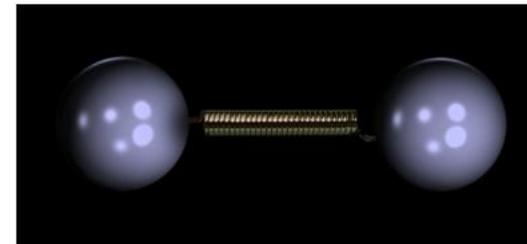
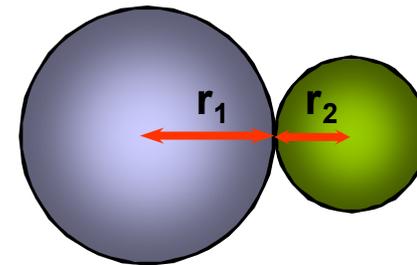
⇒ Radius of sphere correlates with number of residues

* Define spatial restraints

⇒ From NUP-NUP interaction data (also from localizations, copy numbers....)

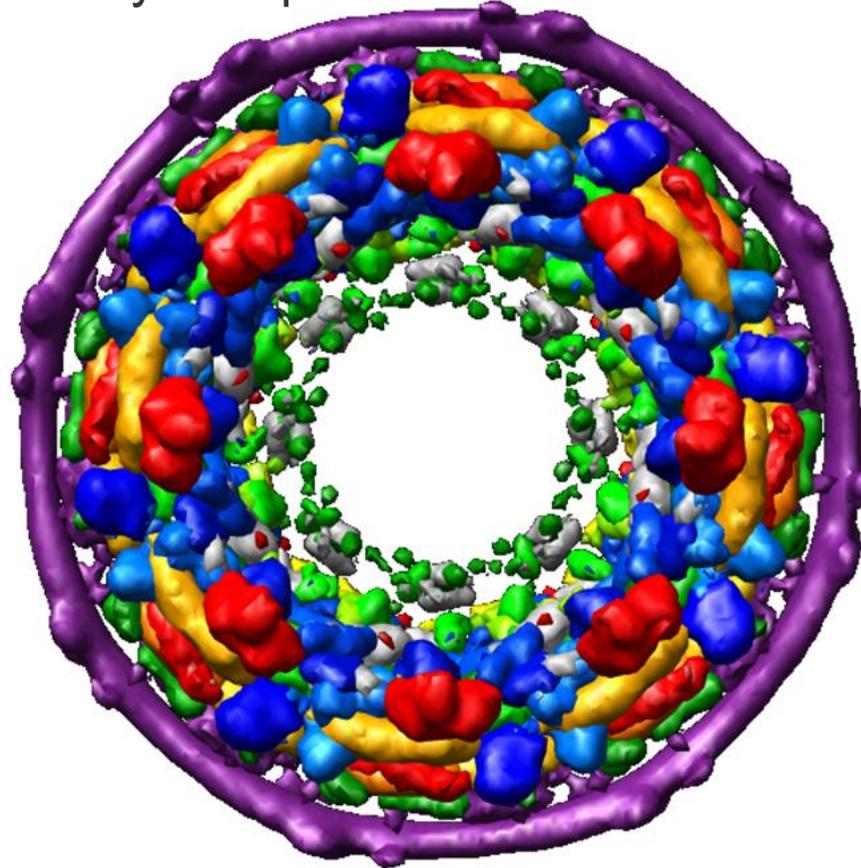
* Randomize protein positions

⇒ Allow model to reform, fulfilling all of the restraints, if possible
⇒ Do this 1000s of times



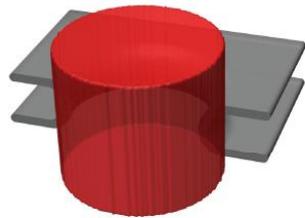
Protein Localization Probability

Given volume is occupied by a given protein.
Calculated from the structural superposition of the ensemble of models that satisfy all input restraints

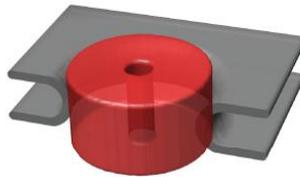


Can See Position of Every NPC Protein

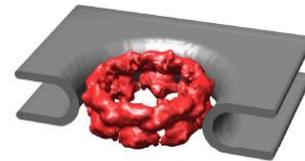
More Experimental Data - Better Localization



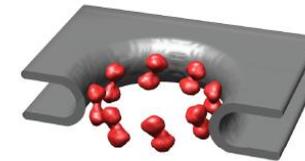
NE Pore Volume



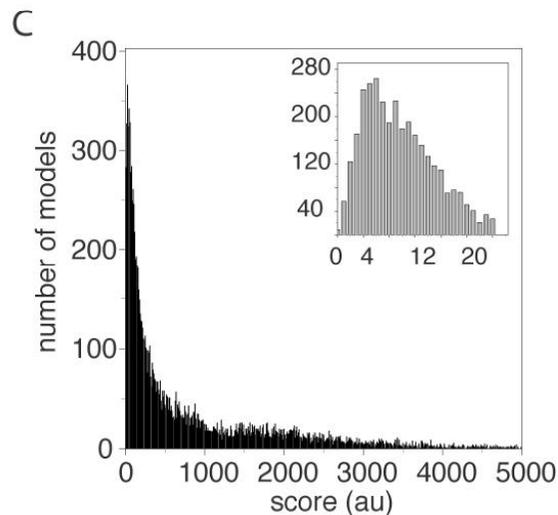
NE Pore Volume
Nup Localization



NE Pore Volume
Nup Localization
Nup Shape
Nup Volume
Nup Stoichiometry
NPC Symmetry



