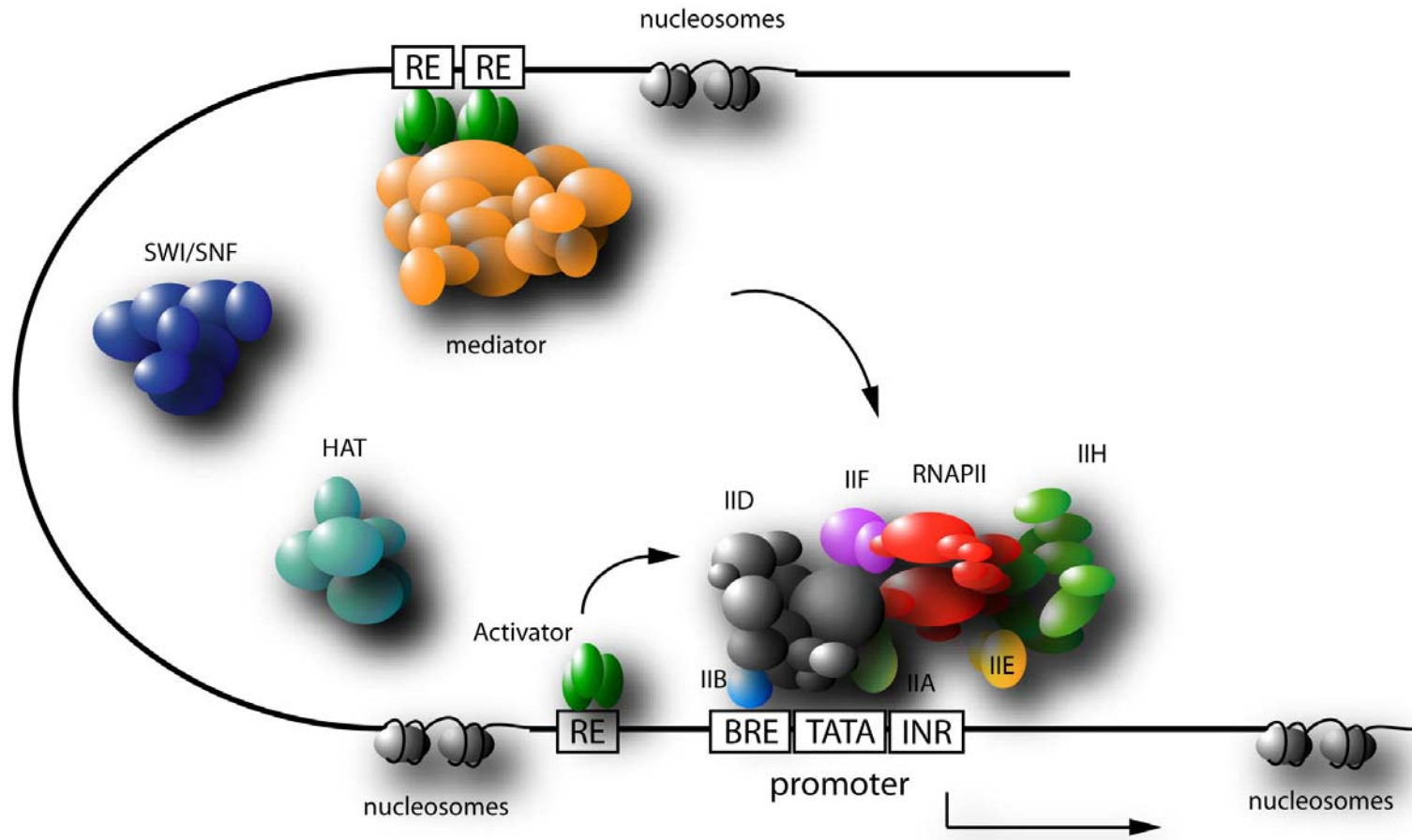


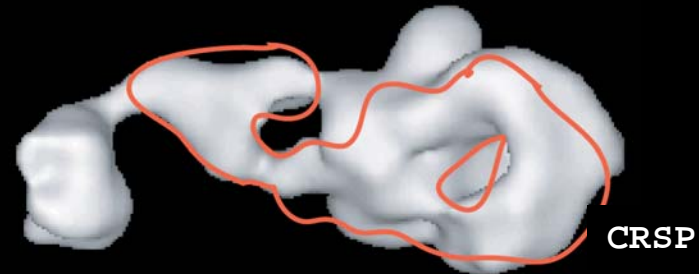
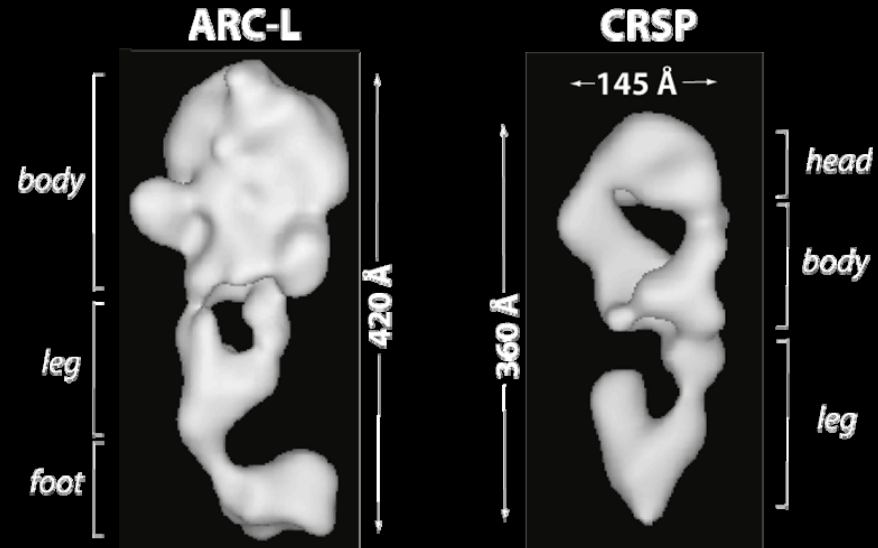
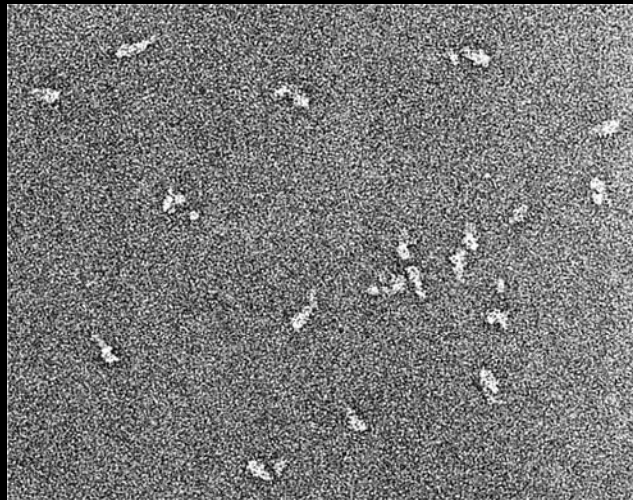
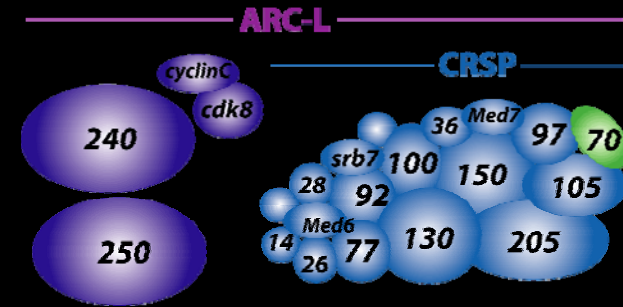
**Detecting and
Characterizing Heterogeneity
(Biochemical or Conformational)
by Cryo-EM**

