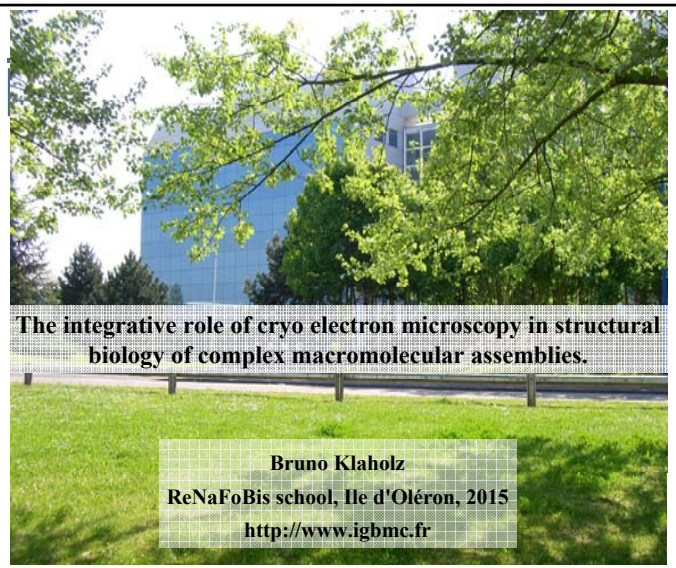

The integrative role of cryo electron microscopy in structural biology of complex macromolecular assemblies.

Bruno Klaholz
ReNaFoBis school, Ile d'Oléron, 2015
http://www.igbmc.fr

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Plan:

I. Some basic concepts of cryo electron microscopy

II. Similarities between structural biology methods

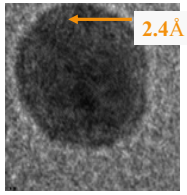
III. Integrated structural biology examples using cryo-EM

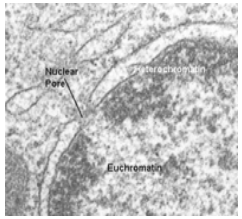
IV. Current & future challenges in cryo-EM

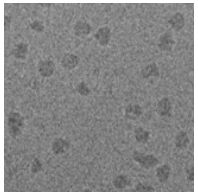
V. Instrumentation & technical highlights towards multi-scale integration

Electron Microscopy:

A) material sciences	B) cellular biology (cell sections)	C) molecular biology (extracted, purified single molecules)







Visual assignment of sample quality, visual annotation of cellular structure

Direct visualization, and more… **3D reconstruction!**
Involves a lot of image processing

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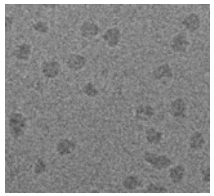
Some basic concepts of cryo electron microscopy

- visualize biological complexes in a hydrated, functional state

- images are 2D projections of a 3D object, i.e. they contain all internal features

- requirement: see the object under different angles to be able to reconstruct it

particles in ~ random orientations



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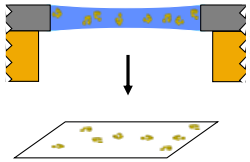
Some basic concepts of cryo electron microscopy

extracted, purified complexes,

preserved in hydrated state:

flash-frozen in the buffer



sample conc.: **~ 0.5 mg/ml**
[compare 3D crystallization: ~2-20mg/ml]

Prioritize cryo-EM over negative staining EM:

- avoids artifacts and limitation in resolution (~30Å) due to staining artifacts and flattening of the structures

- cryo-EM provides best specimen preservation:

no adsorption, no drying

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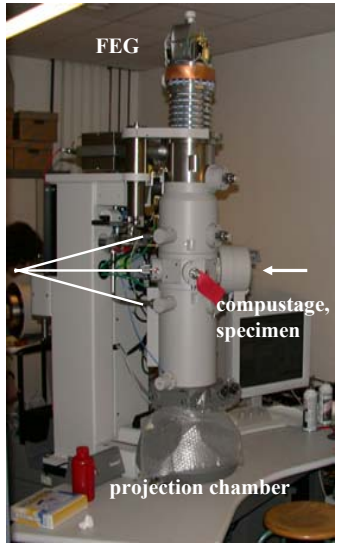
A transmission electron microscope (TEM)



- vacuum: ~10^{-6} Pa
- potentially high electron dose
- potentially high resolution
(λ≈ 0.025Å at 200kV)
i.e. not limited by the wavelength or the optical system

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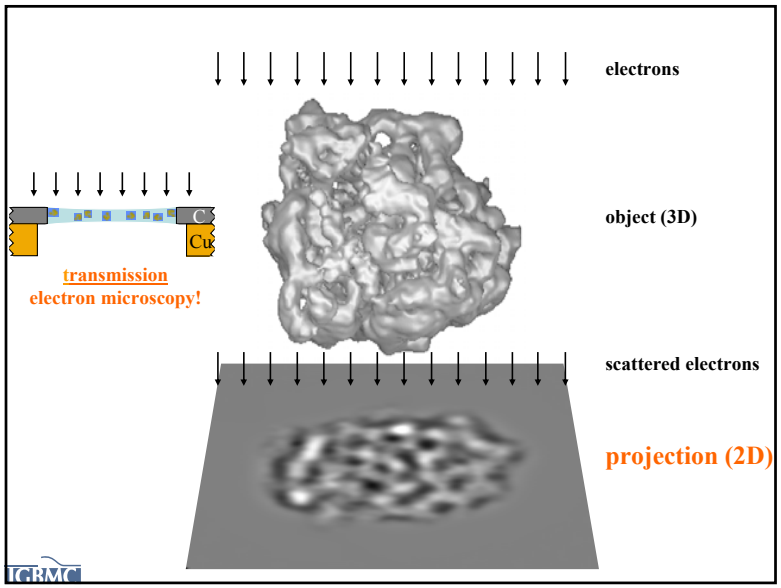
Field emission gun (FEG) electron microscope (Tecnai20, IGBMC)



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Unlike cryo-EM:
here only shadow because of light absorption (amplitude contrast),
information about internal features is lost,
only contours of the object; like for negative staining…

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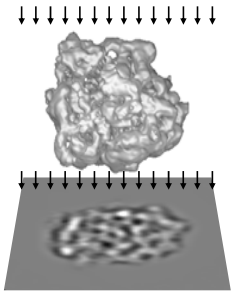
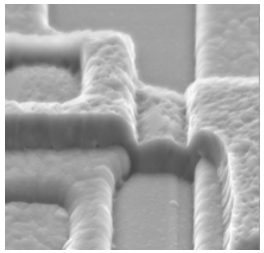
Transmission electron microscopy

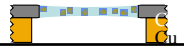


Scanning electron microscopy

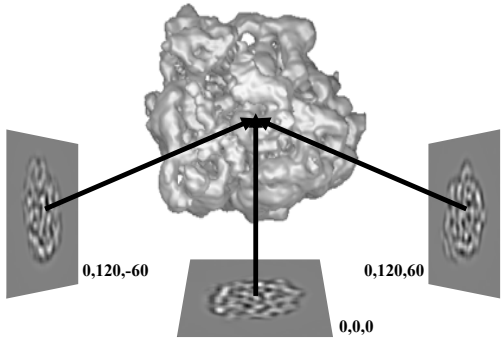


Only surface!

All *internal* features in a 2D view!



Concept of 3D reconstruction: back-projection (franc.: rétro-projection)

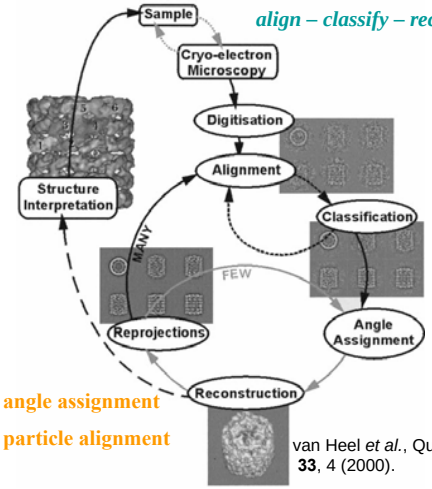


Reconstruction provides a 3D density map, i.e. a **structure** with all internal features

Requires to have **angles** assigned

Structure determination and refinement in cryo-EM

align – classify – reconstruct in 3D



- improve quality of angle assignment
- improve quality of particle alignment

van Heel *et al.*, Quart. Rev. Biophys. **33**, 4 (2000).

Some basic concepts of cryo-EM & 3D reconstruction

Correct terms are important (be precise and rigorous in science :-)

By cryo-EM, we obtain:

- a "3D reconstruction" (initial or refined)
- a "cryo-EM map" or "density map"
- a "structure"

technically:

- back-projection
- angular reconstitution
- random conical tilt
- tilt series / tomogram

NOT:

- an "envelope" (would be SAXS or neg. stain. EM)
- a "volume", units would be Å^3 (e.g. volume of a pocket, volume x density = mol. mass)
- a "surface", units would be Å^2 (e.g. interaction surface between 2 proteins)
- a "model", would be a molecular model *fitted to* the map (crystallography/cryo-EM)
  or a model *compatible with* SAXS data or NMR restraints;
  other "models": "homology model", "hypothetical model", "working model"

Some basic concepts of cryo-EM & 3D reconstruction

Correct terms are important:

A classification is based on a statistical analysis:

- multivariate statistical analysis (MSA) provides information on variance (variability) which serves to merge similar images into class averages (classes); is *independent* of a reference

- classes *are NOT*: the sum of images that correlate best with a reference (through a multi-reference alignment)

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Some basic concepts of cryo electron microscopy

Basic aspects:

- "resolution" corresponds to "spatial frequency" in image processing (1/ Å)

- Nyquist frequency is = 2 x pixel size, e.g. 1 Å / pixel → Nyquist = 2 Å

- interpolations during 2D image alignment and 3D reconstruction limit the possible resolution to about 2/3 of the Nyquist frequency, i.e. here ~ 3 Å *(exception: super-reso)*

pixels in 3D: "voxel"

Consider:

- any correlation calculation (e.g. alignment) is biased by the reference used

- resolution estimation, criteria used:

- 0.5, arbitrary, historically from the virus field, tends to underestimate resol.