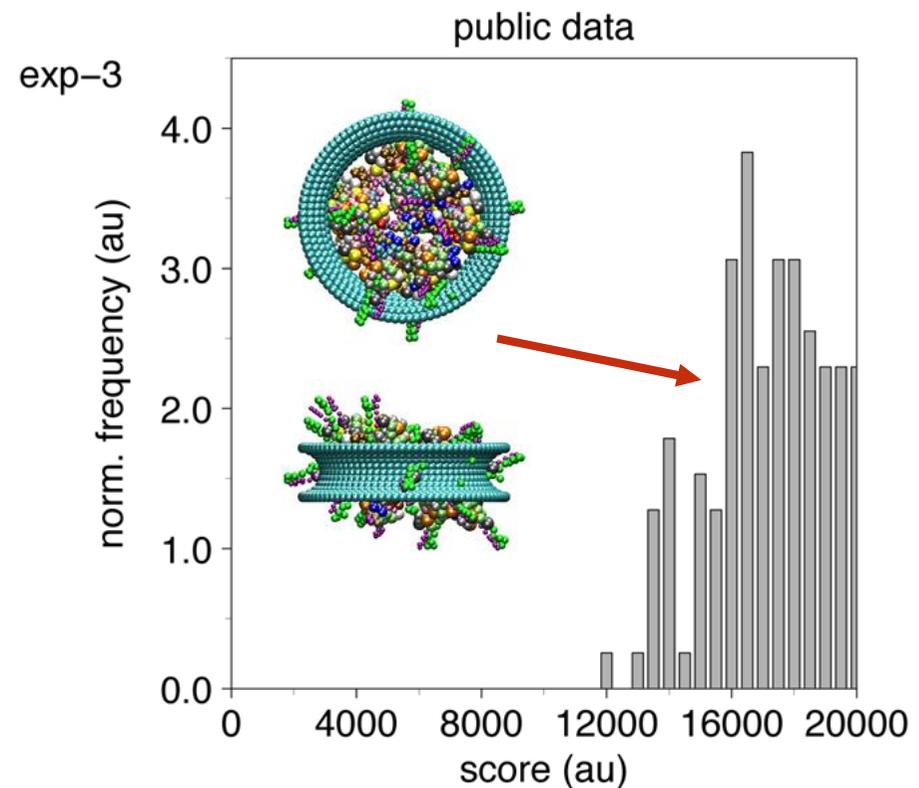
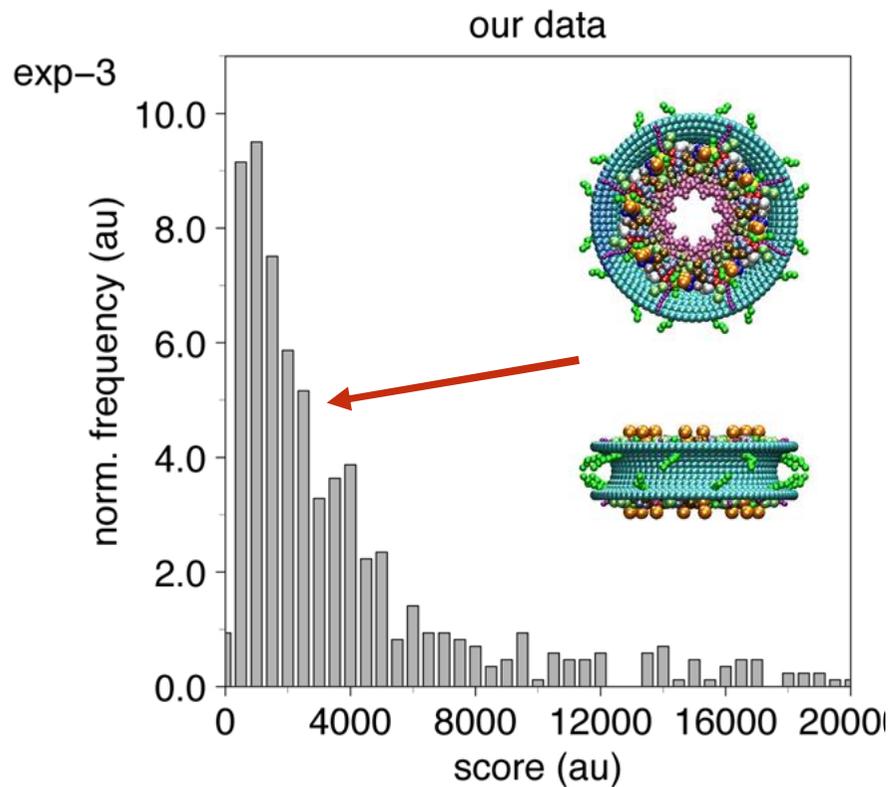
NE Pore Volume
Nup Localization
Nup Shape
Nup Volume
Nup Stoichiometry
NPC Symmetry
Nup Proximities
Nup Contacts
Complex Shape
Complex Connectivity



Score Distribution

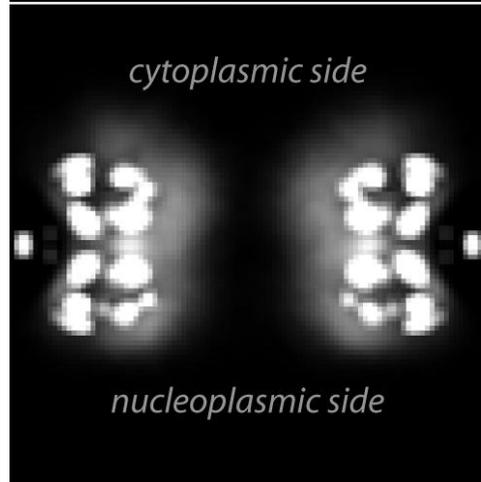
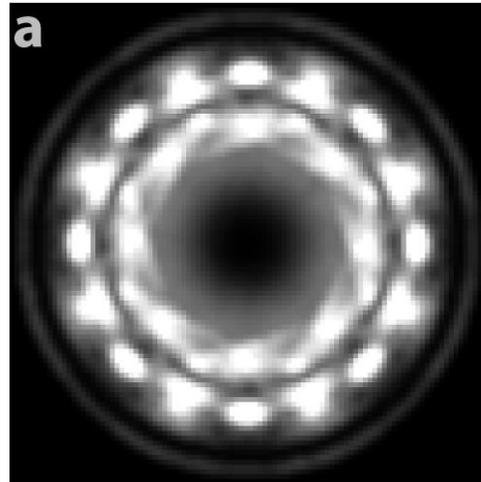
- Symmetry Restraints
- ~~Our ImmuneEM~~
- **Our Interactions**