Eva Nogales
HHMI / UC Berkeley
LBNL

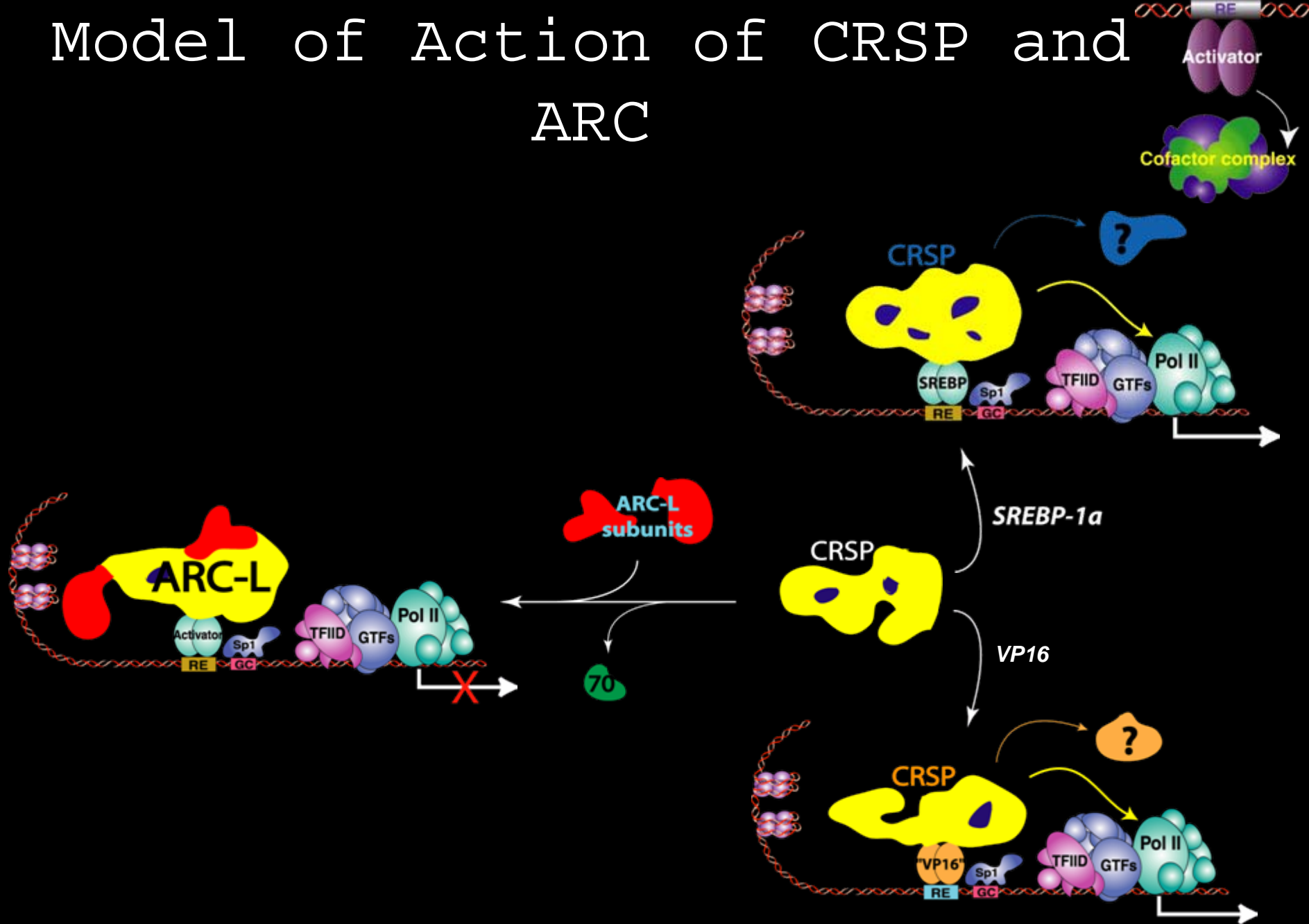
Eukaryotic Transcription



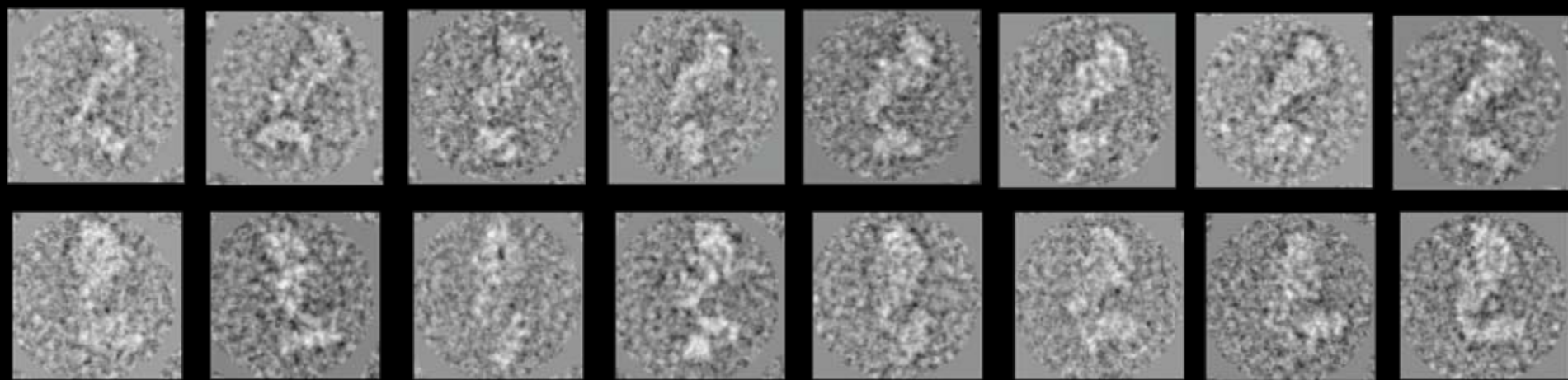
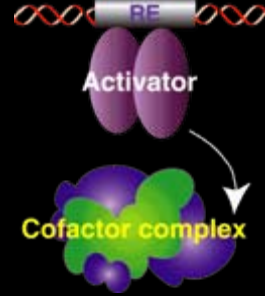
ARC versus CRSP: Structure and Function



Model of Action of CRSP and ARC



Cofactor Flexibility



Spread, ill-defined variability

3-D variance and focused classification

Pawel Penczek



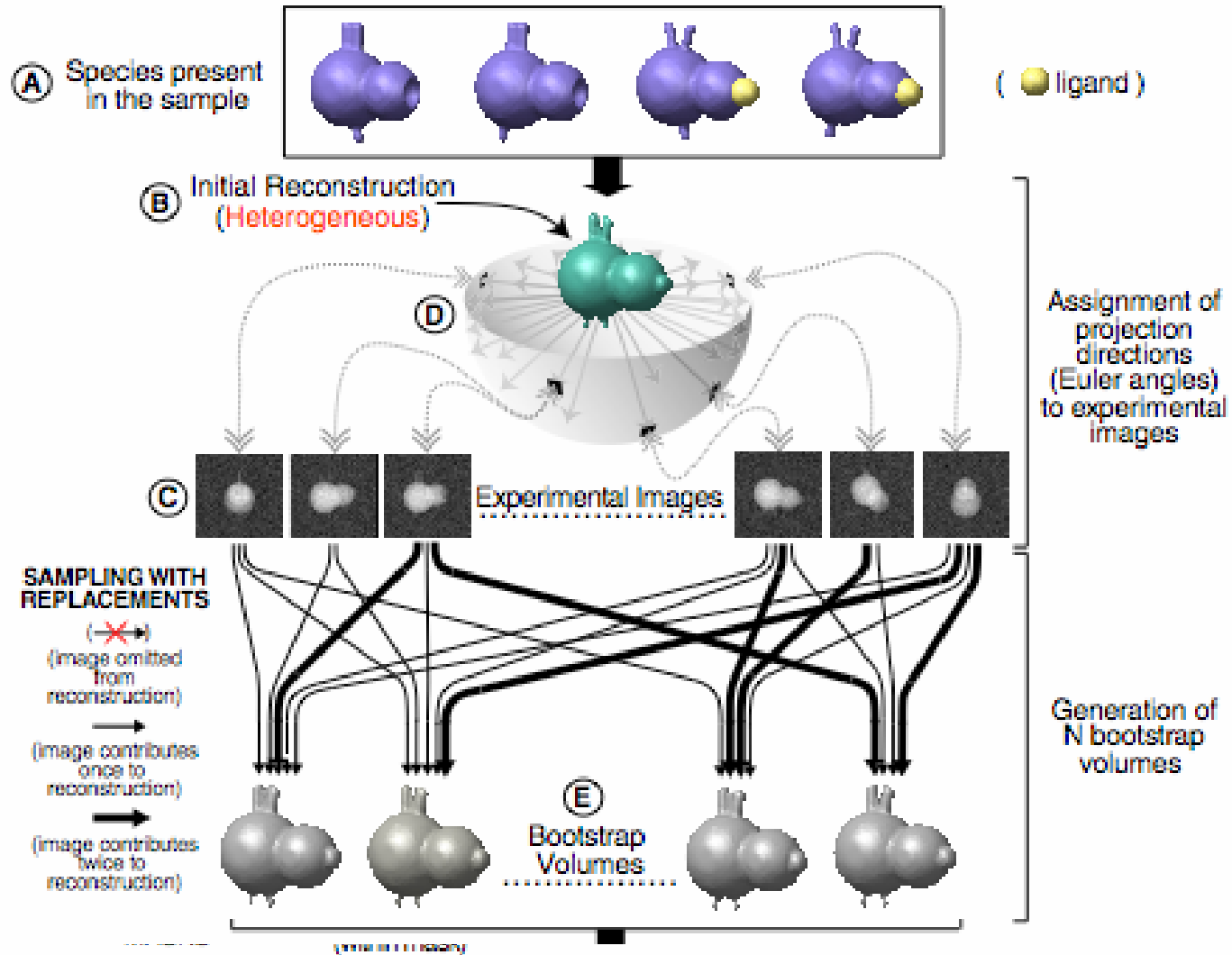
Patricia Grob



Seth Kostek

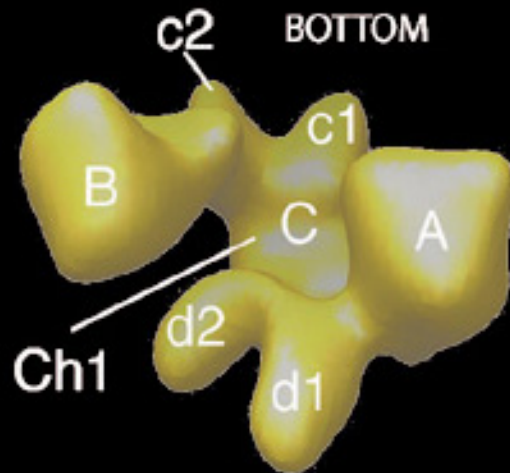
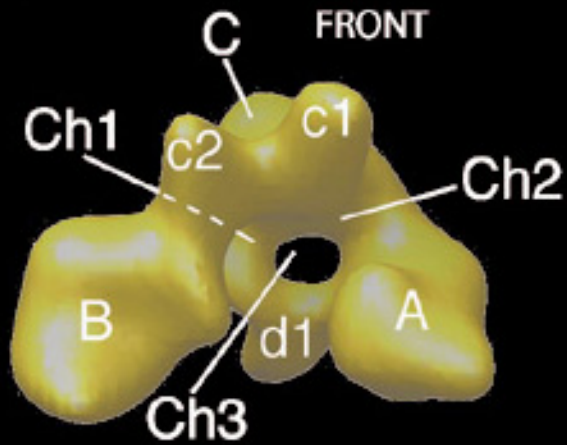


3-D Variance and Supervised Classification: Principles



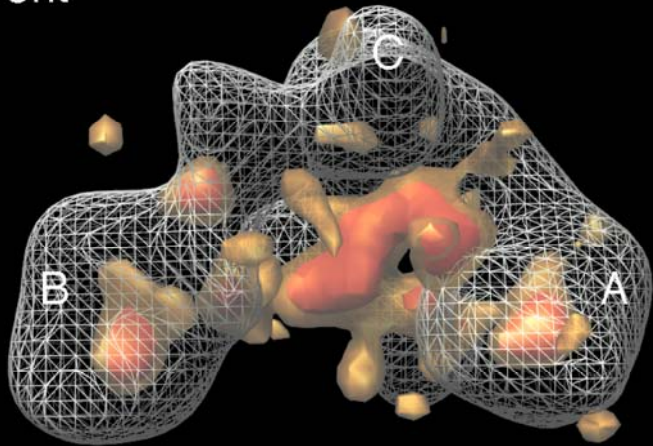
Structure and Flexibility of Human TFIIID