- 0.143 (Henderson) and ½ bit (van Heel)

- 3 σ, not used anymore (over-estimation)

- features in the map: can we see dsRNA helices (~10-12 Å resolution), α-helices (~8 Å), ß-sheets (~5 Å) or side chains (4-2.5 Å depending on size)?

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Single particle cryo-EM image processing and 3D reconstruction

I. Pre-processing

- Digitization of micrographs (negatives); not needed if CCD images

- particle selection, « boxing »

- correction of the contrast transfer function

- band-pass filtering and normalisation of particle images

II. Structure determination

- particle centering / alignments

- MSA (multivariate statistical analysis) + classification

- angle assignment

- angular reconstitution

- projection matching

- 3D reconstruction

- structure refinement

- resolution assessment: criteria + what you can resolve in the 3D map!

- map interpretation ; fitting of crystal or NMR structures, …

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II. Similarities between structural biology methods

Is the purified sample homogeneous?

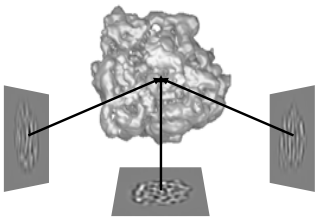
What means homogeneity?
- same composition
- same functional state
- same structural state, i.e. same conformational state

Why do we need homogeneity?

--> **most structural biology approaches are averaging techniques**:
- crystallography
- SAXS
- NMR
- EM and 3D reconstruction

- mass spectrometry (MALDI-TOF etc.)
- dynamic light scattering
- protein / RNA gel electrophoresis
- kinetic studies

exceptions:
- electron tomography
- other single molecule experiments



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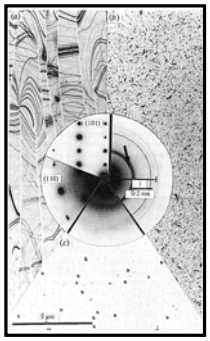
The importance of cryo-approaches

For both crystallography and cryo-EM:

- preservation of the hydrated, functional state
- reduction of irradiation damage
- mechanical stabilization of the sample

cryo-EM:
flash-freezing,
low salt, no cryo-protectants
(would reduce image contrast)



Dubochet *et al.*, 1988

crystallography:
cryo-protectants
glycerol, PEG, high salt, oil, etc.

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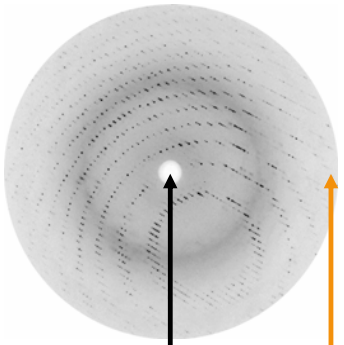
Complementarity of structural approaches: similarities between methods

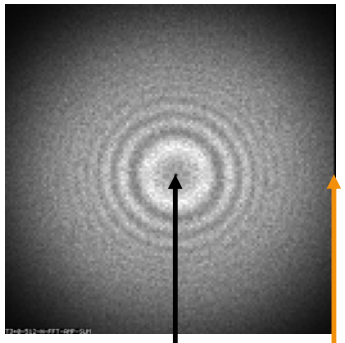
crystallography



low resolution

high resolution

cryo-EM



low resolution
low frequency

high resolution
high frequency

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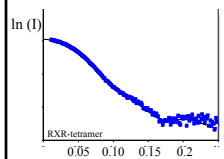
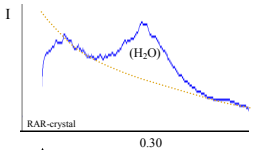
Complementarity of structural approaches: similarities between methods

Profile of the intensity distribution

SAXS

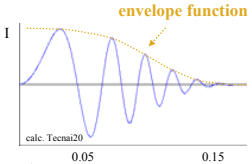


crystal diffraction



beamstop

cryo-EM



very low resolution difficult to measure

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Real space		*Fourier space*
map	3d density distribution ⇄ 3D FFT	3D Fourier transform

$$\rho(\mathbf{x}) = \frac{1}{V}\sum_{\mathbf{h}} \mathbf{F}(\mathbf{h}) \exp(-2\pi i \mathbf{h}\cdot\mathbf{x})$$

Diffraction pattern,
[h, k, l, phases]

Patterson (molecular replacement)
/ auto-correlation function in imaging

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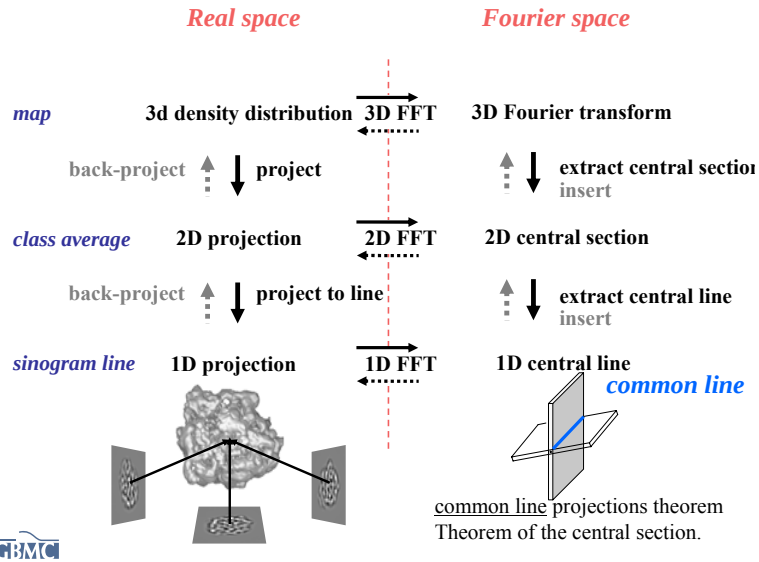

common line projections theorem
Theorem of the central section.

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Common problem: determination of the handedness

- crystallography: depends on correct processing of phased reflections
- SAXS: ambiguity cannot be resolved easily (unless clear fitting of crystal structure etc.)
- NMR: ambiguity solved by using chirality constraints
- single (cryo-)EM images are projections, i.e. mirrors are indistinguishable

Determination of handedness in EM:

- random conical tilt (Radermacher *et al.*, J. Microsc. 1987)
- tomography (technically tricky on single particles)
- phase residual error using a tilt pair (Rosenthal & Henderson, JMB 2003)
- fitting of crystal structures (requires reasonable resolution)
- high-resolution features: right-handed protein and DNA/ARN helices!

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III. Integrated structural biology examples using cryo-EM

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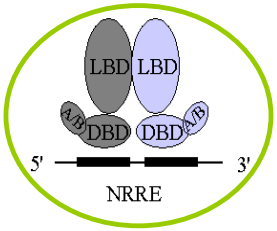
Structure and function of full nuclear receptors

- architecture of full-length DNA-bound NR complexes
- topology of full-length NR's bound to different response elements
- mechanism of ligand- and DNA-dependent activation and co-regulator recruitment
- important targets for biomedical research (steroids, vitamin D etc.)



"textbook drawing"

Possible structural approaches:
- NMR
- crystallography
- SAXS
- cryo-EM

molecular weight: ~100-150kDa

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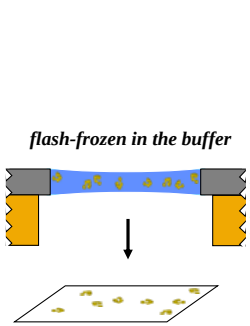
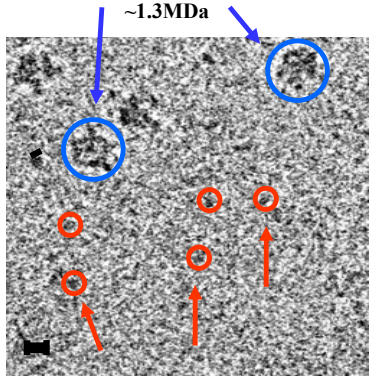
Architecture of the RXR/VDR DR3 DNA complex



50S ribosomal subunit
~1.3MDa



100 Å

NR complex
~100 kDa

200kV data

Avoid a priories…:

was usually considered too small for cryo-EM… (limit >250kDa)

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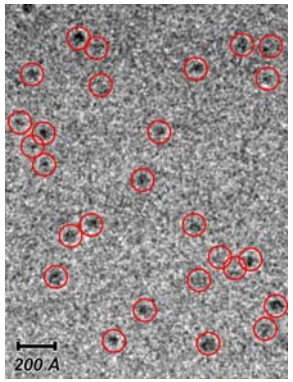
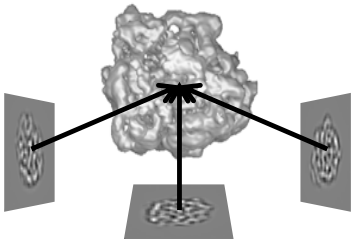
Image processing: particle selection, classification, structure determination





angle determination and 3D reconstruction

20 000 particles selected, defocus used: -2 to -4 µm

MSA, classification, common-line angle assignment and refinement (Imagic)

(no projection matching)

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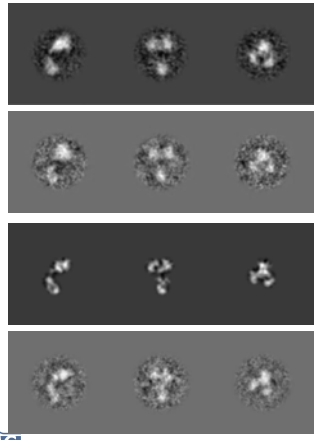
Two independent *ab initio* structures:



100kV data
Re-projections

4k CCD, Polara

100kV data
Class averages

200kV data
Re-projections

film, Tecnai20

200kV data
Class averages

→ 3D correlation ~90%!