- Symmetry Restraints
- ~~Our ImmuneEM~~
- **Public Interactions**

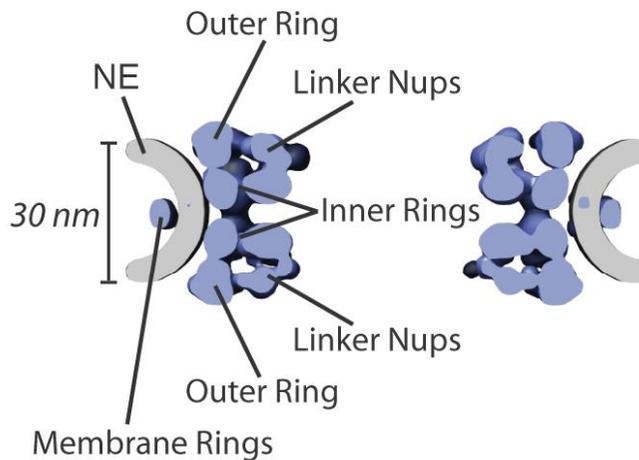
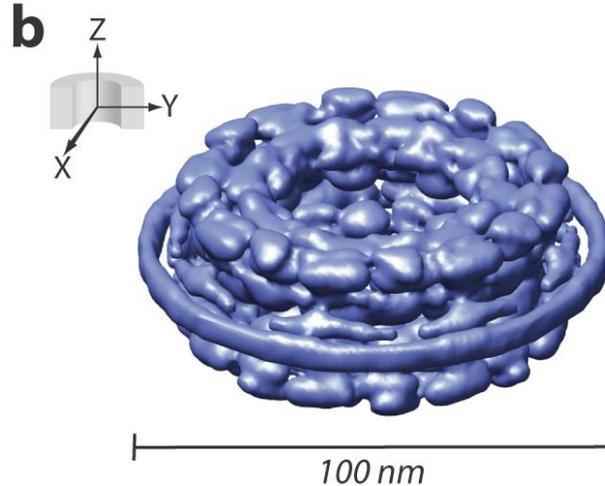


“Quality” of Data is Very Important

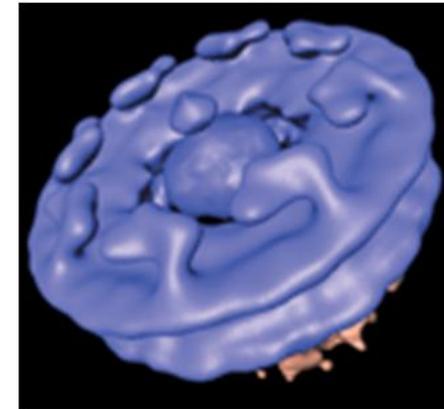
Molecular Architecture of the NPC - Spatial Configuration of Nucleoporins