3D Variance and Covariance

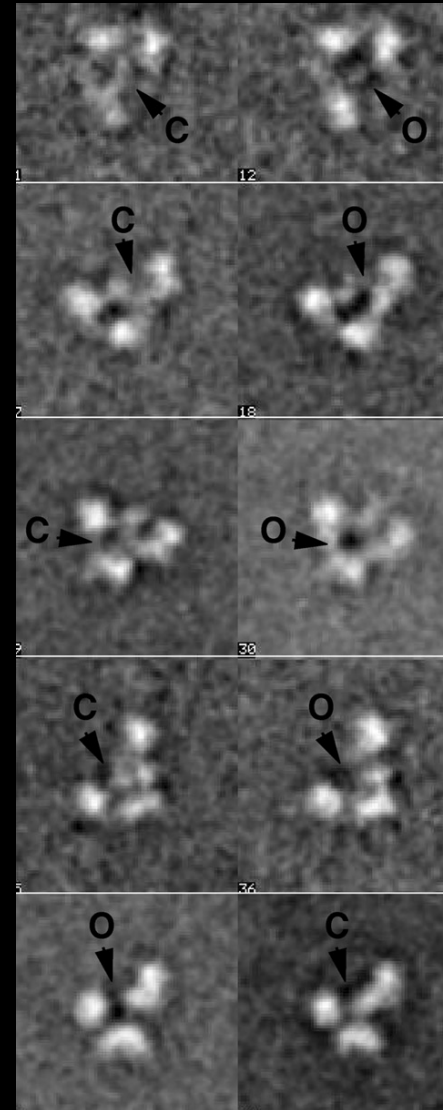
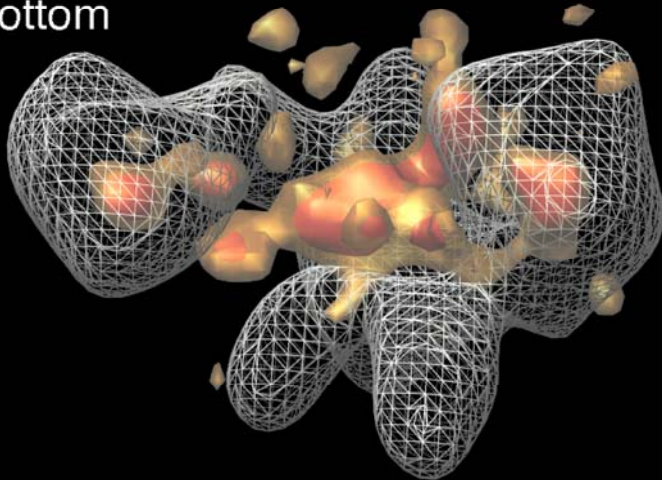


Conformational Flexibility of Human TFIIID

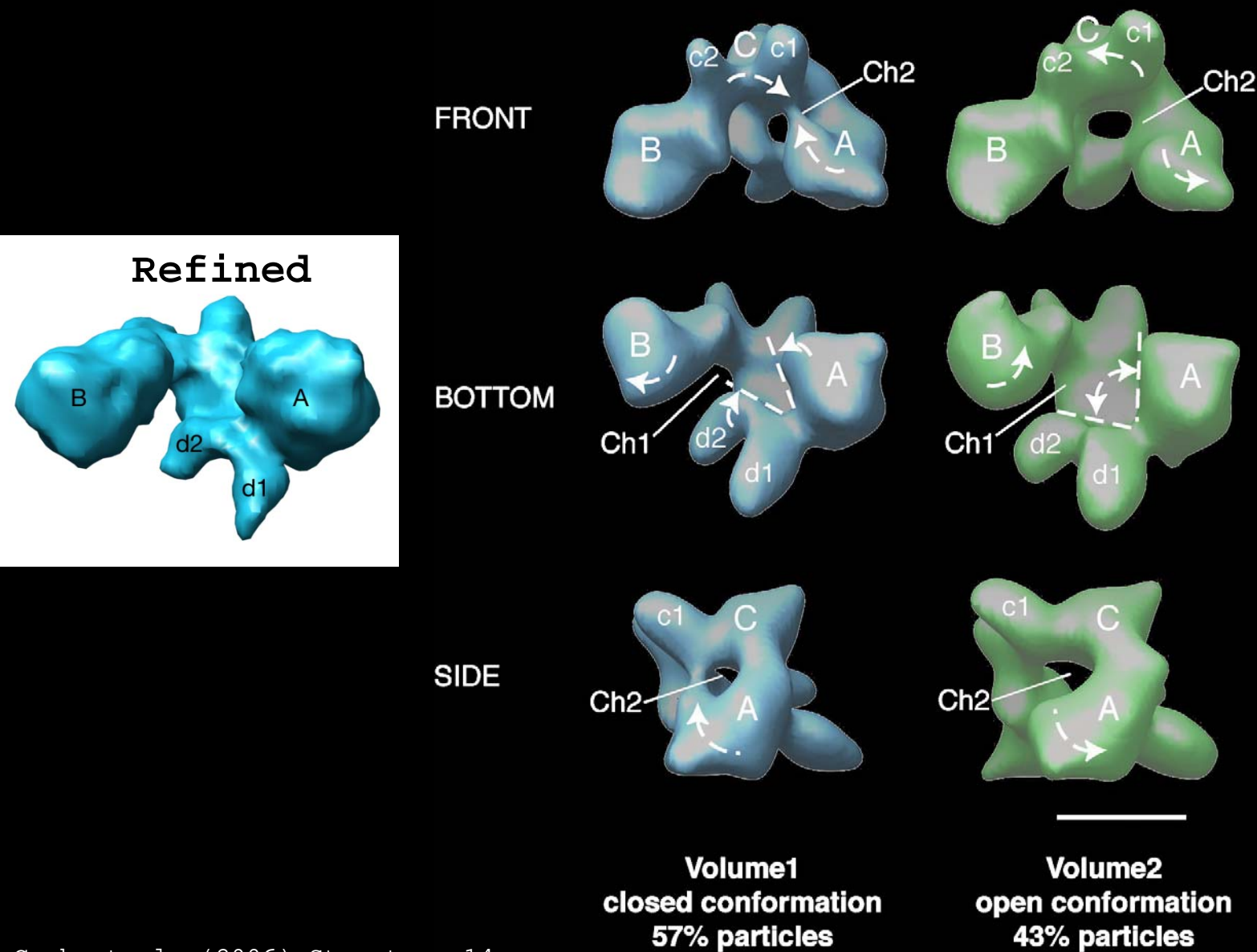
Front



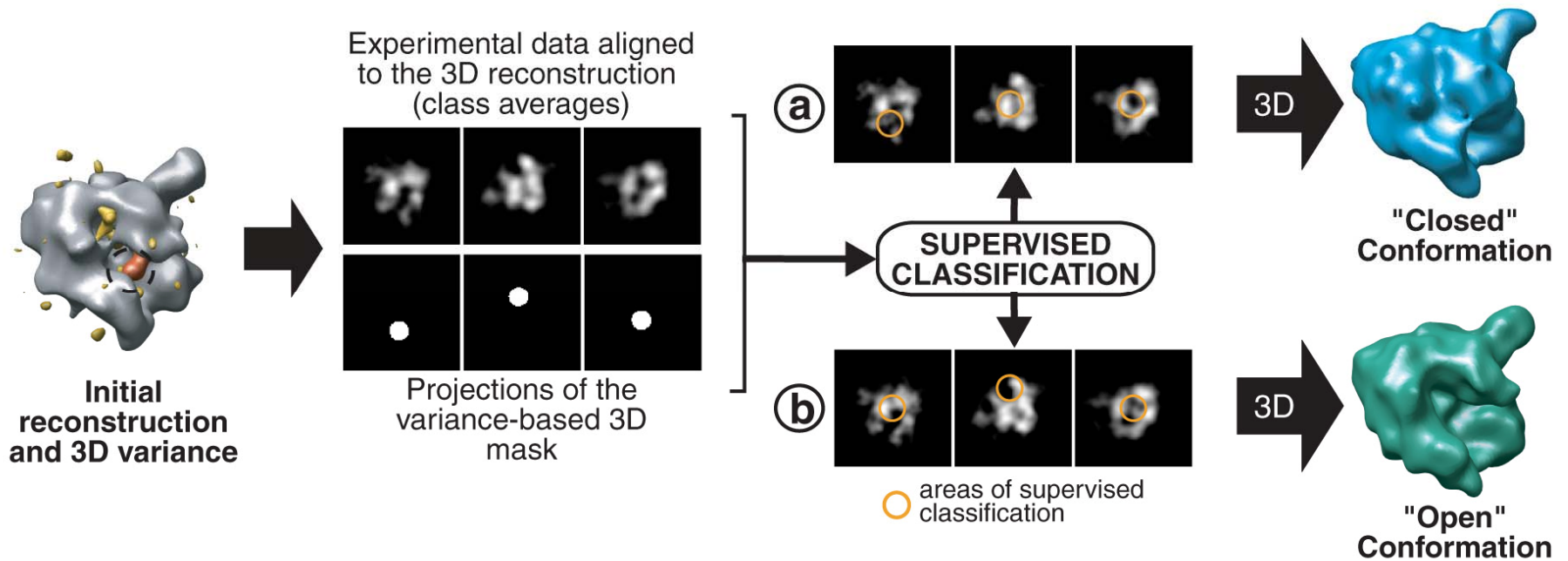
Bottom



Coordinated Motions in "Breathing" of TFIID



Human Pol II Conformational Sorting S



Localized variability of small but significant size

Cross-Correlation of Common Lines (CCCL)