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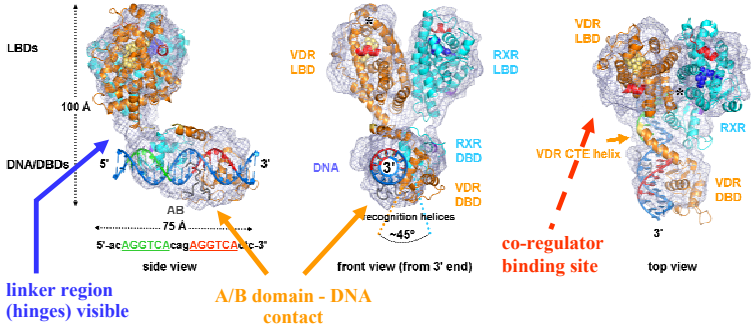
Architecture of the RXR/VDR DR3 DNA complex



linker region (hinges) visible

A/B domain - DNA contact

co-regulator binding site

Orlov *et al.*, *EMBO J.* 2012.

key concepts: - asymmetric topology
- open conformation

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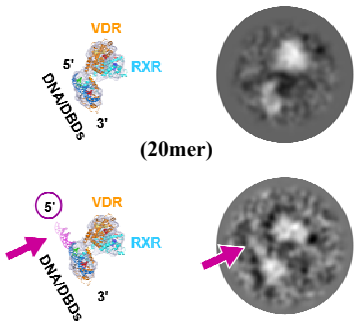
Architecture of the RXR/VDR DR3 DNA complex

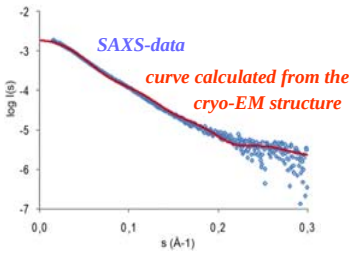
Assignment of DNA polarity:



5'-extended DNA
(35mer)

→ *independent structure validation*

Consistency of cryo-EM and SAXS data:



Orlov *et al.*, *EMBO J.* 2012.

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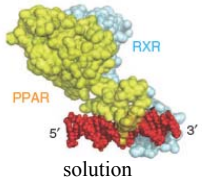
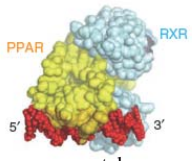
Architecture of nuclear receptors on DR1 response elements

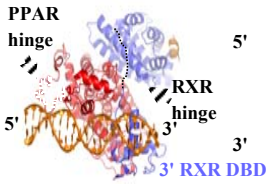
PPAR/RXR DR1



solution

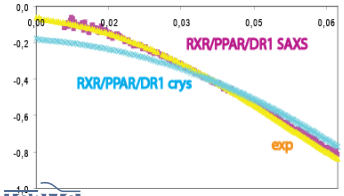


crystal



PPAR/RXR DR1 crystal structure
(Chandra *et al.*, *Nature* 2008)

SAXS-derived PPAR/RXR model



different protein conformation in the crystal and in solution

Rochel et al., Nat. Struct. Mol. Biol., 2011.

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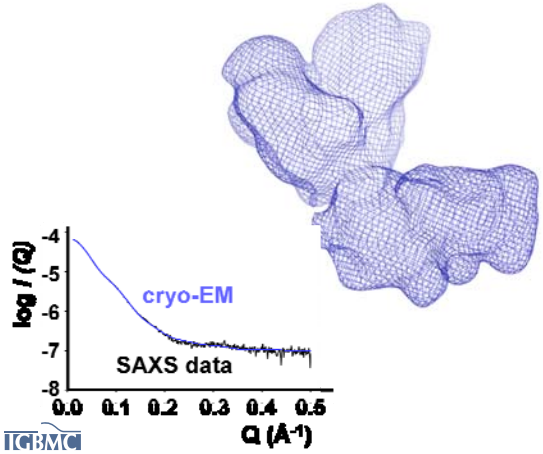
Structure of the USP/EcR complex on a natural DNA inverted repeat (IR1)



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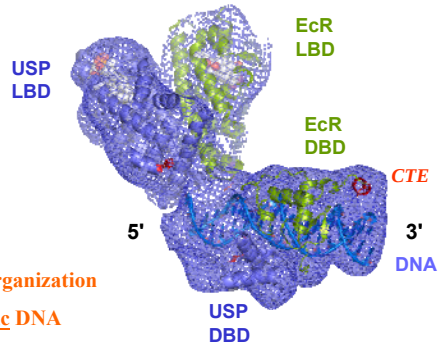
Structure of the USP/EcR complex on a natural DNA inverted repeat (IR1)



key concept:

asymmetric organization

on a symmetric DNA

IR1: CAAGGGTTCAATGCACTTGT
GTTCCCAAGTTACGTGAACA

hsp27

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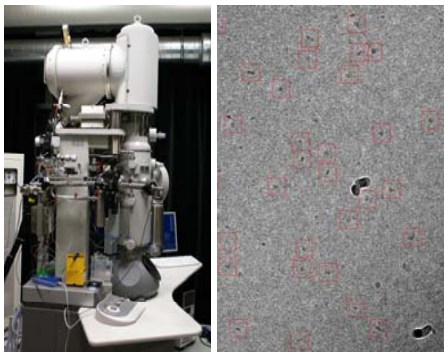
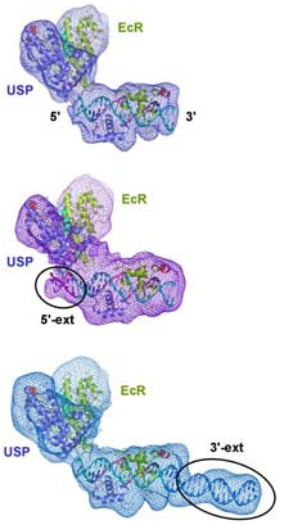
3 cryo-EM structures:



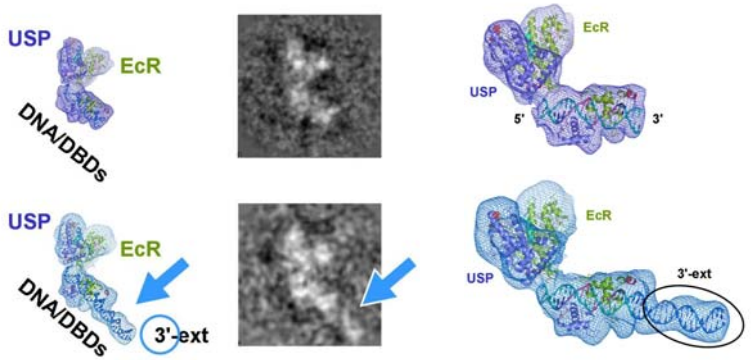
Polara electron microscope data, CCD, 100kV, 59k, 50 000 particles



→ *independent structure validation*

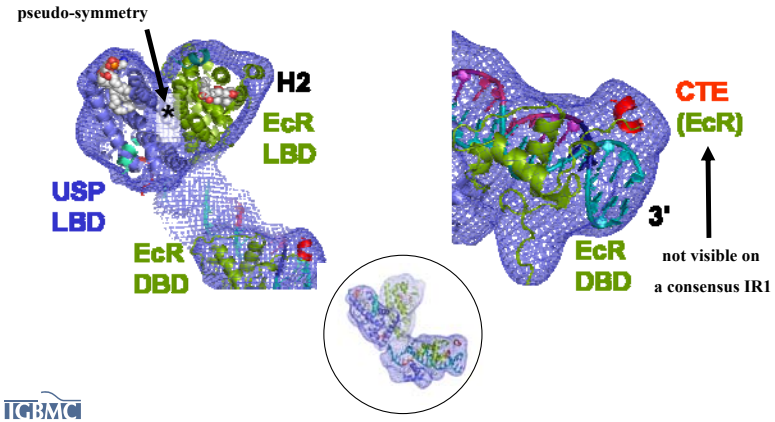
Maletta *et al.*, *Nature Communications, 2014*.