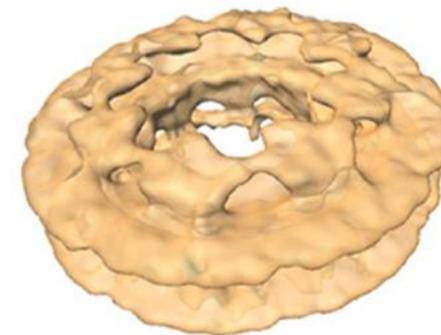
DENSITY PROJECTION



DENSITY CONTOUR

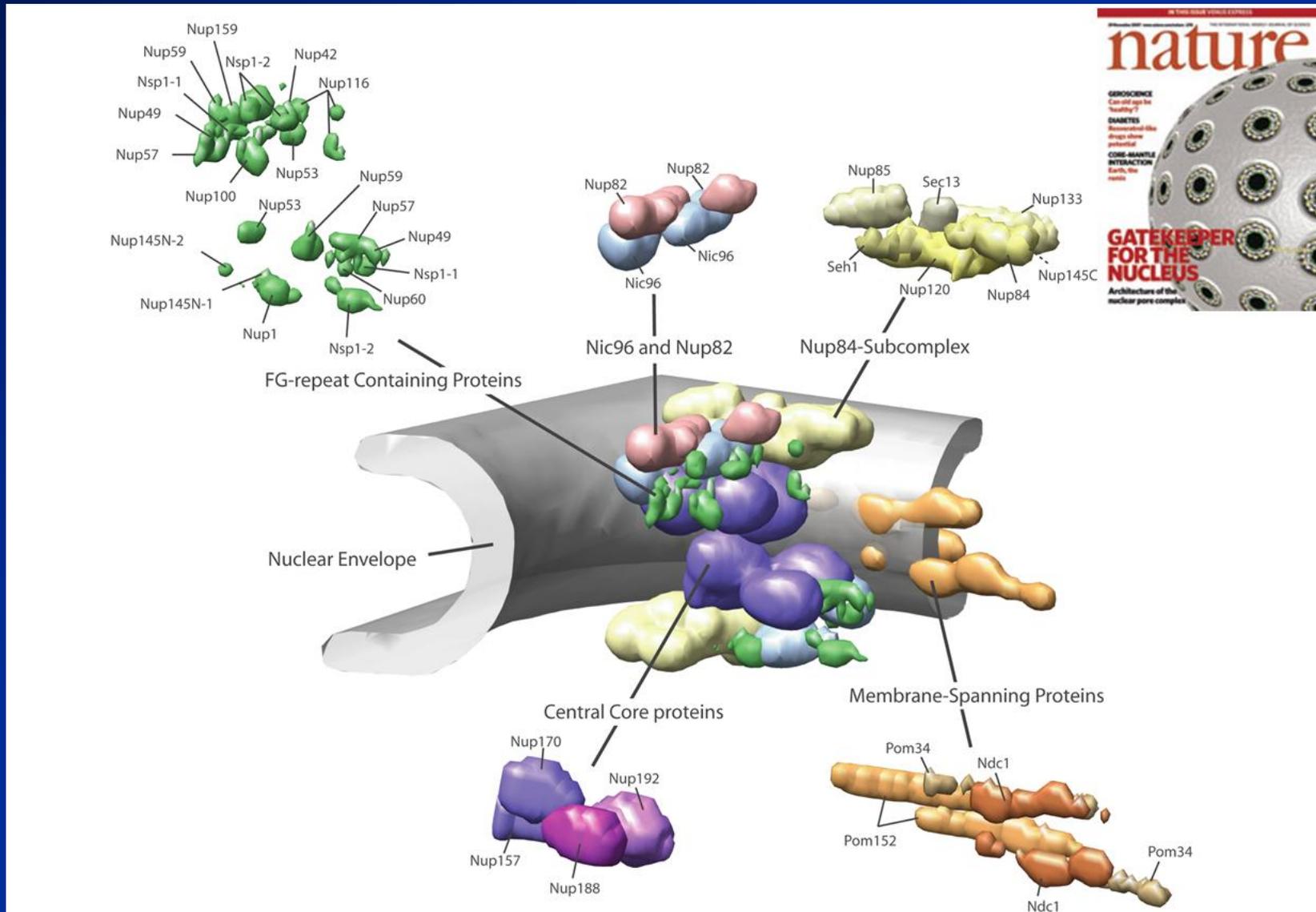


Dictyostelium NPC ~80Å



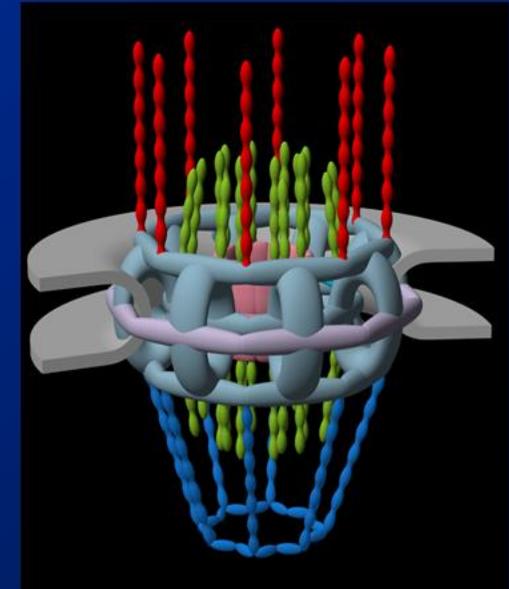
Dictyostelium NPC ~50Å

NPC Structure at the Molecular Level



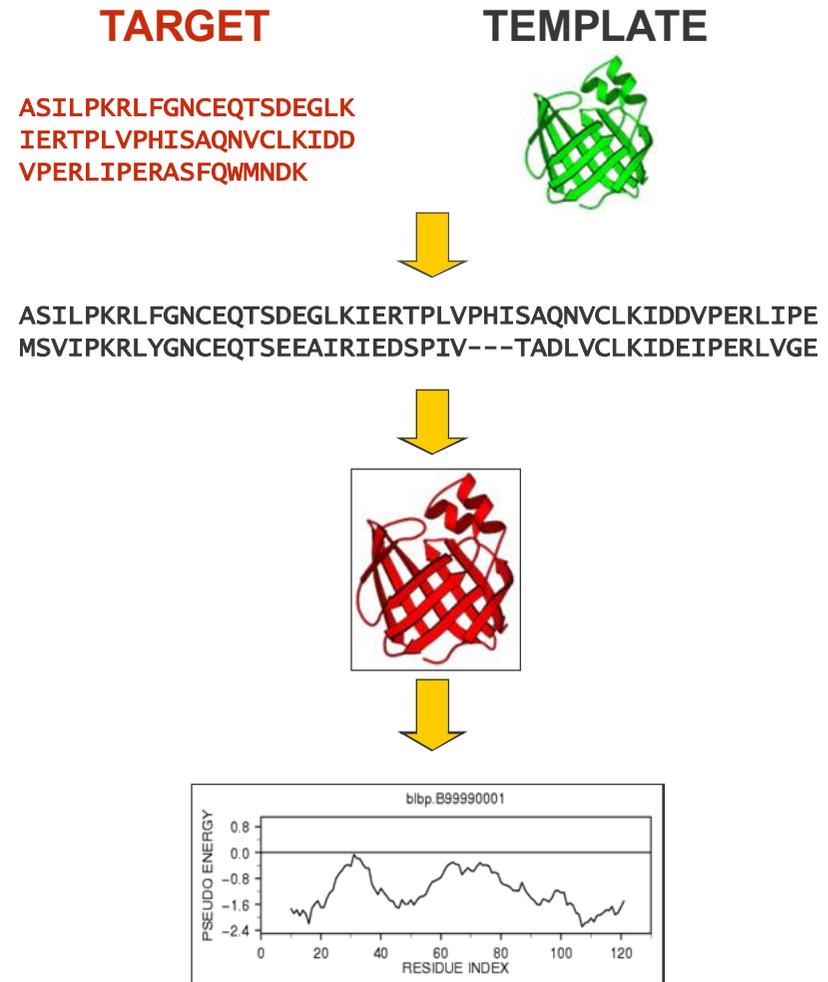
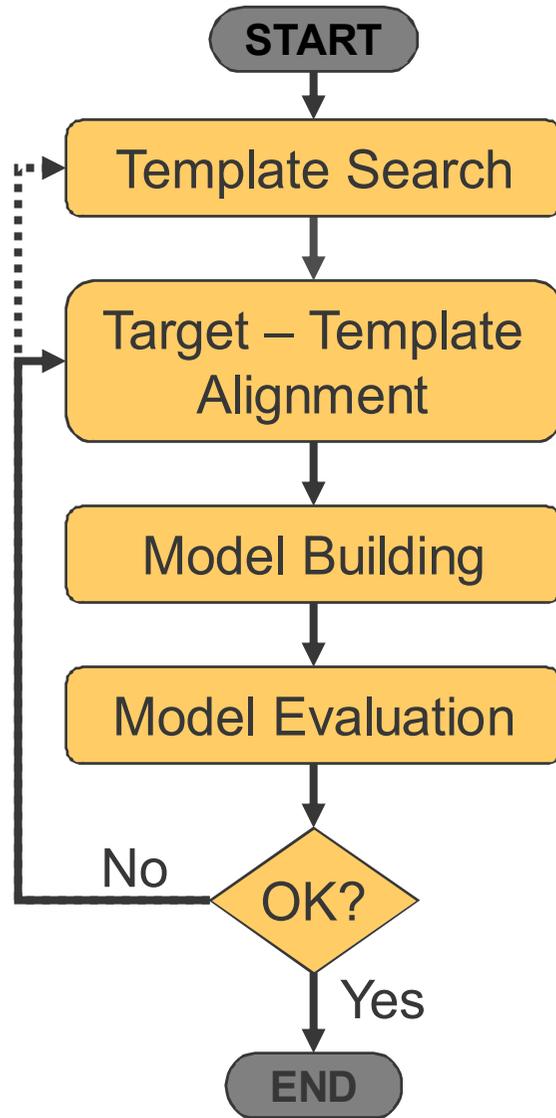
Alber et al., Nature 2007

Working Out the Structure of the NPC - We Need:



- x An NPC Component List X
- x Amount of Each Component in the NPC X
- x Where the Components Go in the NPC X
- x What the Whole NPC Looks Like X
- x **What Each Component Looks Like** X
- x How the Components Fit Together X