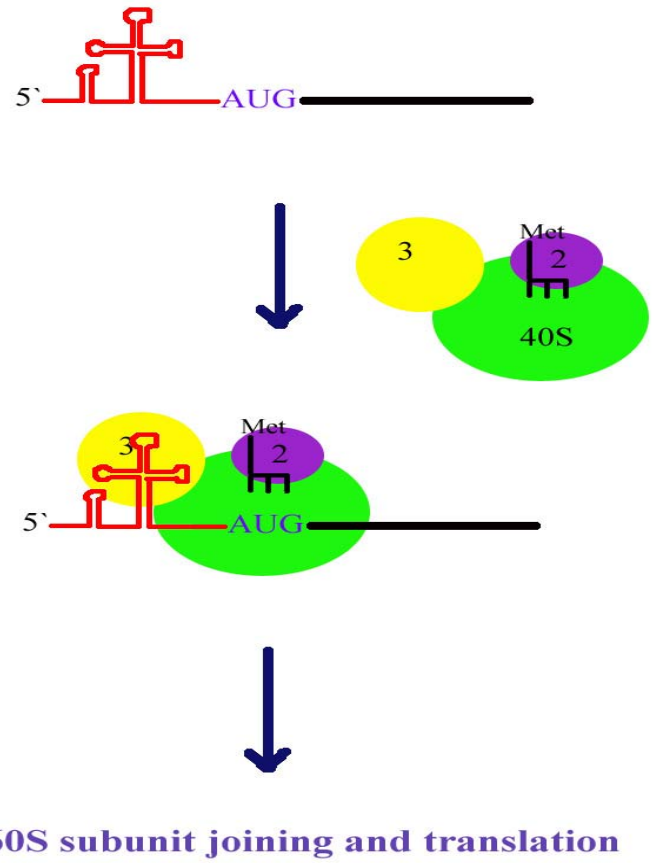
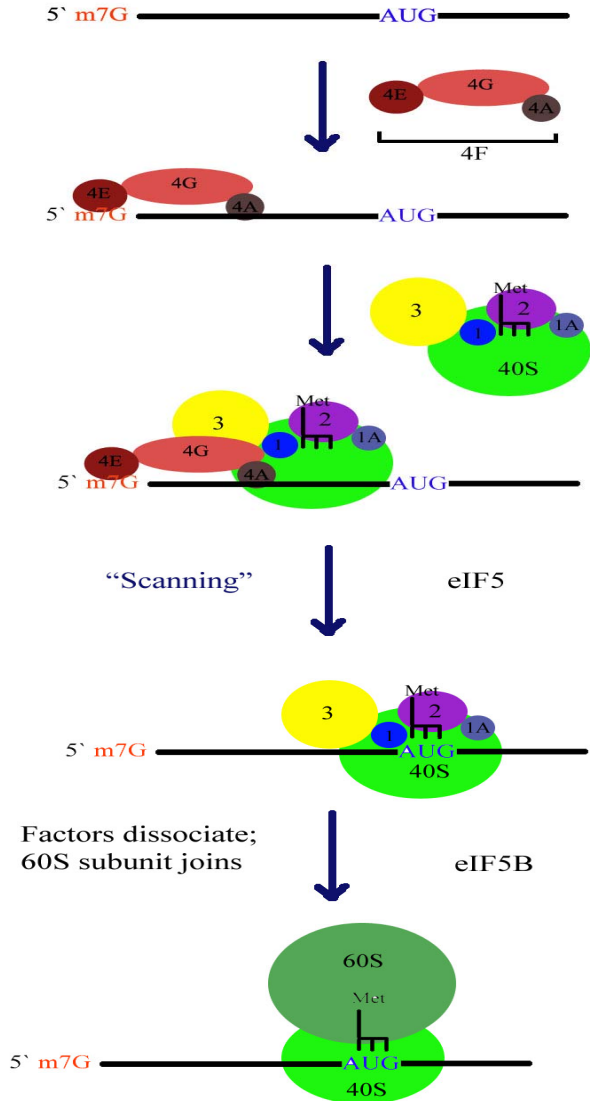


Richard Hall

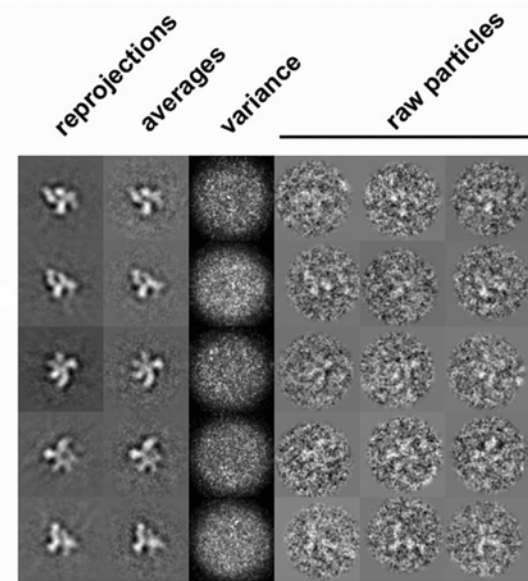
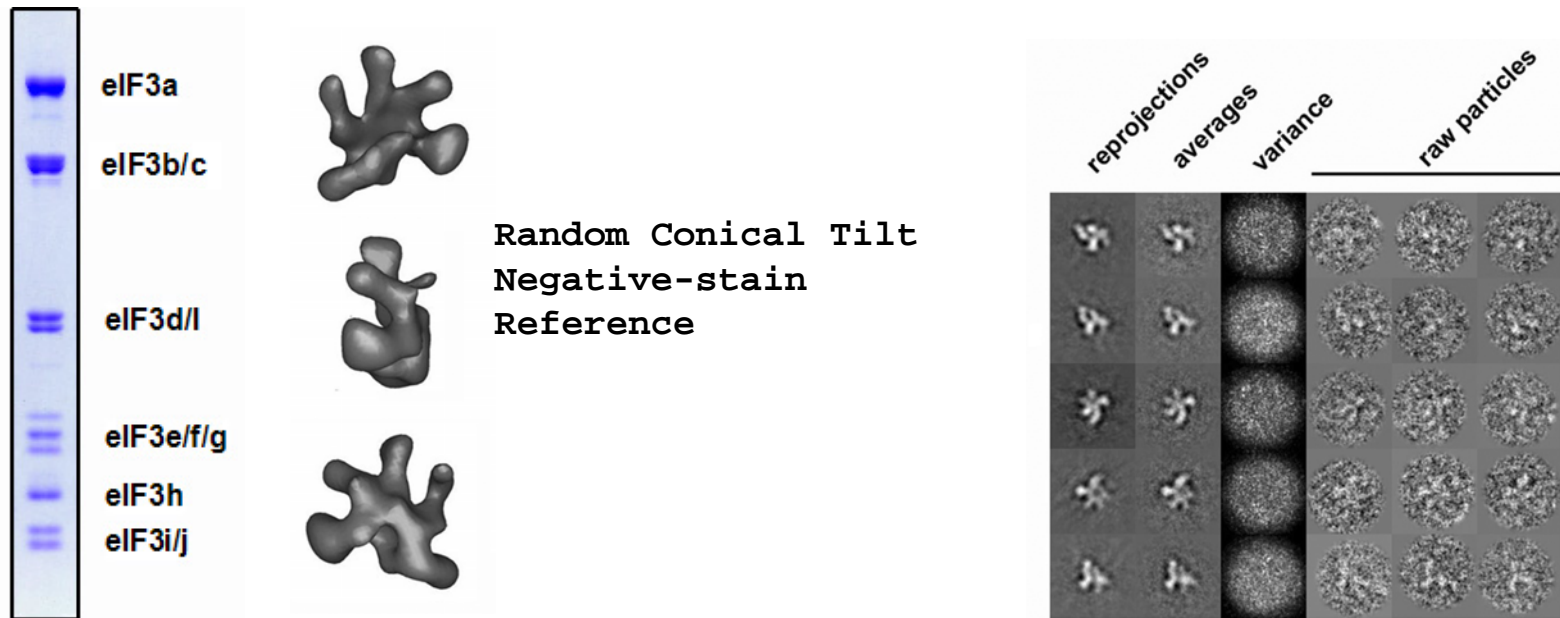


Bunpote Siridechadilok

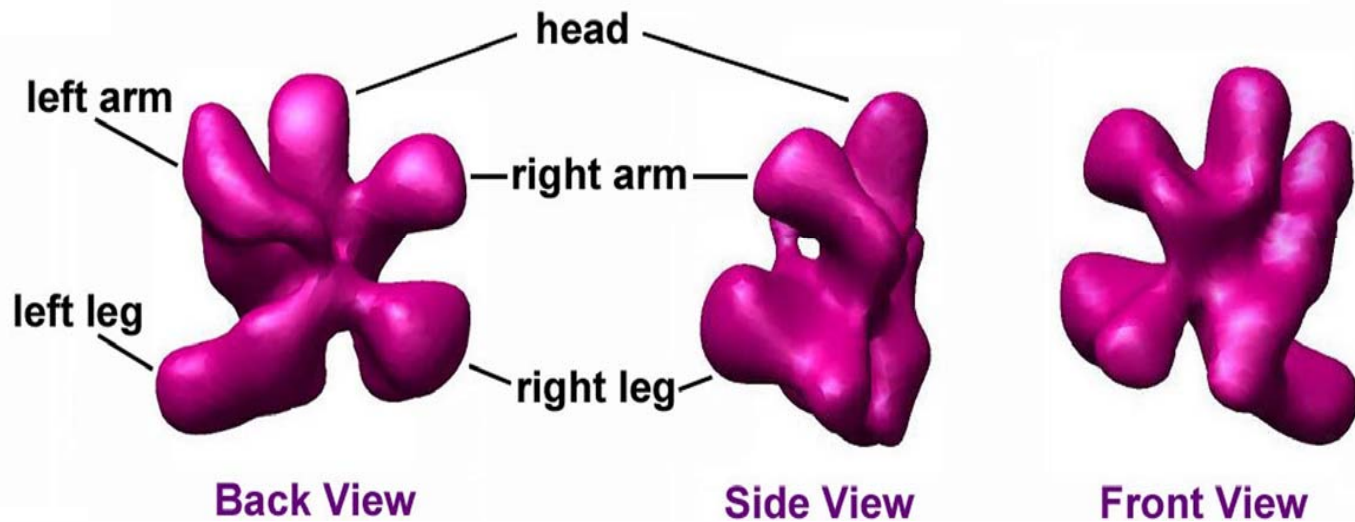
Eukaryotic Translation Initiation: Cap-dependent and Cap-independent Pathways