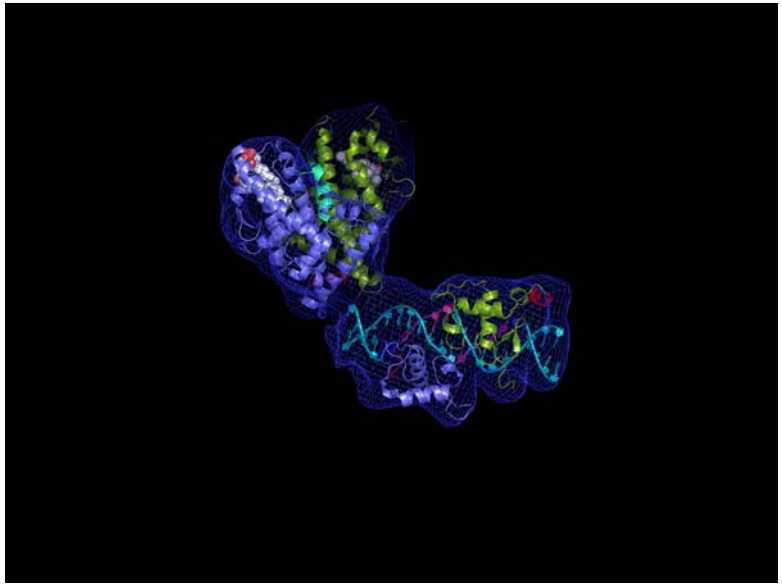
Assignment of the polarity on the DNA:



3'-DNA extended complex (35mer, +18mer)

Identification of key structural features in the USP/EcR complex





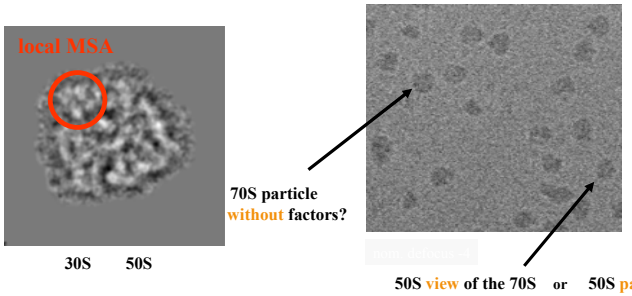
IV. Current & future challenges in cryo-EM:
- how to push resolution to the atomic level?
- how to analyze flexible complexes?
- how to integrate towards the cellular level?

→ instrumentation
→ software developments for image processing

Conformational changes of cats?

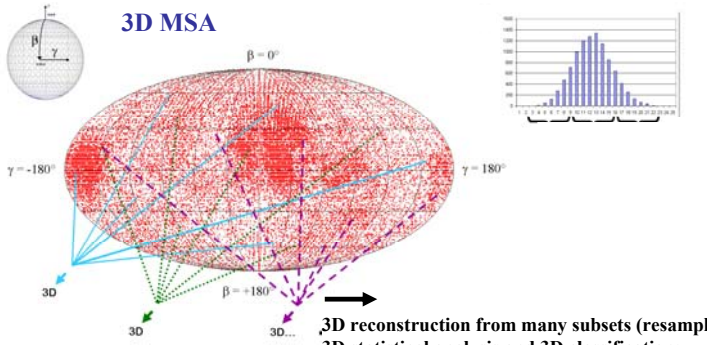
Determining structures of multiple conformational states in a single sample

local 2D MSA (multi-variate statistical analysis)



Klaholz et al., *Nature* 2004; see Suppl. Mat.

Determining structures of multiple conformational states in a single sample



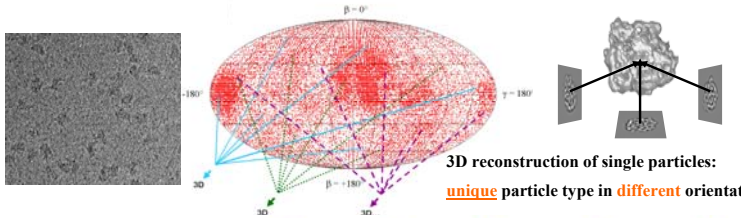
,3D reconstruction from many subsets (resampling)
3D statistical analysis and 3D classification:
3D sampling and classification (3D-SC)

Simonetti *et al.*, *Nature,* 2008.

→ does both re-sampling and 3D classification;
see also work by P. Penczek (bootstrapping (re-sampling), used primarily to find region of variance)
see also S. Scheres/J-M Carazo (maximum likelihood parameter refinement and classification)

Sorting out heterogeneity of the complexes:

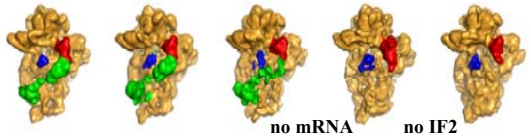
3D statistical analysis and 3D classification:
3D sampling and classification (3D-SC)



3D reconstruction of single particles:
unique particle type in different orientations

2D → 3D → 4D

Structural states in equilibrium with each other

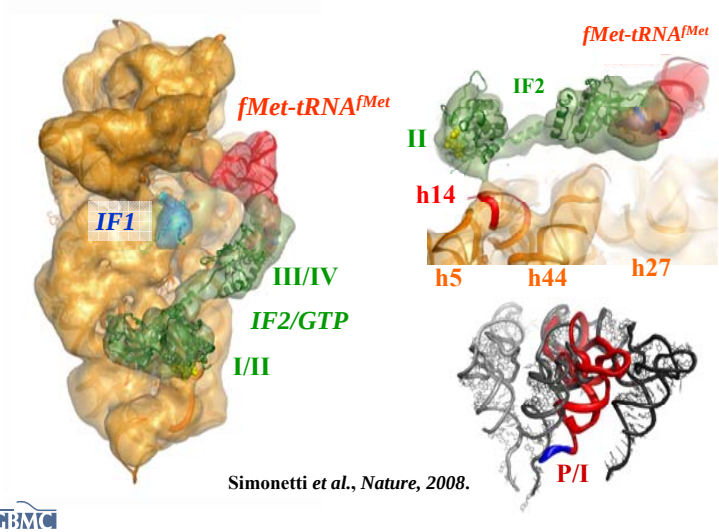


40 % **28 %** **no mRNA 10 %** **8 %** **no IF2 14 %**

Multiple states in the 30S initiation complex **Simonetti *et al.*, *Nature*, 2008.**

used by Fischer et al., *Nature*, 2010; Papai et al., *Nature* 2010.

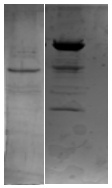
Cooperative binding of the initiator tRNA and IF2 in the 30S initiation complex



Simonetti *et al.*, *Nature*, 2008.

Structure determination of translation initiation factor IF2

crystals

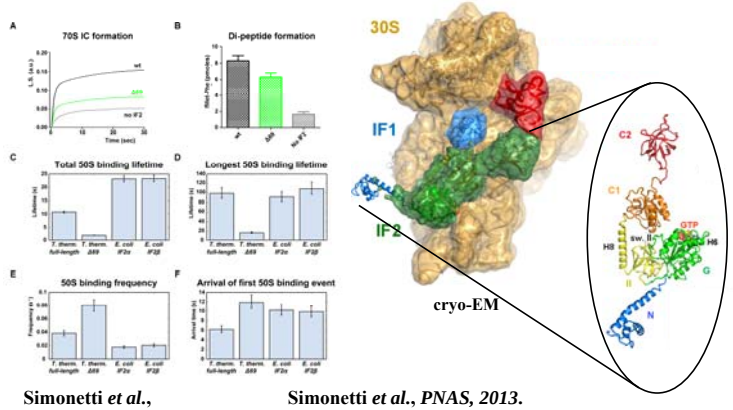


1 - 571
1 - ~378

	Native		Se-Met	
Data collection				
X ray source	SLS (Pilatus Detector)		SLS (Pilatus Detector)	
Space group	$P2_12_12_1$		$P2_12_12_1$	
Cell dimensions				
a, b, c (Å)	45.42, 61.46, 162.4		45.19, 60.93, 160.74	
α, β, γ(°)	90, 90, 90		90, 90, 90	
Molecules/A.U	4		4	
Solvent Content (%)	50		50	
		Peak	***Inflection***	***Remote***
Wavelength(Å)	0.9194	0.9792	0.9796	0.9537
Resolution (Å)	50 – 1.95	50 - 2.4	50 - 2.4	50 - 2.4
Distance (mm)	200	450		
Exposure time (sec)	2	2		
R_{sym} (%)	9.7 (43.7)	7.1 (30.9)	6.5 (28.3)	15.6 (60.4)
Reflections	36, 743	33,368	33,414	88,585
Completeness (%)	100 (100)	99.6 (99.7)	99.5 (99.4)	99.6 (98.7)
Redundancy	10.96	4.67 (4.3)	4.77 (4.53)	4.98 (4.91)
Refinement				
Resolution (Å)	1.95			
No of reflections	32, 857			
Protein atoms	2886			
Solvent atoms	235			
R_{work}/R_{free}	18.6/22.5 (21.6/27.4)			

Simonetti *et al.*, *Acta Cryst D, 2013.*

Involvement of IF2 N domain in ribosomal subunit joining revealed from architecture and function of the full-length initiation factor