Comparative Protein Structure Modeling



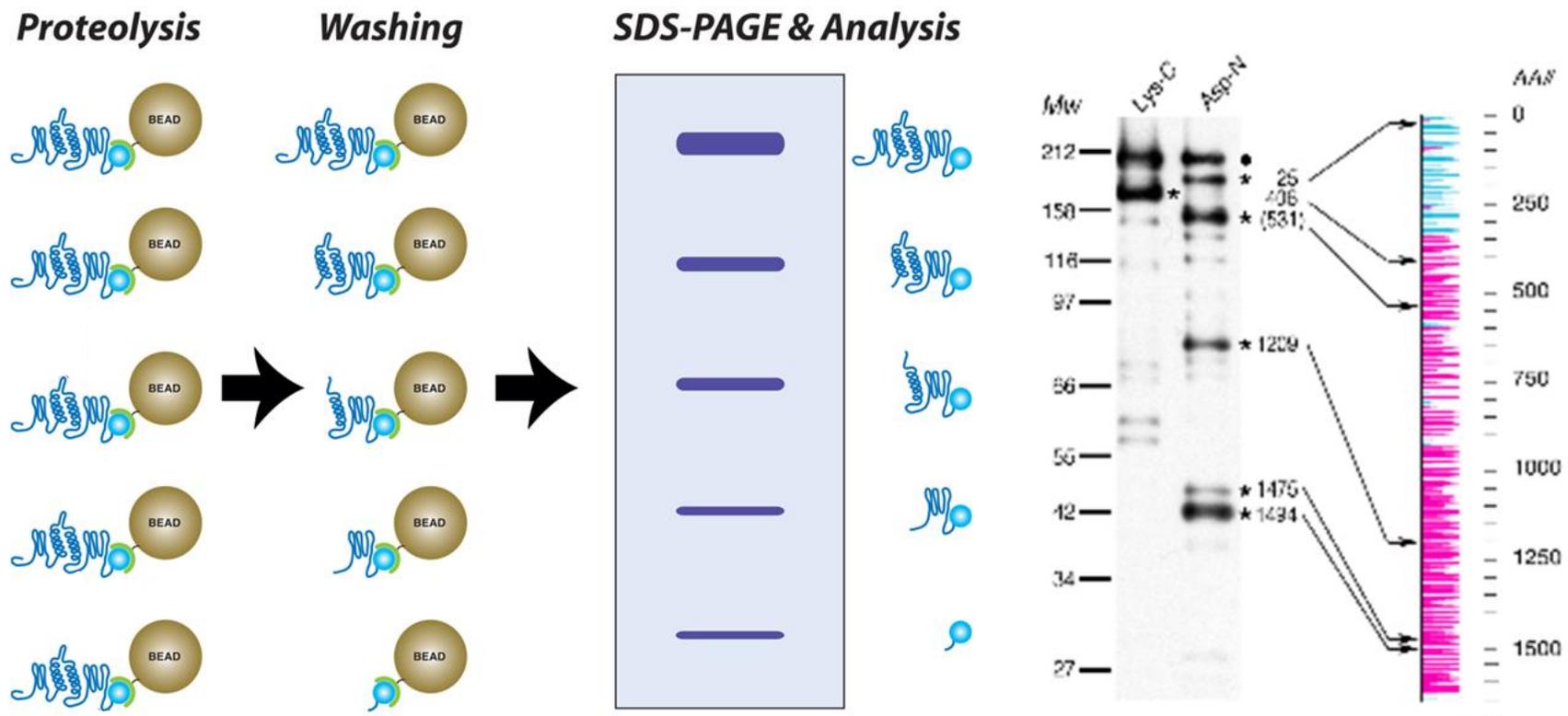
A. Šali, *Curr. Opin. Biotech.* 6, 437, 1995.