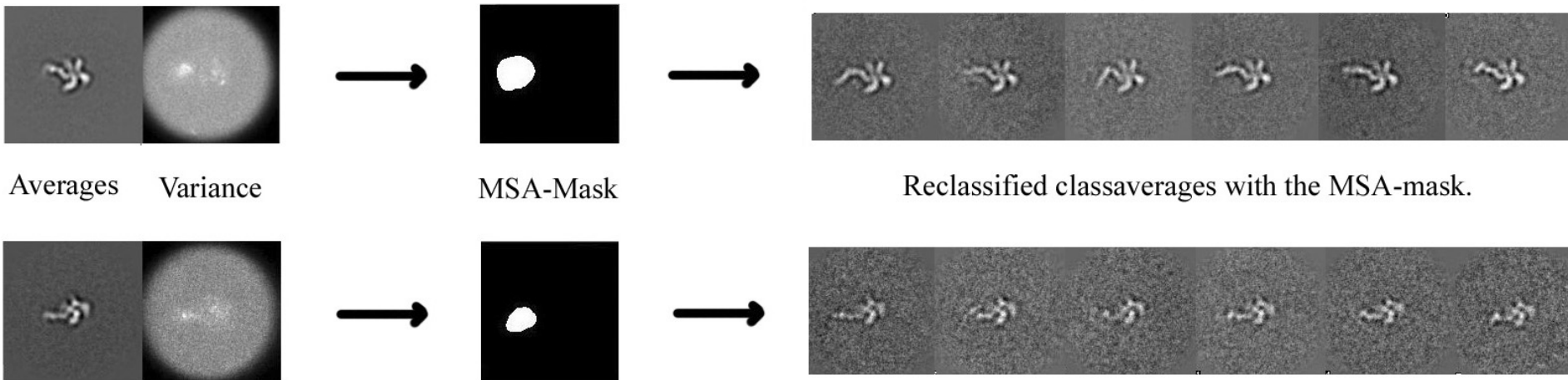
Structure of Human eIF3



CRYO-EM DATA

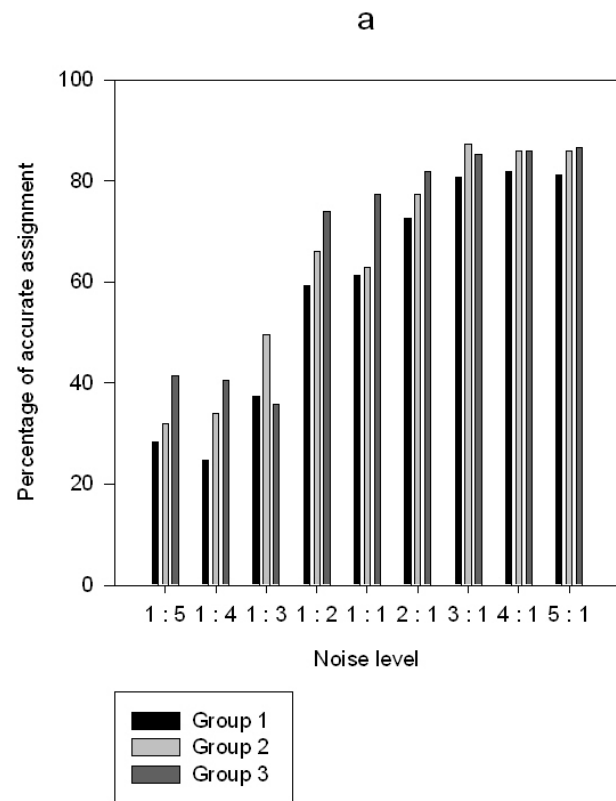
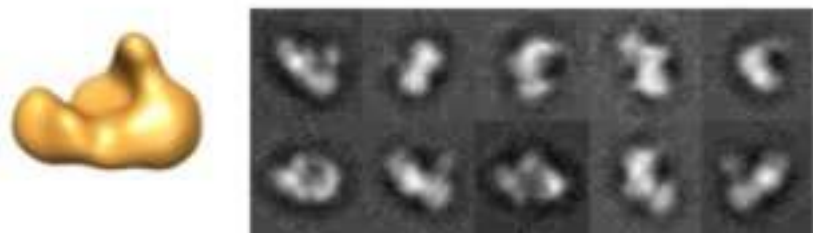
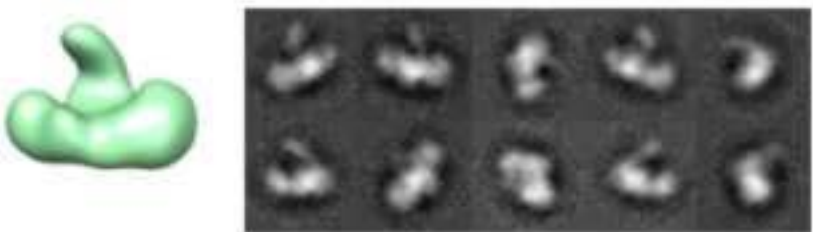
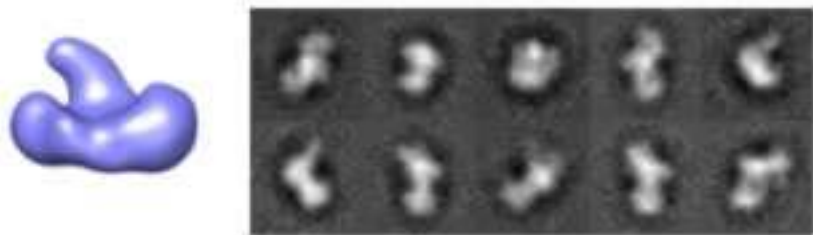


Interaction with HCV IRES: The RNA is in Multiple Conformations

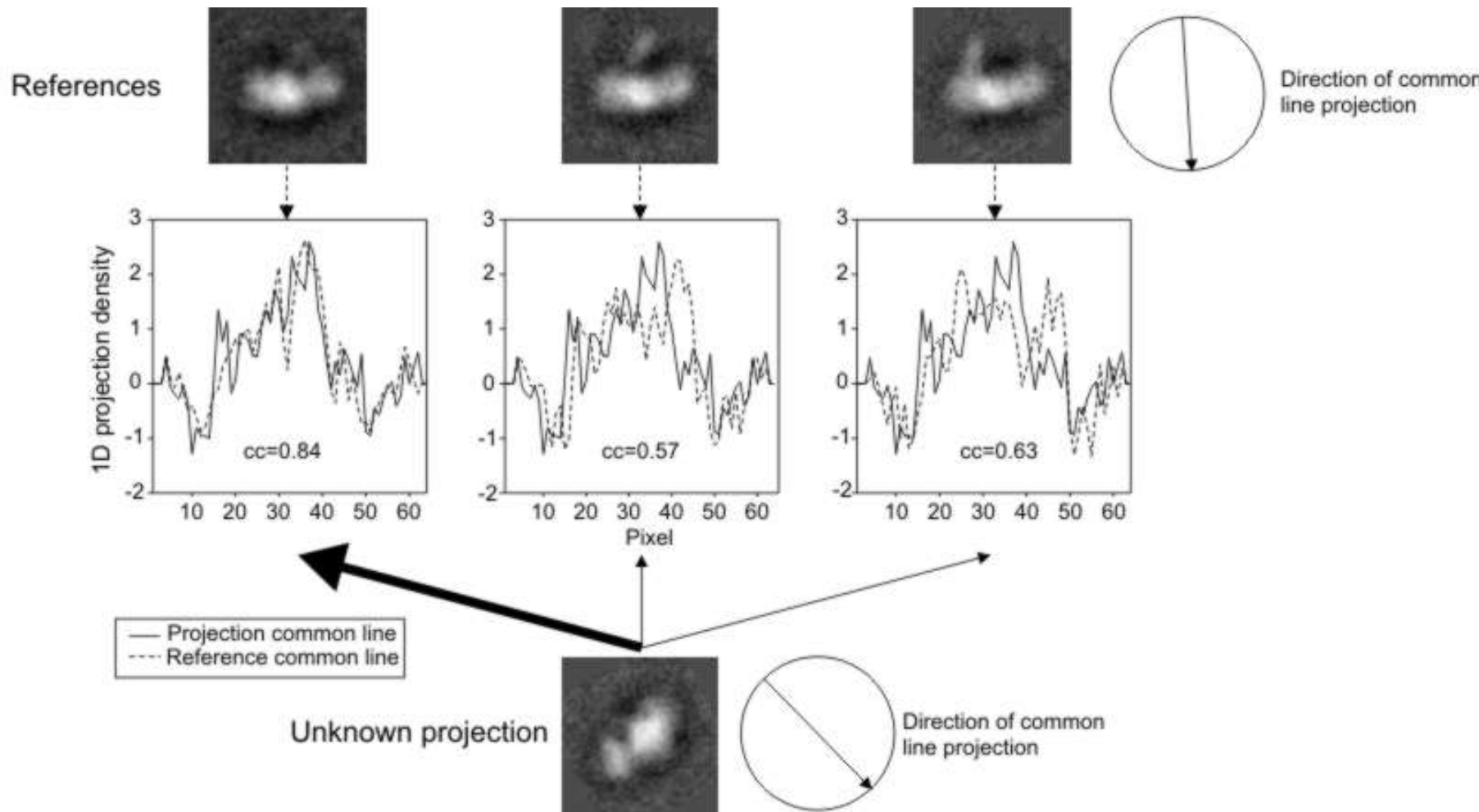


Testing the Principle of CCC with Synthetic Data

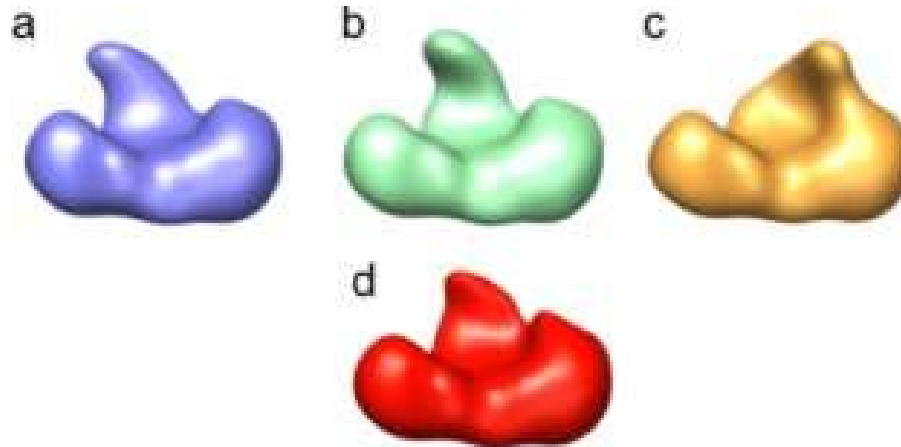
a



Comparison of Common Lines



3D Sorting and Statistics

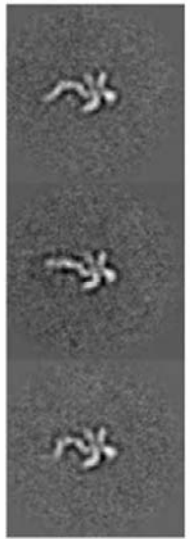
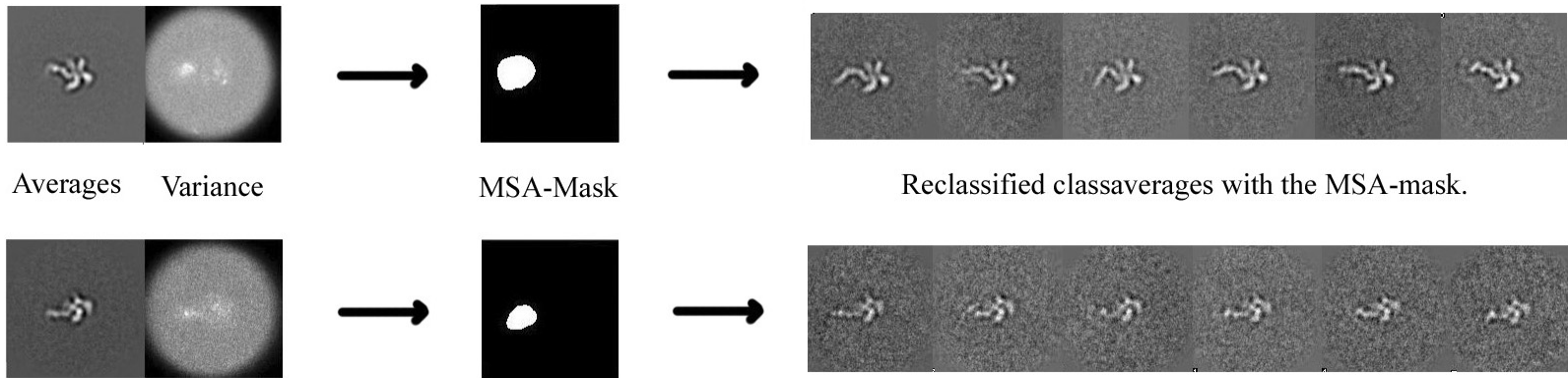