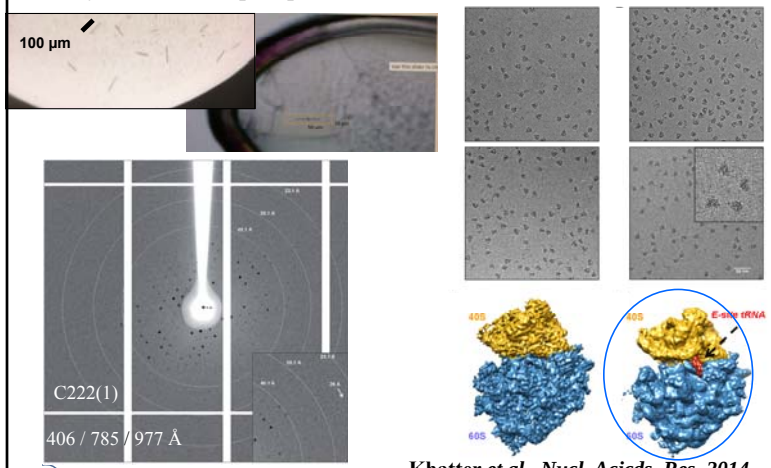
Simonetti *et al.*, *Acta Cryst D, 2013.*

Simonetti *et al.*, *PNAS, 2013.*
Eiler *et al.*, *PNAS, 2013.*

crystallography, SAXS, cryo-EM, kinetics and single molecule fluorescence

Crystallography of (large) macromolecular complexes:

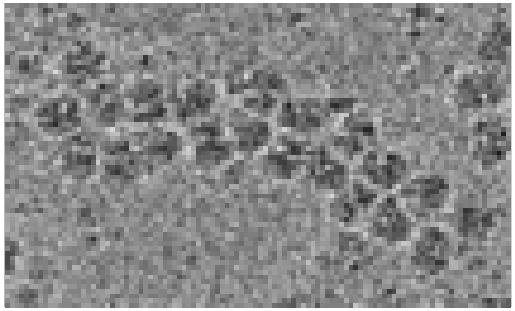
use cryo-EM for sample optimization: first human 80S ribosome crystals



IGBMC SLS; (+rt tests microfocus Diamond)

Khatter *et al., Nucl. Acicds. Res. 2014.*

Towards higher complexity: molecular assemblies



Cryo electron tomography (cryo-ET) of

- purified complexes

- cell sections

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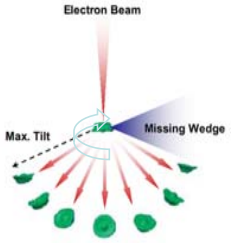
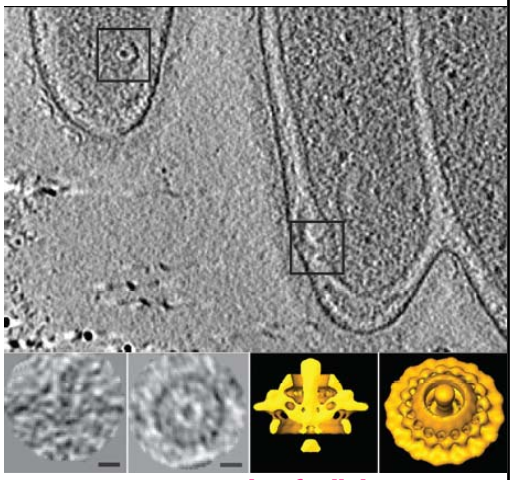
Cryo electron tomography





→ tomogram
(i.e. a 3D reconstruction from tilt images)

flagellar motor

Murphy et al,. Nature 2006, 442, 1062–1064.

Tomography of cellular structures

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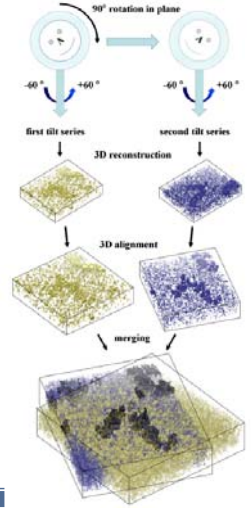
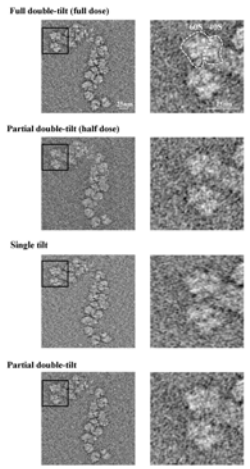
3D polysome reconstruction from single- / dual-tilt cryo electron tomography





full dual-tilt

partial dual-tilt (half-dose)

single-tilt

partial dual-tilt (3° versus 12°)

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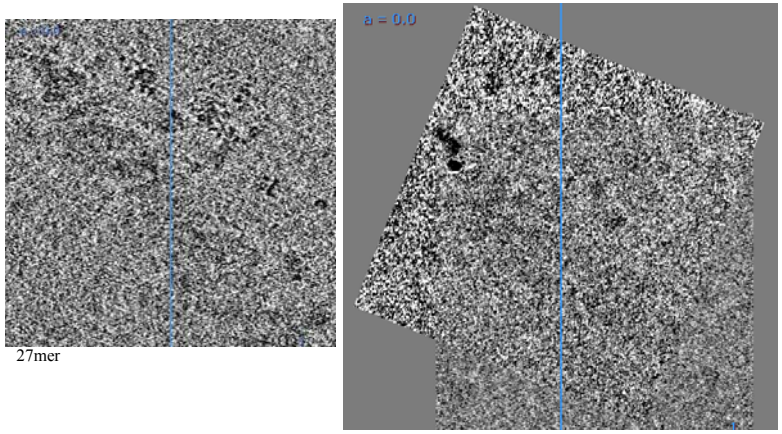
Myasnikov *et al.*, *Ultramicroscopy* 2013.

3D polysome reconstruction from single- / dual-tilt cryo electron tomography



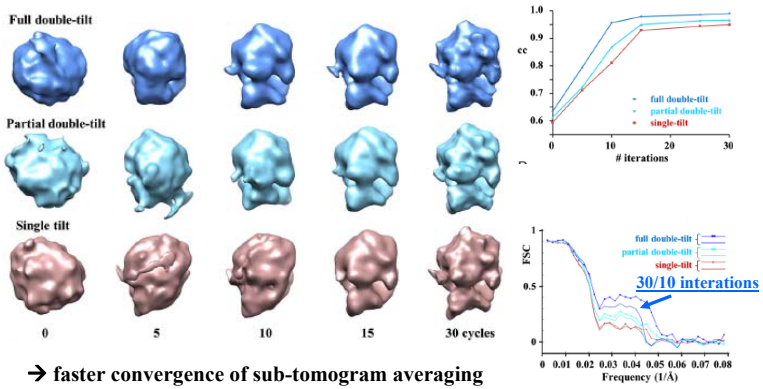
27mer

single tilt *Polara, 4k CCD, 150kV* dual tilt

[like a κ-goniometer setup]

inspect3D, IMOD Myasnikov *et al.*, *Ultramicroscopy* 2013.

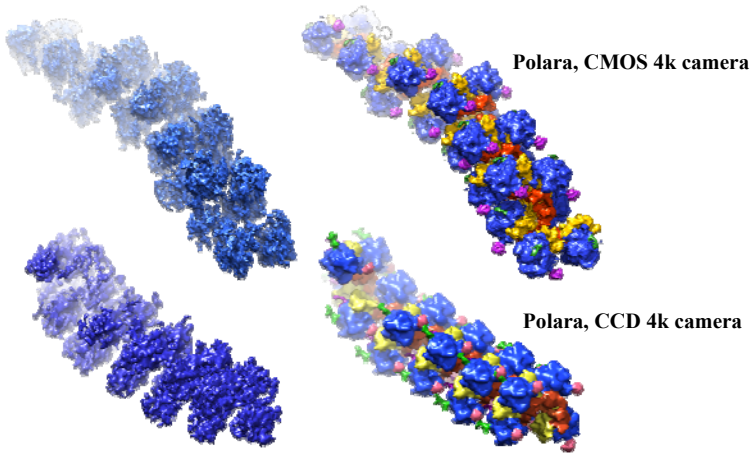
3D polysome reconstruction from single- / dual-tilt cryo electron tomography



→ faster convergence of sub-tomogram averaging when using dual-axis data (even partial)

Myasnikov *et al.*, *Ultramicroscopy* 2013.

Architecture of eukaryotic polyribosomes

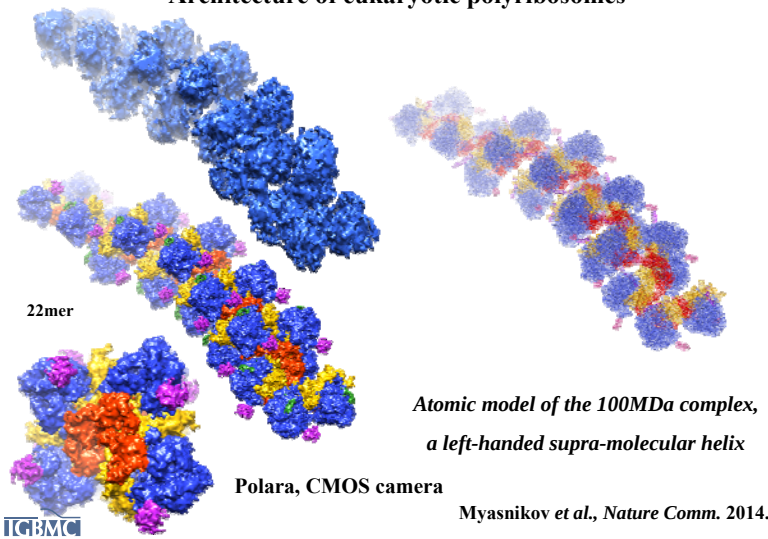


Polara, CMOS 4k camera

Polara, CCD 4k camera

raw tomogram sub-tomogram averaging

Architecture of eukaryotic polyribosomes



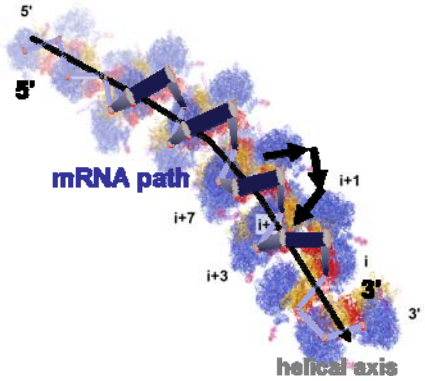
22mer

Atomic model of the 100MDa complex, a left-handed supra-molecular helix

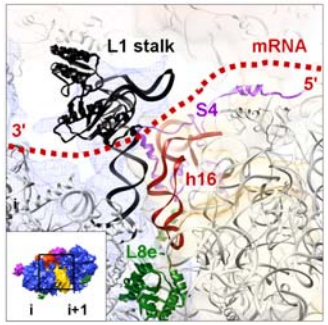
Polara, CMOS camera

Myasnikov *et al.*, *Nature Comm.* 2014.