R. Sánchez & A. Šali, *Curr. Opin. Str. Biol.* 7, 206, 1997.

M. Marti et al. *Ann. Rev. Biophys. Biomolec. Struct.*, 29, 291, 2000.

<http://salilab.org/>

Protease Accessibility Laddering (PAL) & Fold Prediction



Dokudovskaya et al, Structure 2006

Fold Types in the NPC



• **Alpha-Solenoid** ~38%



• **Beta-Propeller** ~16%



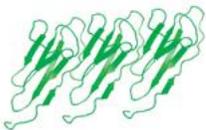
• **FG Repeats** ~29%



• **Coiled-Coil** <5%



• **Transmembrane** <5%



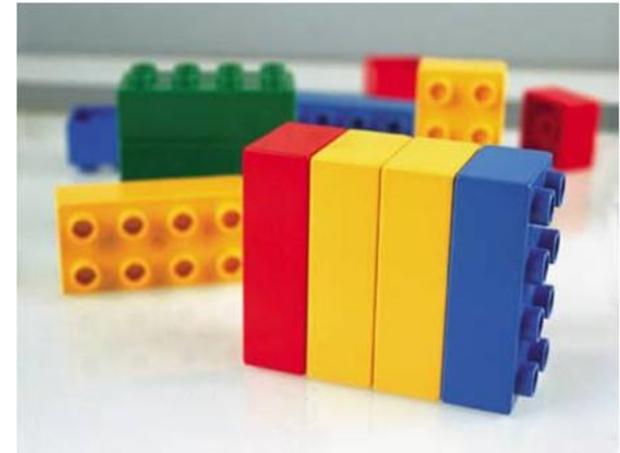
• **Cadherin-like** <5%



• **Beta-Prism** <5%

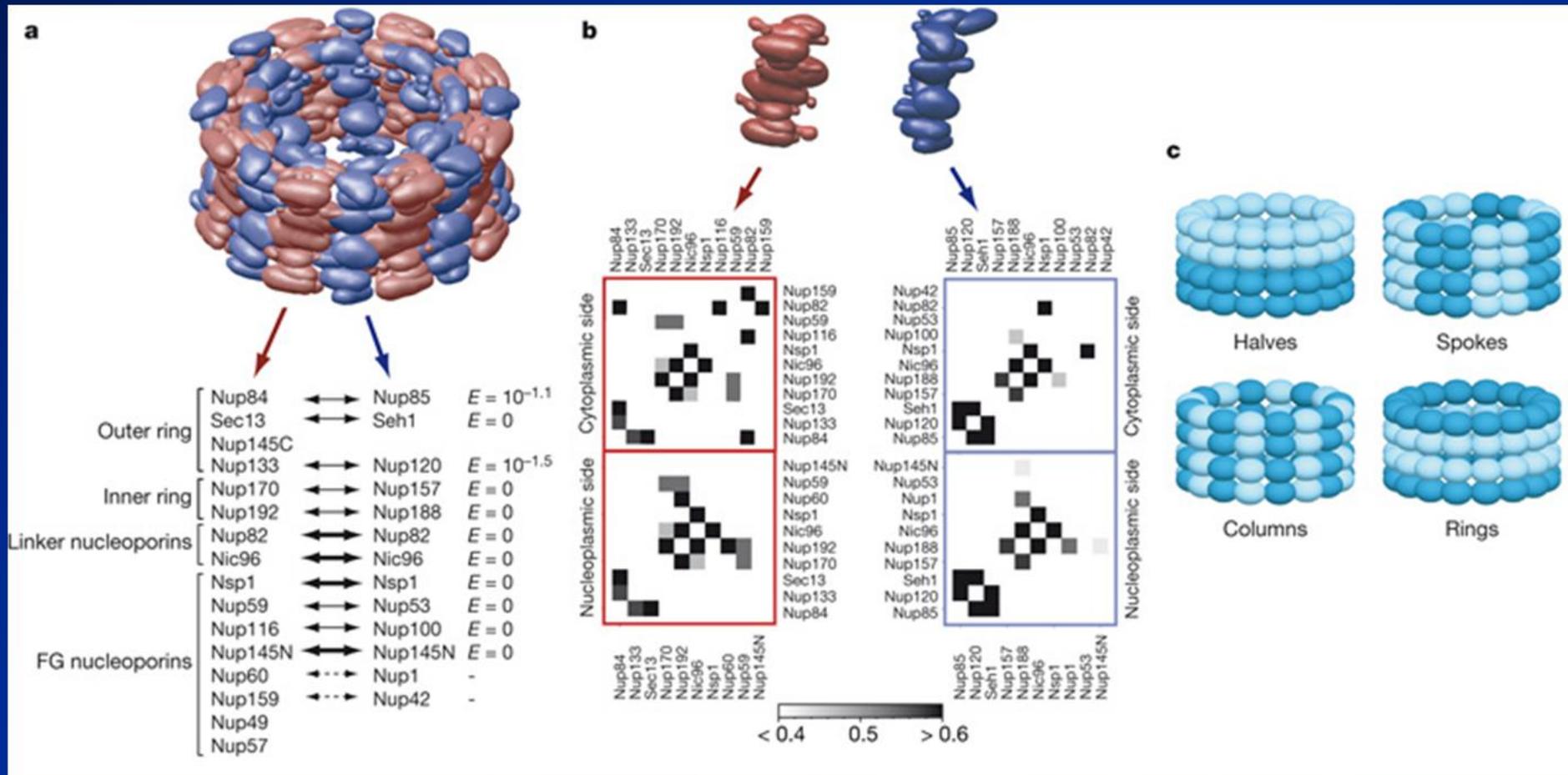


• **RRM-like** <5%

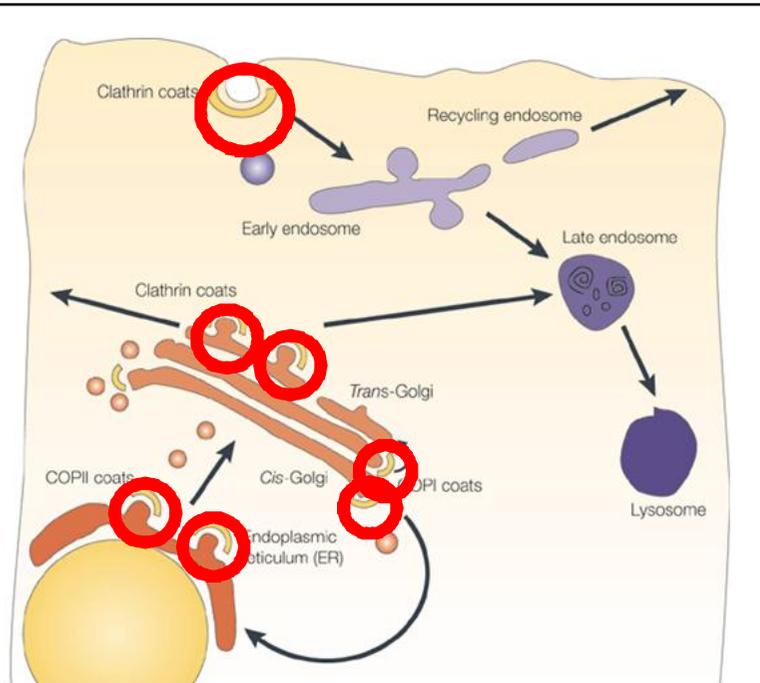


“LEGO-like”:
Small number of repetitive motifs

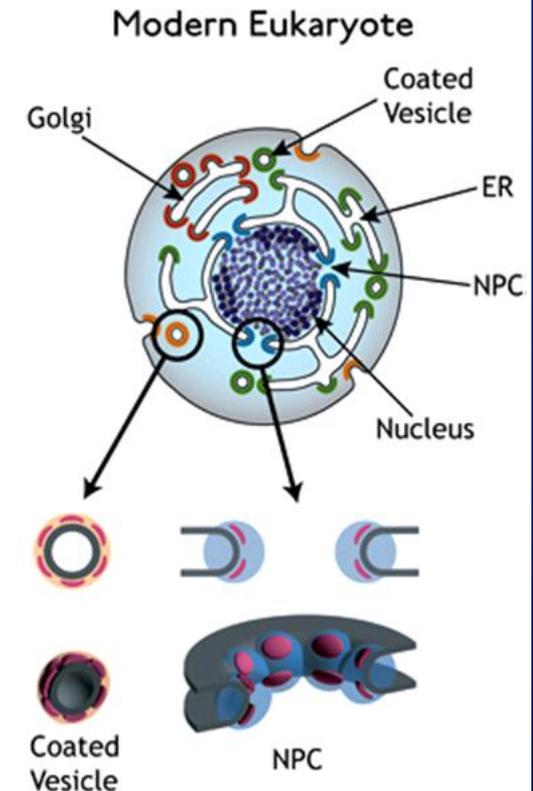
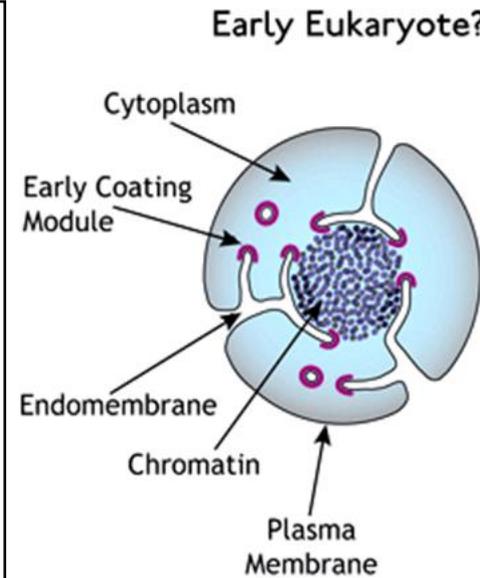
Modular Duplication in the NPC