Number of projections	Group 1	Group 2	Group 3
Conformation 1	100	35	13
Conformation 2	41	88	19
Conformation 3	22	17	110
Assignment	61%	63%	77%

b

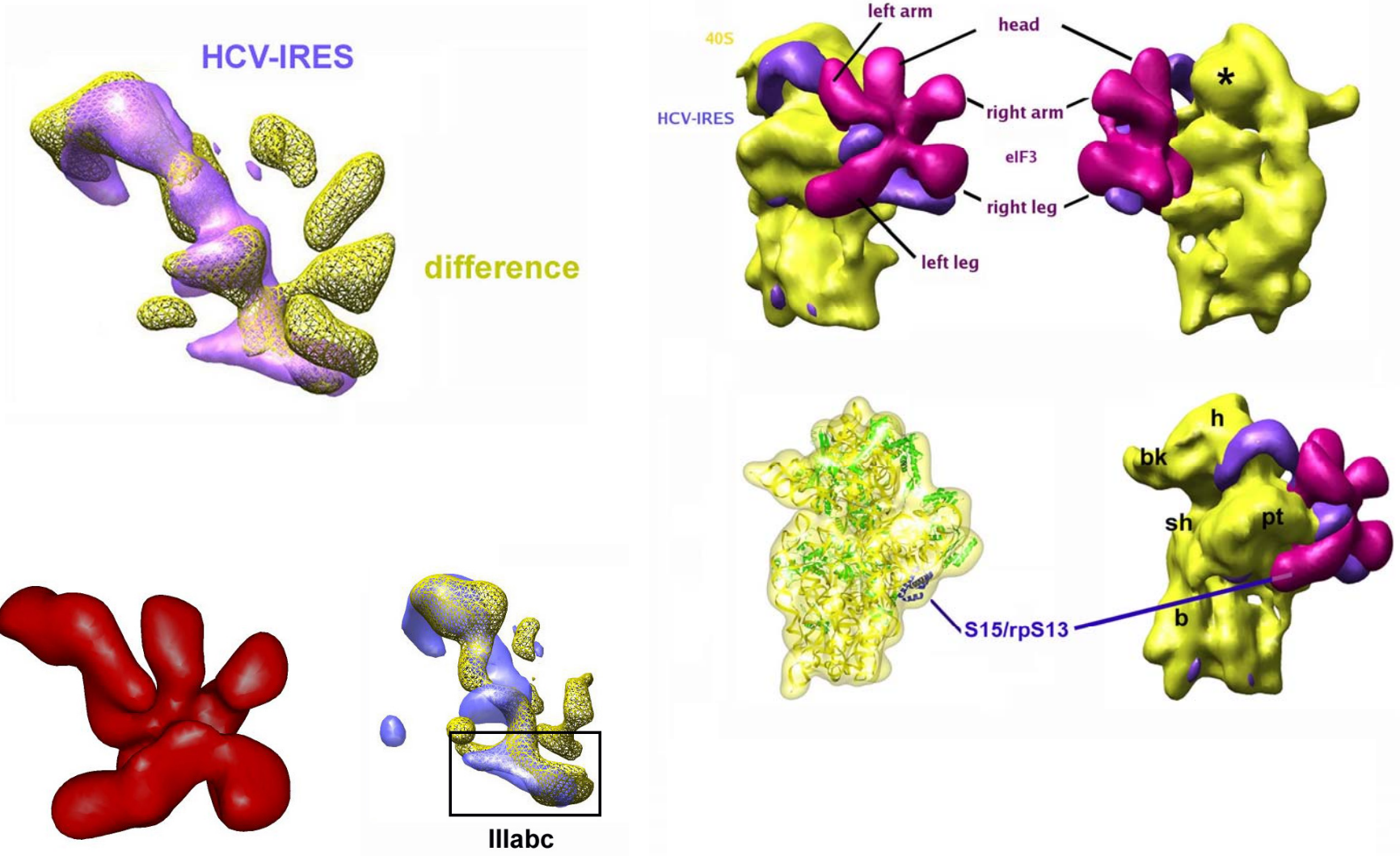
Number of projections	Group 1	Group 2	Group 3
Conformation 1	127	19	4
Conformation 2	7	137	6
Conformation 3	2	2	146
Assignment	93%	87%	94%

Interaction with HCV IRES: The RNA is in Multiple Conformations



Interaction with the 40S Ribosome

Christian Spahn, Kieft, Grassucci, Penc Zhou, Doudna, and Frank (2001) Science



Interaction with eIF4G:

Model



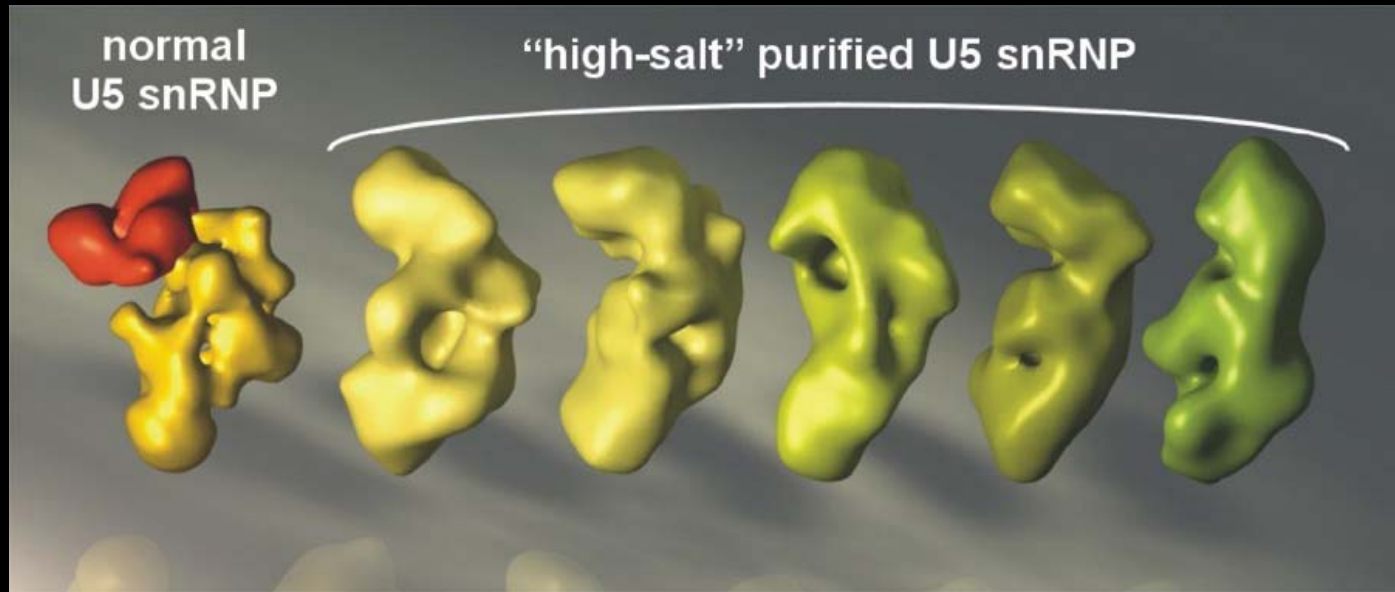
**Potentially large, widespread variability
requiring ab initio separation**

Orthogonal Tilt Reconstruction (OTR)