Architecture of eukaryotic polyribosomes



Formation of a continuous helical mRNA channel that protects from degradation



mRNA-transfer from exit to entry sites

Myasnikov *et al., Nature Com., 2014.*

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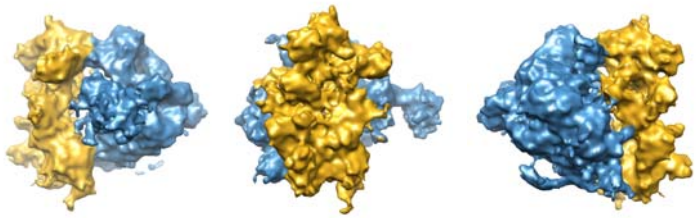
Architecture of eukaryotic polyribosomes



sub-tomogram average structures from Polara + direct electron detector (Falcon 1),

→ single particle cryo electron tomography

Myasnikov *et al., Nature Comm.* 2014.

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V. Instrumentation & technical highlights towards multi-scale integration

<u>High-resolution electron microscopes:</u>

- ultra-stable specimen holders,
- high-resolution optics,
- parallel electron beam,
- aberration correction,
- use lower voltage for better contrast of small complexes,
- automatic data collection for single particle cryo-EM and cryo electron tomography (cryo-ET),
- standardize sample preparation (cryo-EM freezing, high-pressure freezing and ultra-microtomy for cell section)
- phase plates (Zernike, Volta etc.)

<u>High-sensitivity cameras:</u>

direct electron detectors, CMOS camera, counting events;
is part of a "revolution" in cryo-EM and structural biology, like for Pilatus/Eiger detectors in X-ray crystallography

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High-resolution electron microscopes and direct electron detectors

Polara electron microscope

CMOS camera

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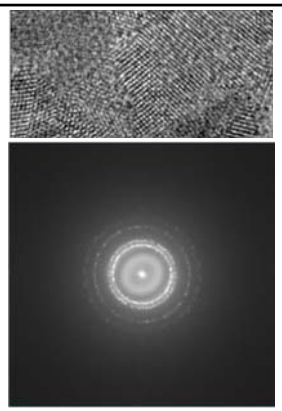
Titan Krios installation 10-12.2013, CBI,

Instruct/FRISBI-infrastructure access



Reaching the atomic level…?

- large data sets,
- image processing to high-resolution

→ see data set for the cryo-EM practicals

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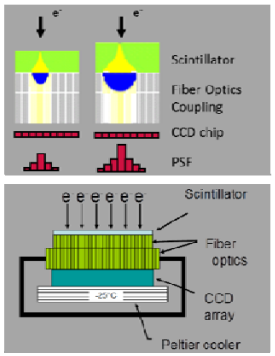
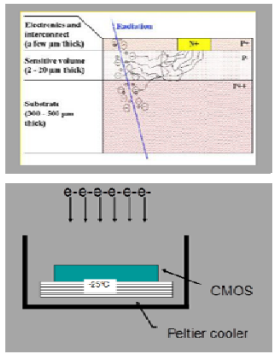
High-resolution electron microscopes and direct electron detectors

CCD: multi stage conversion of electron energy via fiber or lens optics



CMOS: direct conversion of electron energy without fiber or lens optics



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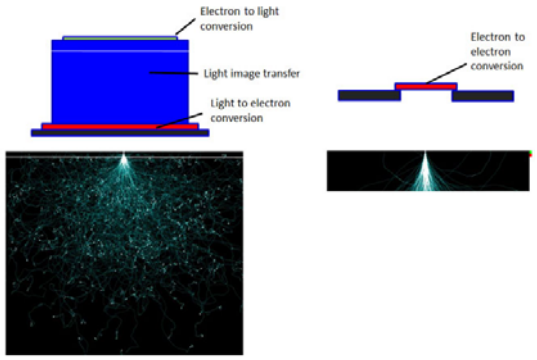
FEI website

High-resolution electron microscopes and direct electron detectors



Monte-Carlo simulation of e- scattering

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Gatan website

High-resolution electron microscopes and direct electron detectors

- back-thinning of Si-layer
- counting mode
- super-resolution mode
- dose fractionation
- movie processing
- beam-induced specimen drift correction
- exposure filtering (dose optimization / frame selection)

Brilot *et al.*, *JSB*, 2012.
Campbell *et al.*, *Structure*, 2012.
Li *et al.*, *Nat Methods*, 2013.
Ruskin *et al.*, *JSB*, 2013.
Liao *et al.*, *Nature*, 2013.
Fernández *et al.*, Science 2013.
McMullan *et al.*, *Ultramicr.*, 2014.
Allegretti *et al.*, *eLife*, 2014.
Wong *et al.*, *eLife*, 2014.
Bartesaghi *et al.*, *PNAS*, 2015.
Scheres, *eLife*, 2014.
Fischer *et al.*, *Nature*, 2015.
Khatter *et al.*, *Nature*, 2015.
Greber *et al.*, Science, 2015.
Bartesaghi *et al.*, *Science*, 2015.
Grant *et al.*, *eLife*, 2015.
…

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Atomic interpretation of cryo-EM maps

2 levels:

- global positioning of crystal/NMR structures, protein domains etc.

- (*ab initio*) atomic modelling

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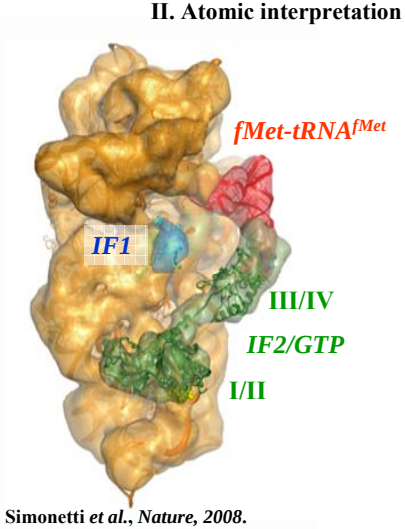
II. Atomic interpretation of cryo-EM maps



Simonetti *et al.*, *Nature, 2008*.

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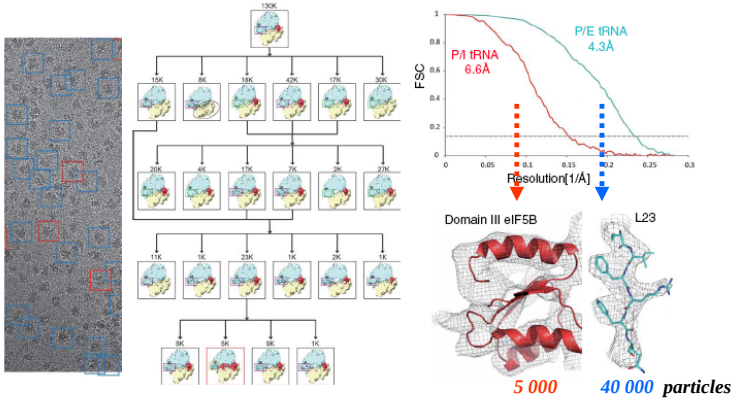
II. Atomic interpretation of cryo-EM maps



Strong heterogeneity of a reconstituted eukaryotic translation initiation (eIF5B) complex:

sorting → 5143 particles, representing 3% of the population in the sample, 6.6 Å reconstruction.

Fernández *et al.*, Science 2013; V. Ramakrishnan & S. Scheres.

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Fitting of crystal structures into cryo-EM maps and atomic model building

Fitting procedures:

1) global search

2) refinement

- manual fitting (e.g. O, Coot, Pymol, Chimera…)
- real space fitting
- reciprocal space fitting

At ~8-20 Å resolution:

- fit complete structures, protein or RNA domains, factors; usually backbone is enough.