Nup84 Complex: Structure & Origin



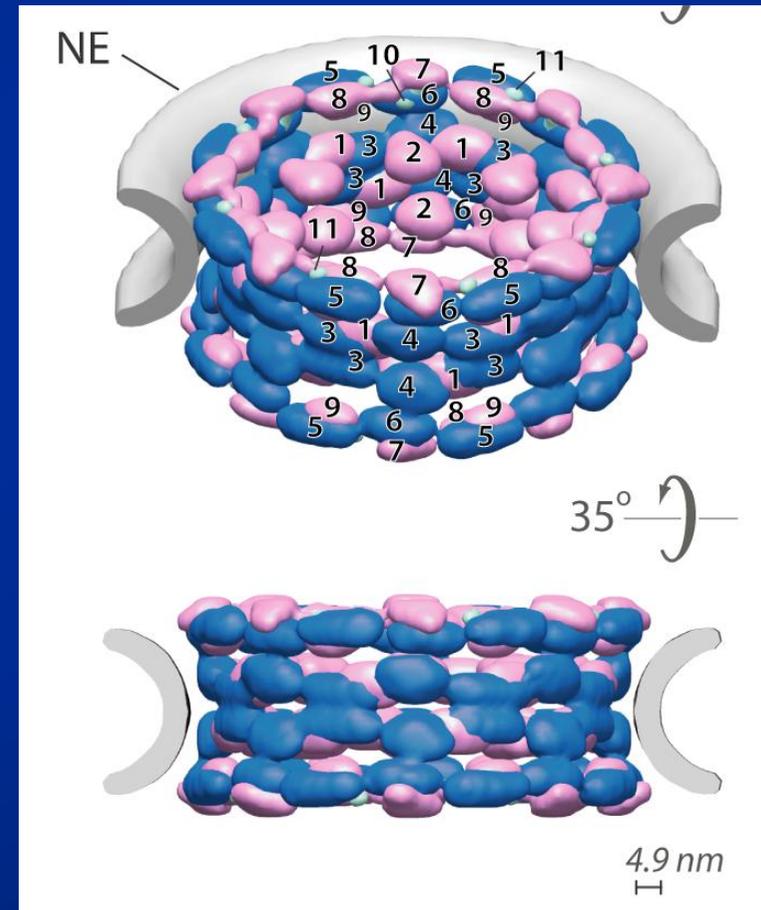
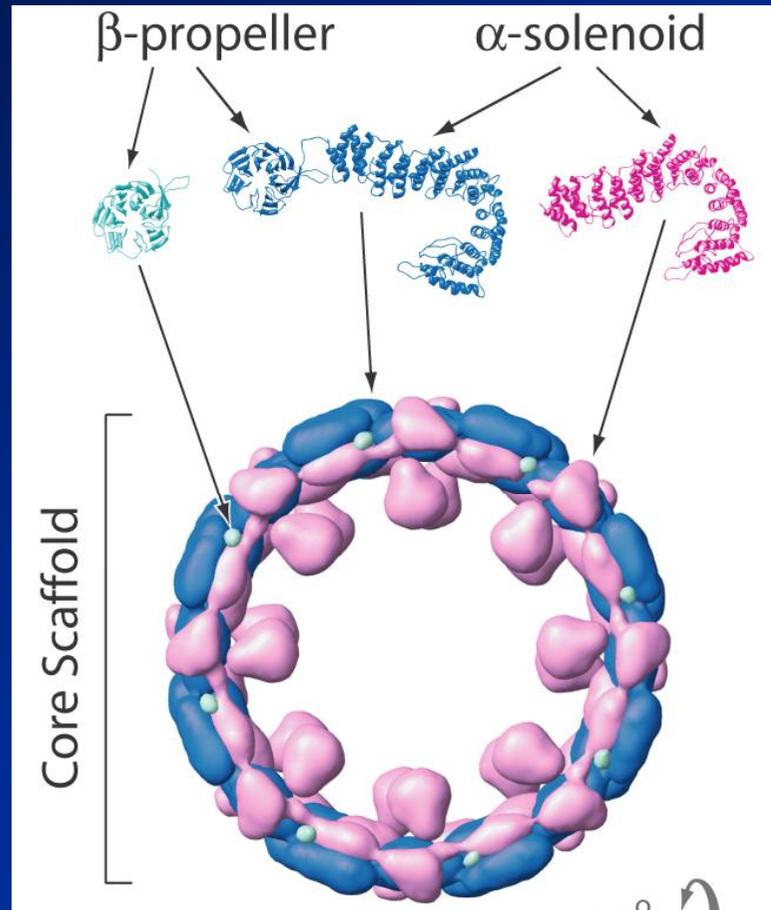
**Clathrin / Adaptin
Homologs found in
Different
Coated Vesicles**