Andres Leschziner

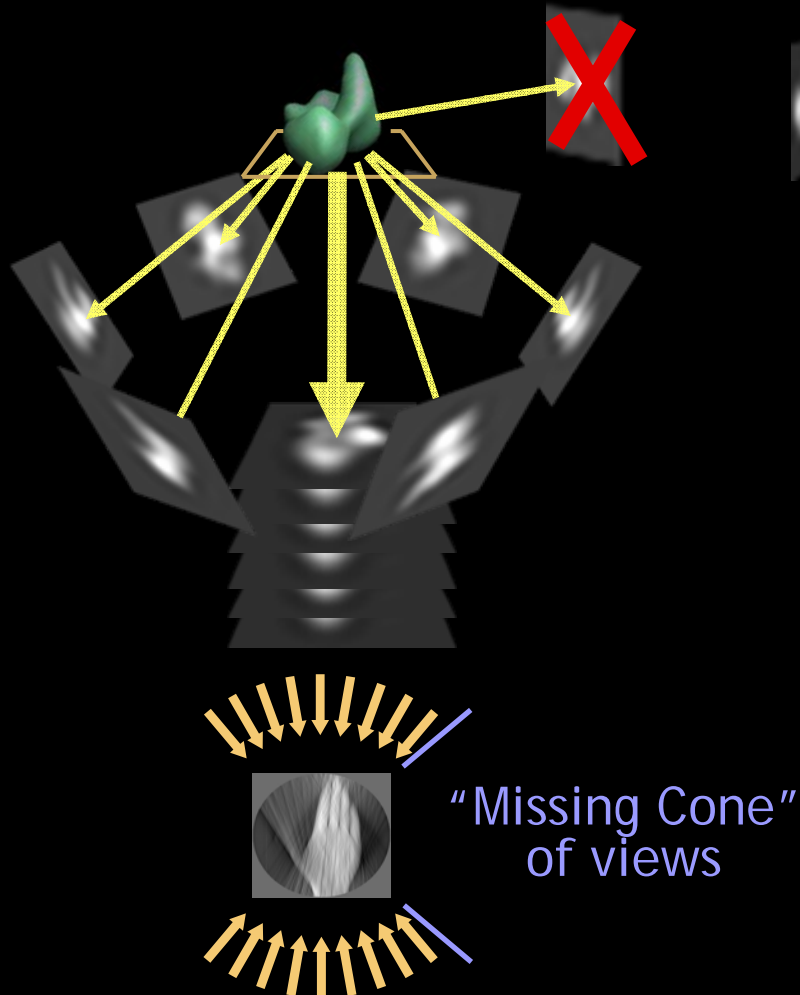


RCT followed by 3D MSA

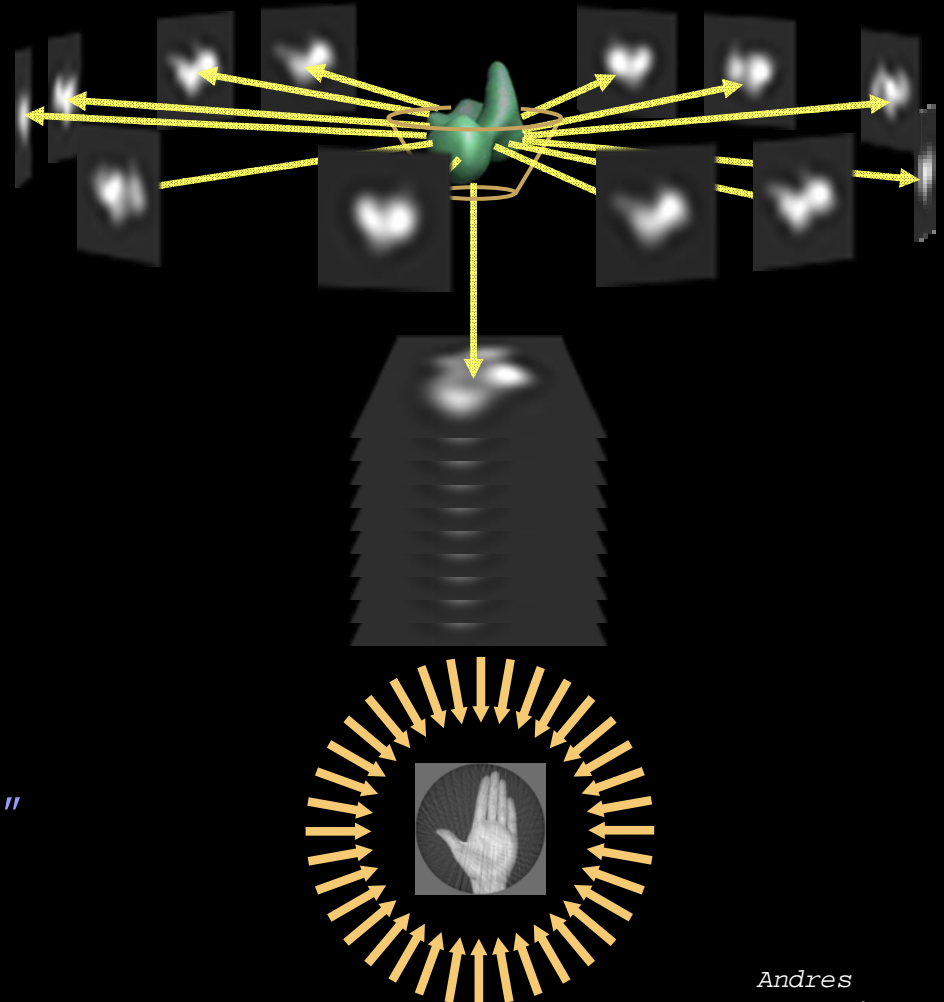


B. Sander, M.M. Golas and Holger Stark

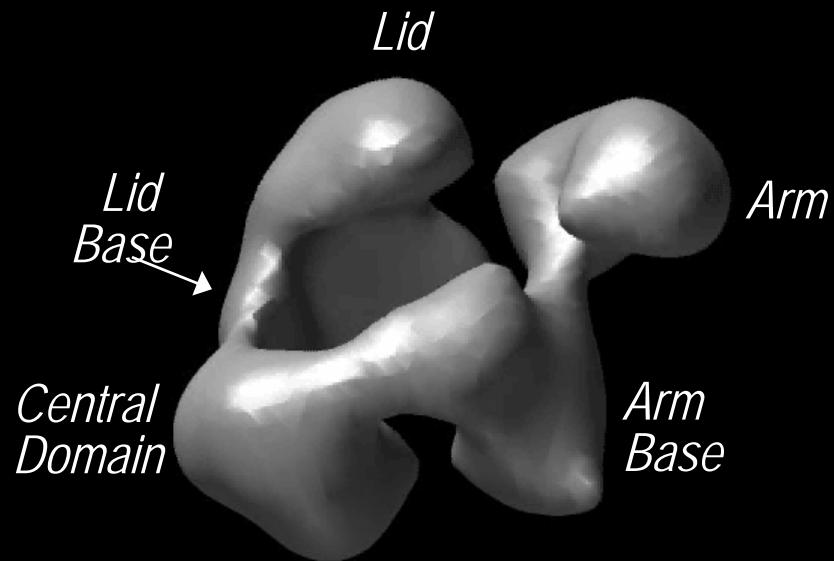
Random Conical Tilt (RCT)



Orthogonal Tilt Reconstruction (OTR)