Rigid body or flexible fitting (e.g. Situs, MDFF, Flex-EM, iMODfit, …)

- use full maps or difference maps

At ~3-5 Å resolution:

- atomic model building: start with poly-Ala model, check register (position of Cα atom), check secondary structure elements (e.g. direction of α-helices), refine with crystallography programs (CNS, Buster, Phenix, CCP4,…), add side-chains if clearly visible,

use information from multi-sequence alignments; check geometry with Ramachandran plot

In general: be careful with local minima and over-fitting/over-interpretation!

modelling

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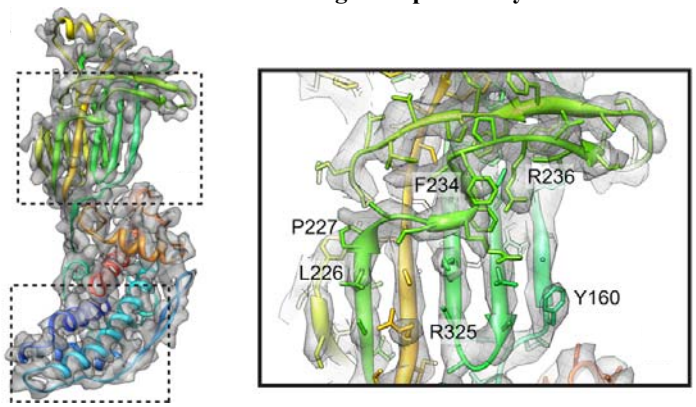
Atomic model building examples in cryo-EM



Rotavirus VP6 cryo-EM structure; 3.8 Å resolution; α-helices, β-sheets, bulky side-chains;
Individual stands in the β-sheet region are separated, loops connecting the strands are defined.

Near-atomic-resolution cryo-EM for molecular virology.

Hryc CF, Chen DH, Chiu W. Curr Opin Virol. 2011.

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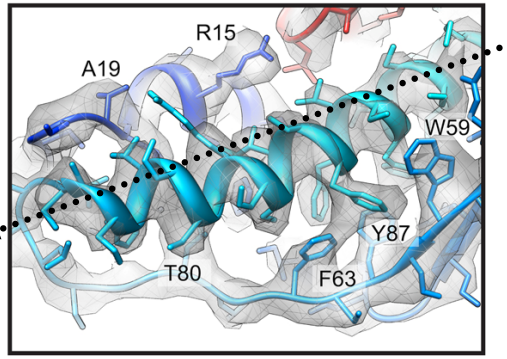
Atomic model building examples in cryo-EM

side-chains point to the N-ter



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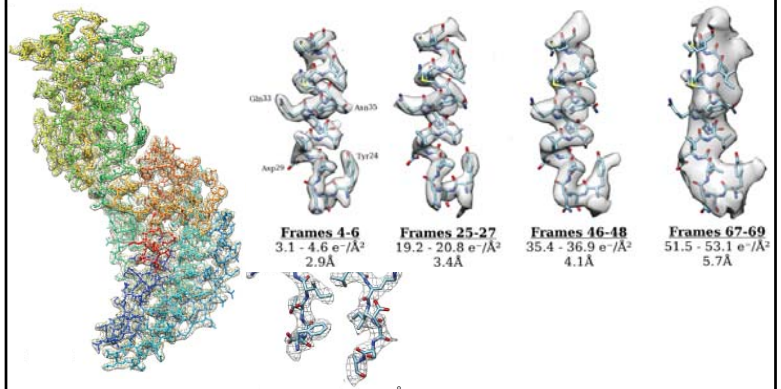
Atomic model building examples in cryo-EM



Rotavirus VP6 cryo-EM structure; 2.6 Å resolution; side-chains are defined.

optimize exposure dose to select movie frames

Grant, T., Grigorieff, N., *eLife*, 2015.

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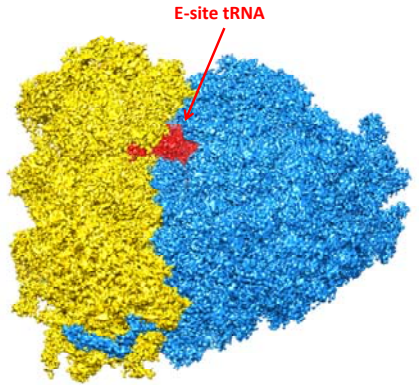
Human 80S structure

Titan Krios
Cs corrector
CMOS Falcon II

High Tension: 300kV
Defocus: from -0.6 to –1.4 (4.5) µm
Magnification: 59k
Pixel Size: 1.08Å
Quantifoil Grids
R2/2
Conc.: 0.5mg/ml
Final no. of particles: 24,000
Movie processing (3 frames only)



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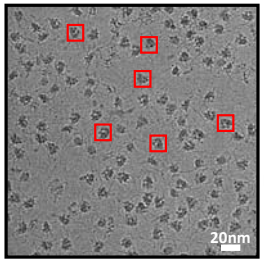
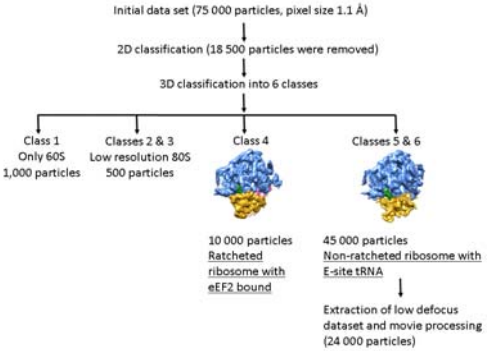
Atomic structure of the human ribosome

Structure determination at < 3 Å resolution by single particle cryo-EM





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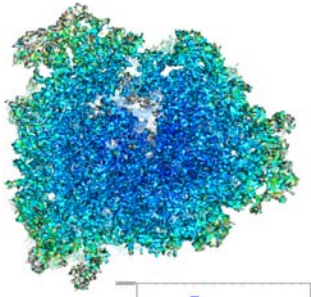
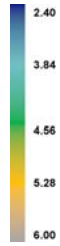
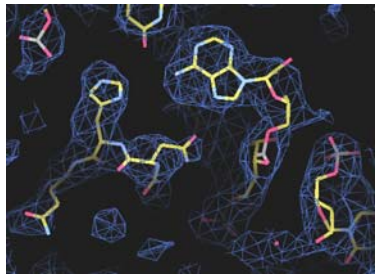
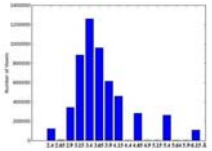






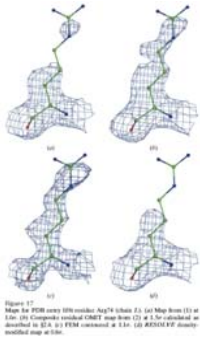


IGBMC

Usage of feature enhanced map for cryo-EM

FEM calculation protocol

1. OMIT map filter (section 2.4)
   - Compute composite residual OMIT map: M_{cromit}
   - Scale M_{cromit} by rms deviation (σ)
   - Compute filter: M_{filter}=0 if M_{cromit}<1σ else M_{filter}=1
2. Initialize collector of integer maps, IMC (section 2.5)
3. For j in j=1,16:
   a. Map randomization and averaging (sections 2.3, 2.5)
      For i, i=1,10:
      - Compute 100 map coefficients (1,3) and average them: $MC_{average}$
      - Randomly remove 5% of terms from $MC_{average}$: $\overline{MC}_{average}$
      - Compute Fourier map M_i from $\overline{MC}_{average}$
      - Scale M_i by rms deviation
      - Truncate low values: set M_i =0 if M_i <0.5σ
      - Eliminate regions in M_i with small volume (section 2.3)
   b. Sharpen $M_i \rightarrow M_{sharp}$ (section 2.6)
   c. Histogram equalize $M_{sharp} \rightarrow M_{HE}$
   d. Filter M_{sharp} by OMIT map: $M_{filtered} = M_{HE} * M_{filter}$
   e. Add $M_{filtered}$ to IMC (section 2.5)
4. Compute median map M_m from 16 maps in IMC (section 2.5), which is resulting Feature Enhanced Map, $M_{FEM} = M_m$



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FEM: feature-enhanced map

Pavel V. Afonine,[a]* Nigel W. Moriarty,[a] Marat Mustyakimov,[b] Oleg V. Sobolev,[a] Thomas C. Terwilliger,[c] Dusan Turk,[d,e] Alexandre Urzhumtsev[f,g] and Paul D. Adams[a,h]

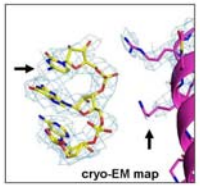
Afonine *et al., Acta Cryst D, 2015.*

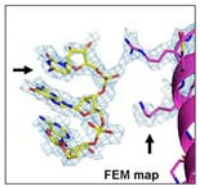
IGBMC

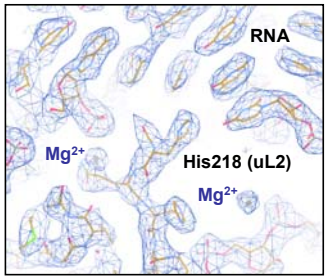
Atomic structure of the human ribosome

Atomic model building:

combining cryo-EM and X-ray crystallography refinement procedures





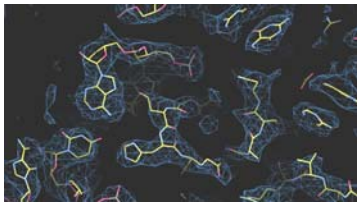


Fourier coefficients from cryo-EM map
and phases from refined atomic model
(= crystal structure refinement, 2mFo-DFc, σ-weighted)