**Protocoatomer
Hypothesis**

***Coated Vesicles and NPCs
Originated in a Common Precursor***

The Core Scaffold as a Membrane Coating Complex

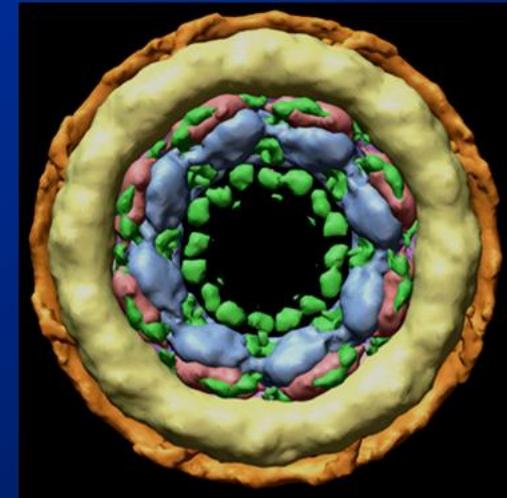
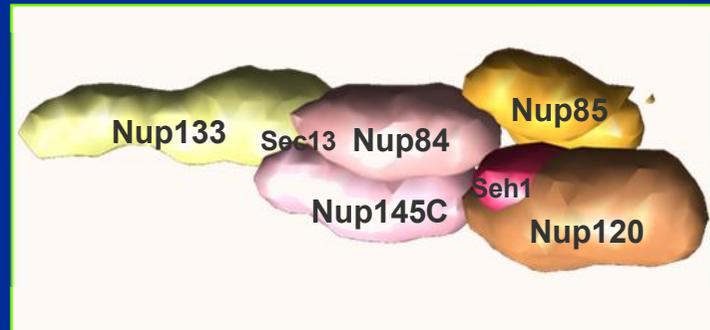
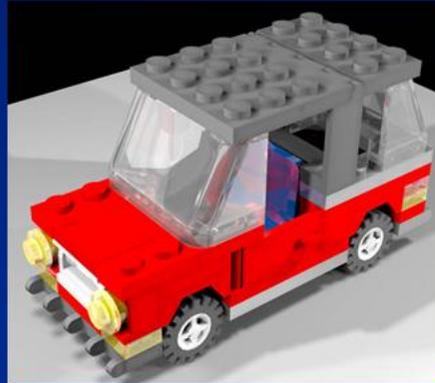
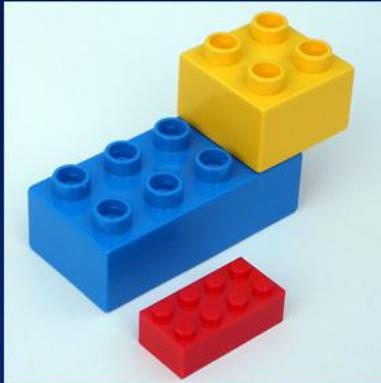


“Take Home” Messages

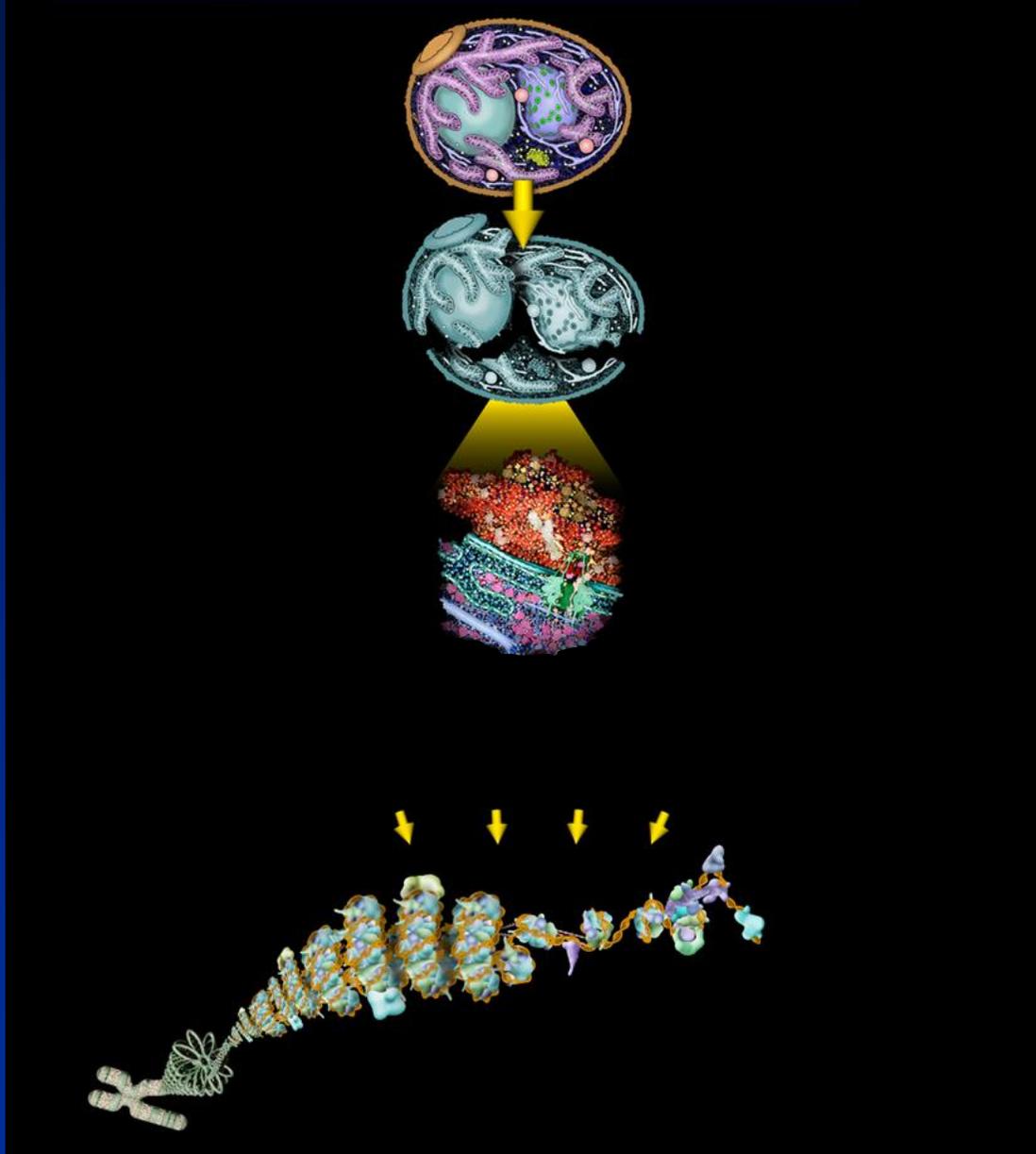
Molecular Architecture of the Nuclear Pore Complex is:

- ✓ Simple
- ✓ Modular
- ✓ Contains coating complex
- ✓ Formed by duplications and divergence of simple ancestral module

Molecular Architecture of the NPC - Welcome to the Legoland!



Integrative Approaches to Study Protein Complexes



- Tagged macromolecular complexes
- Complexes preserved by freezing & cryolysis
- Rapid isolation preserves complexes
- Determine complex:
 - COMPOSITION
 - STOICHIOMETRY
 - MODIFICATIONS
 - INTERACTIONS
 - MORPHOLOGY
 - DYNAMICS
- Integration of data in spatial & temporal models

Orit Karni
Julia Kipper

Caterina Strambio de
Castillia

Svetlana Dokudovskaya

Yingming Zhao

Mario Niepel

Joe Fasolo

Ben Timney

Liesbeth Veenhoff

Wenzhu Zhang

Marlene Oeffinger

Frank Alber

Tari Suprpto

Kelly Hjertaas

Damien Devos

Jackie Novatt

Rosemary Williams

Brian Chait

Vincent Archambault

John Aitchison

Beth Hatton

Andrej Sali

Fred Cross

Mike Rout

Marcelo Magnasco