OTR Reconstruction of the Chromatin Remodeling complex RSC



50Å

Distinct Conformations of RSC

“Open”
Conformation

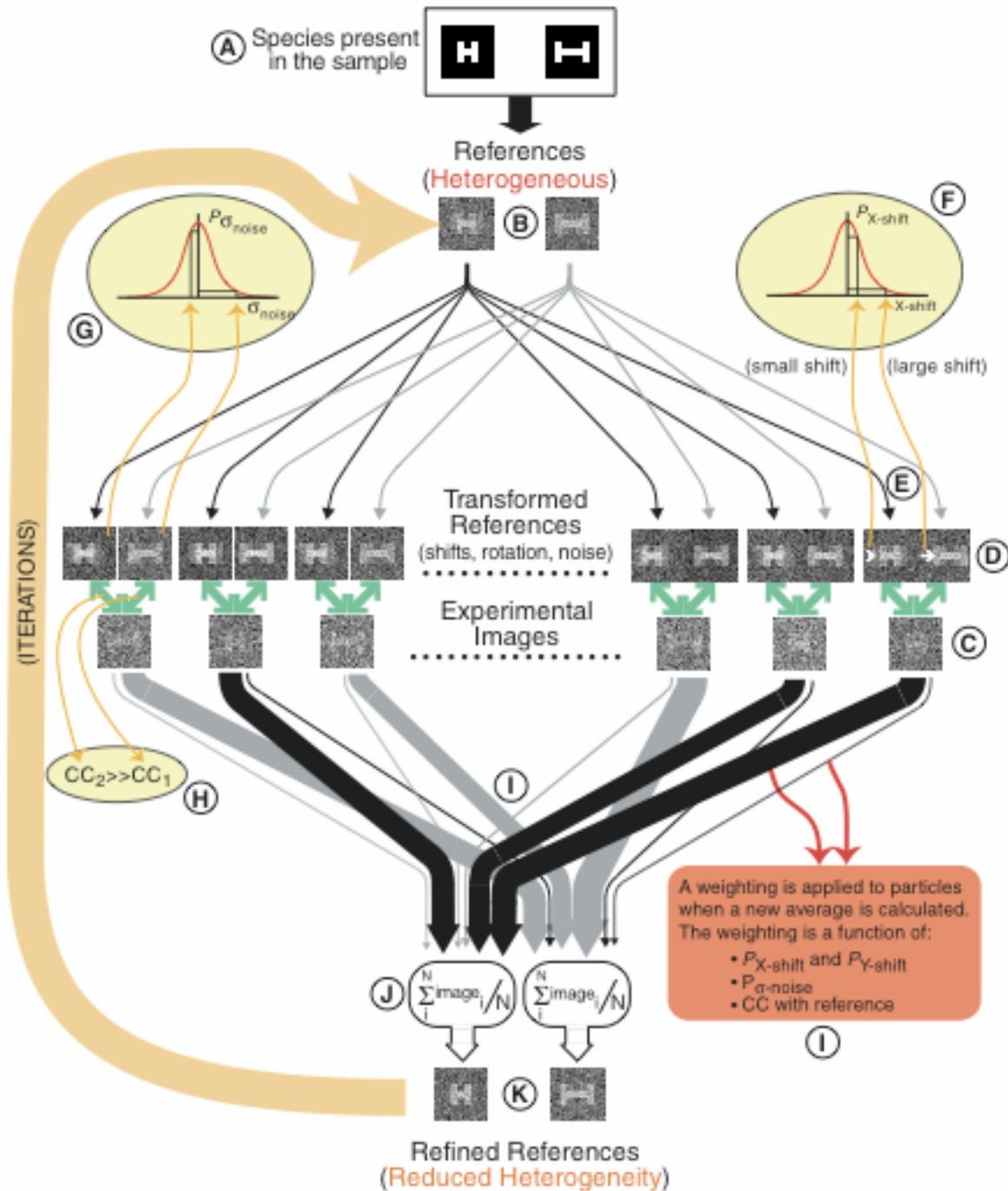


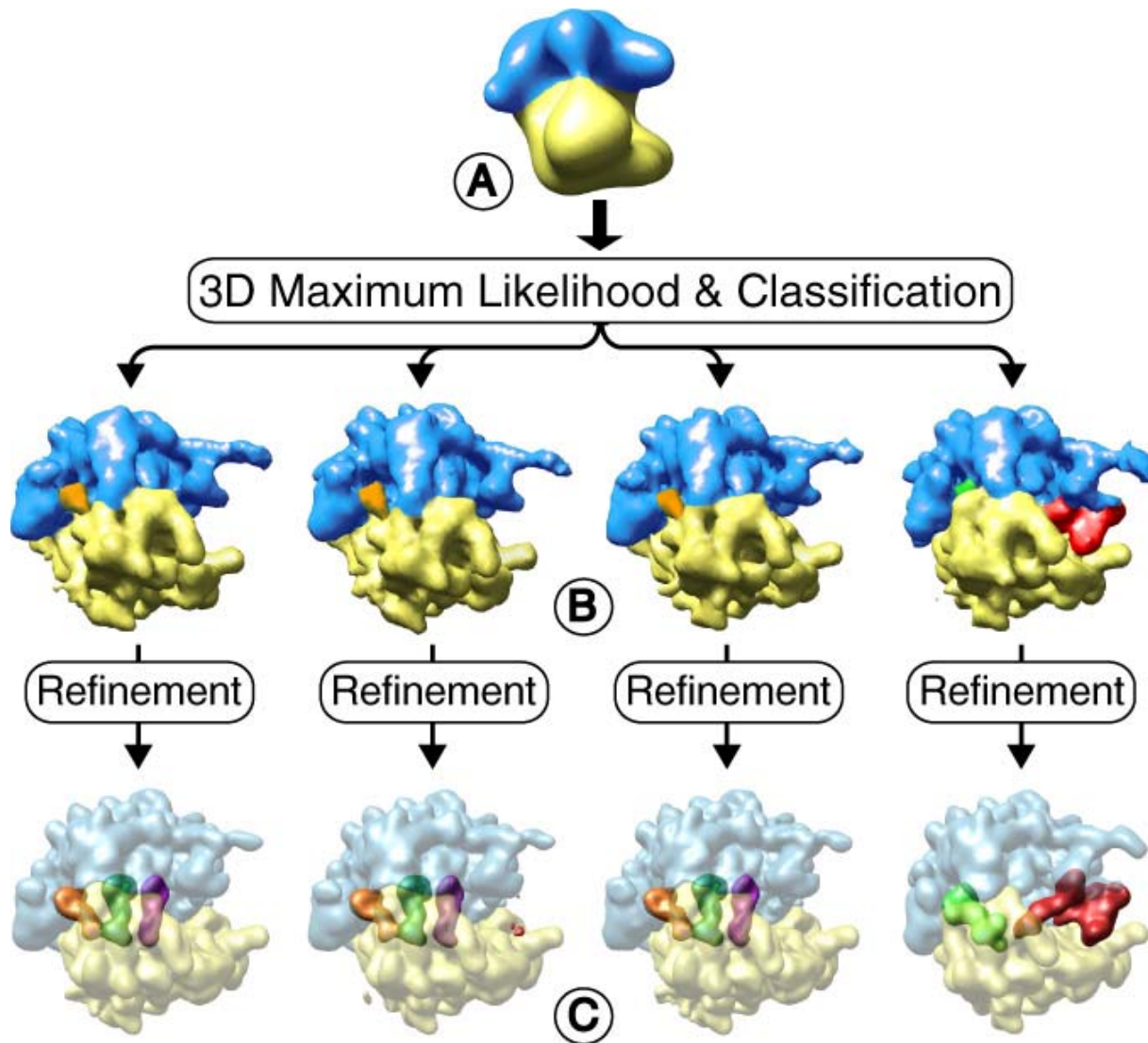
“Closed”
Conformation



The two major RSC conformers accommodate a nucleosome

Maximum Likelihood





S.H. Scheres, H. Gao, M. Valle, G.T. Herman, PPB Eggermont, J. Frank, JM Carazo (2007) Nature Methods 4

Acknowledgements

ARC/CRSP-Dylan Taajes

(Boulder University)

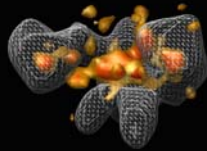
HHMI)



Frank Andel

Robert Tjian (UC Berkeley,

TFIID-Patricia Gro



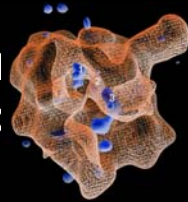
Frank Andel

Pawel Penczek (University of Texas)

Carla Inouye / Robert Tjian

PolIII - Seth Kostel

Patricia (



Sacha de Carlo, Jie Fang

RSC - *Andres Leschz

(University of Utah,

(Harvard University)



Anjanabha Saha / Brad Cairns

eIF3 - Richard Hall

*Bunpote Siridechadilok

Doudna (UC Berkeley, HHMI)



Chris Fraser / Jennifer
Agouron, DOE, HHMI, NCI, NIH

TUESDAY

7:30 am - 8:30 am Breakfast

9:00 am - 12:30 pm

Better Resolution of Structures with Low Symmetry

Discussion Leader: **Melissa Jurica**
(University of California, Santa Barbara)

9:00 am - 9:10 am

Introduction and overview

9:10 am - 9:35 am

Roger Craig (University of Massachusetts)

"Atomic model of the 'OFF' state of a myosin filament determined by cryo-EM"

9:35 am - 9:50 am

Discussion

9:50 am - 10:15 am

Thomas Marlovits (Yale University)
"The type III protein secretion machinery: structure, assembly and conformational changes"

20-fold symmetry

10:15 am - 10:30 am

Discussion

10:30 am - 11:10 am

Coffee Break

11:10 am - 11:35 am

Helen Saibil (Birkbeck College)
"Single particle studies of protein machinery"

7-fold symmetry

11:35 am - 11:50 am

Discussion

11:50 am - 12:15 pm

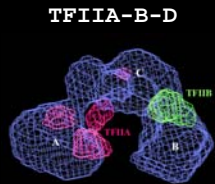
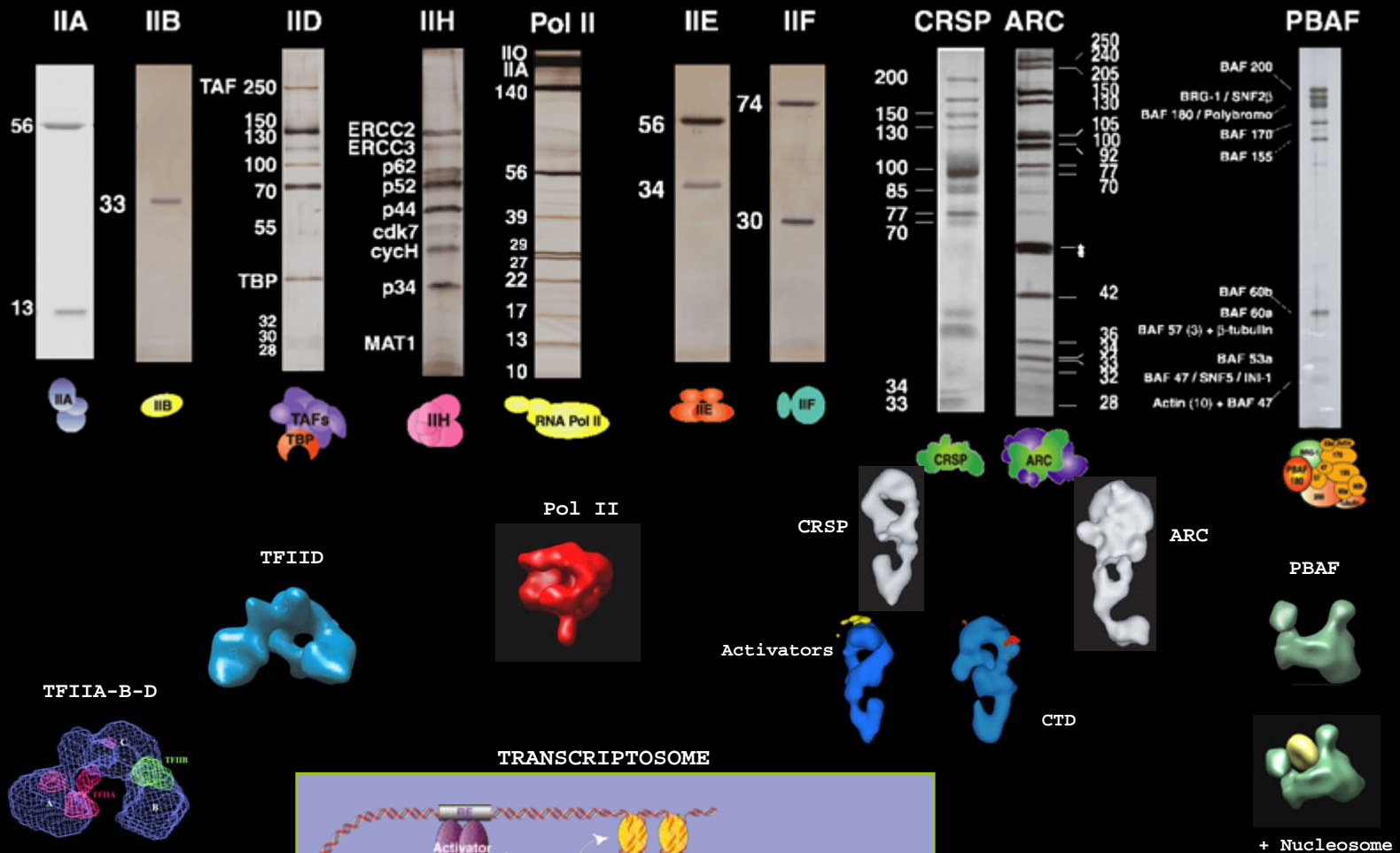
Christian Spahn (Universitätsklinikum Charité, Berlin)
"Visualization of secondary structure elements in cryo-EM maps of ribosomal complexes at sub-nanometer resolution"

ASYMMETRIC!

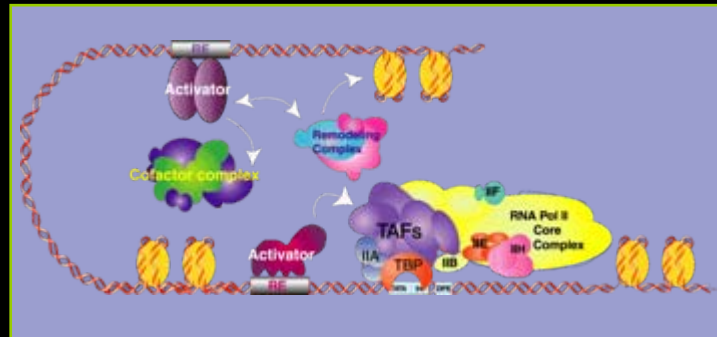
12:15 pm - 12:30 pm

Discussion

Putting it all together



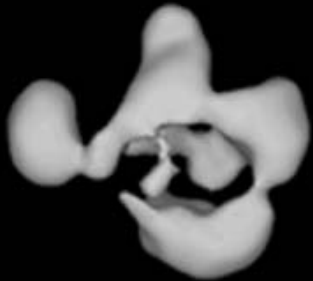
TRANSCRIPTOSOME



How do this new reconstruction compare with the previously published ones?

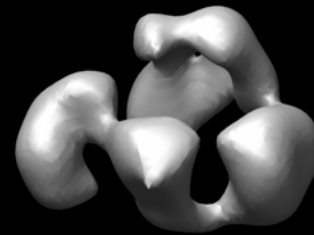
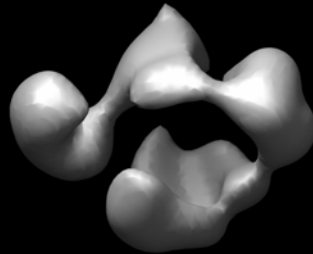
RSC

(RCT)



RSC

(OTR)



PBAF

(RCT)

Asturias *et al.* (2002)
PNAS 99: 13,477