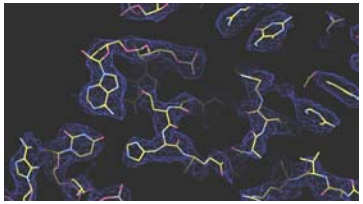
IGBMC

Atomic structure of the human ribosome

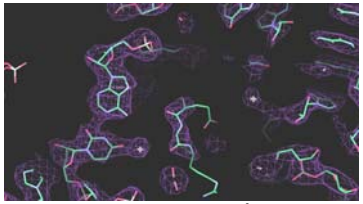
Comparison of maps determined by cryo-EM and X-ray crystallography



human 80S
cryo-EM map



human 80S, Phenix map, 2mFo-DFc



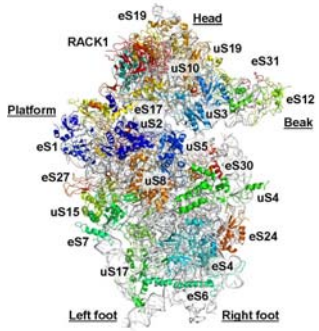
70S crystal structure at 2.8 Å resolution
(Selmer *et al.*, *Science* 2006)

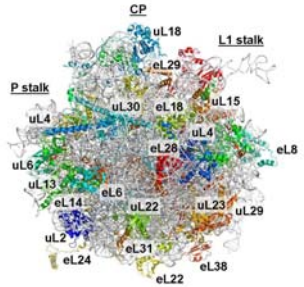
ICBMC

Atomic structure of the human ribosome

Overall structure: 80 proteins and 4 rRNA's (28S, 5, 5.8 and 18S),

~220 000 atoms (5866 nucleotides, ~11590 amino acids)





Ramachandran plot: preferred regions 67.9%; allowed regions 22.5%; 9.5% outliers, r.m.s. bond deviations of 0.008Å and angle deviations of 1.24º

ICBMC

Latest cryo-EM structures in the 3 Å range or better:

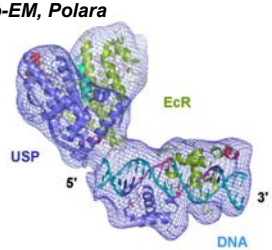
2015 (likely incomplete selection):
Fischer *et al.*, 2015
Amunts *et al.*, 2015
Greber *et al.*, 2015
Khatter *et al.*, 2015
Bartesaghi *et al.*, 2015
Grant *et al.*, 2015
…

ICBMC

V. Instrumentation & technical highlights towards multi-scale integration

small, challenging complexes

cryo-EM, Polara



100 kDa
nuclear receptor complex
Maletta *et al.*, *Nature Comm.*, *2014*.

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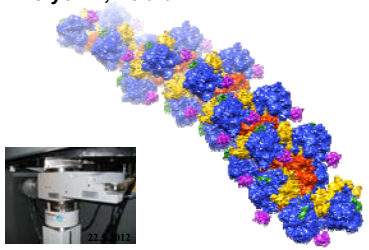
V. Instrumentation & technical highlights towards multi-scale integration

large, challenging complexes

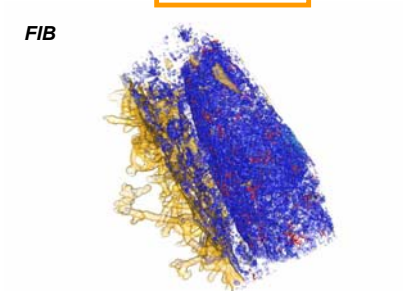
feasibility of FIB

cryo-ET, Polara



100 MDa polyribosome complex
Myasnikov *et al.*, *Nature Comm.*, 2014.

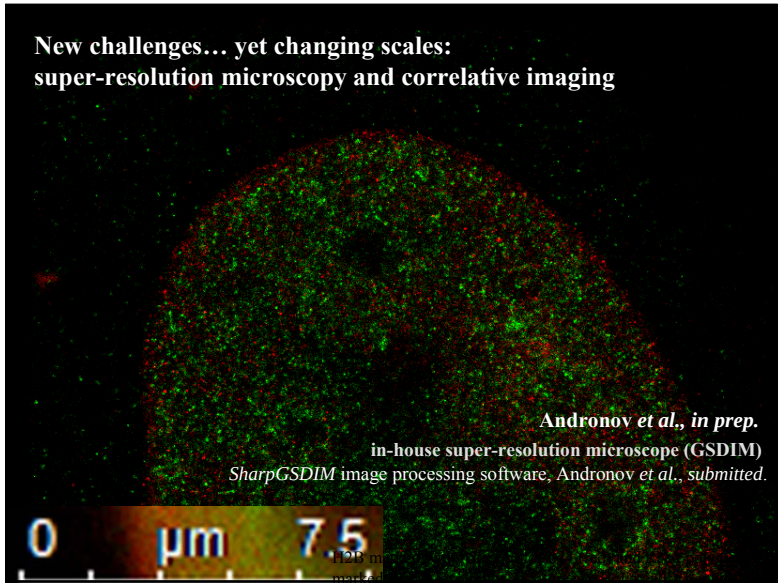
FIB



12 nm 3D reconstruction of the nucleus
Orlov *et al.*, *Sci. Rep., 2015.*

IGBMC

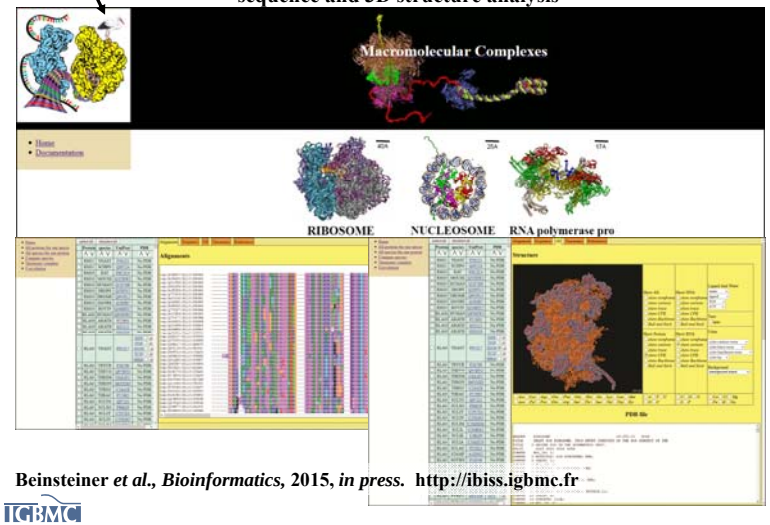
New challenges… yet changing scales: super-resolution microscopy and correlative imaging



Andronov *et al., in prep.*
in-house super-resolution microscope (GSDIM)
SharpGSDIM image processing software, Andronov *et al., submitted.*

IBiSS, a versatile and interactive tool for integrated sequence and 3D structure analysis

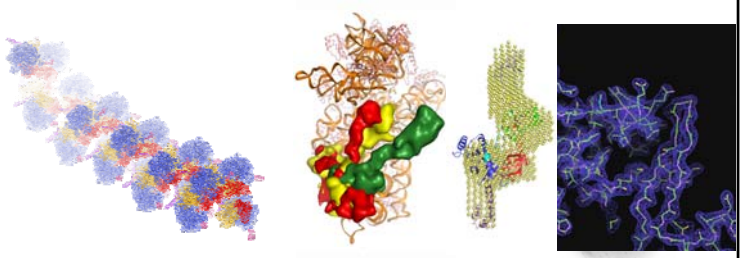
"Alsatian Ibis"



Beinsteiner *et al., Bioinformatics,* 2015, *in press.* http://ibiss.igbmc.fr

IGBMC

Multi-resolution integration of gene expression regulation



Molecular assemblies ← Single particles → Crystal structure

Myasnikov *et al.*, *Ultramicroscopy* 2013
Myasnikov *et al.*, *submitted.*
Simonetti *et al.*, *Nature* 2008.
Simonetti *et al.*, *Acta Cryst. 2013*
Simonetti *et al.*, *PNAS 2013.*

fluorescence microscopy ***cryo electron tomography*** ***cryo-EM*** ***SAXS*** ***crystallography***

1cm 1mm 100 µm 10 µm 1µm 100nm 10nm 1nm 0.1nm (1Å)

IGBMC

Integrative structural biology of (large) macromolecular complexes

synergy-core for data integration:

- **electron microscopy**
- **crystallography**
- **NMR**
- **SAXS**
- **bio-informatics**
- **biochemistry (purification, functional tests)**

structure ⇄ **function**

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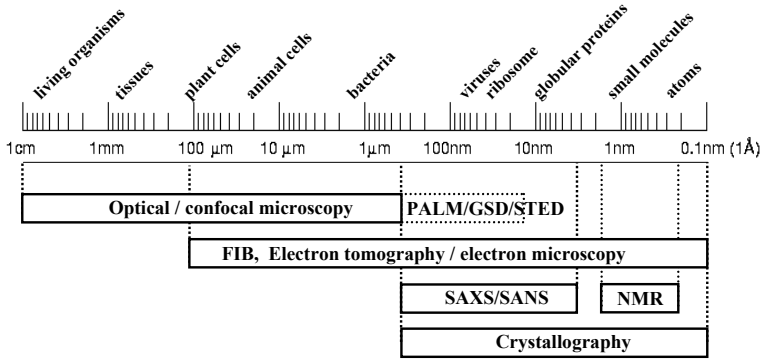
Integrated Structural Biology



Challenging objects require multi-scale multi-resolution integration

→ integrative role of electron microscopy

IGBMC

Ménétret *et al.*, RNA structure and folding (book), de Gruyter, 2013.