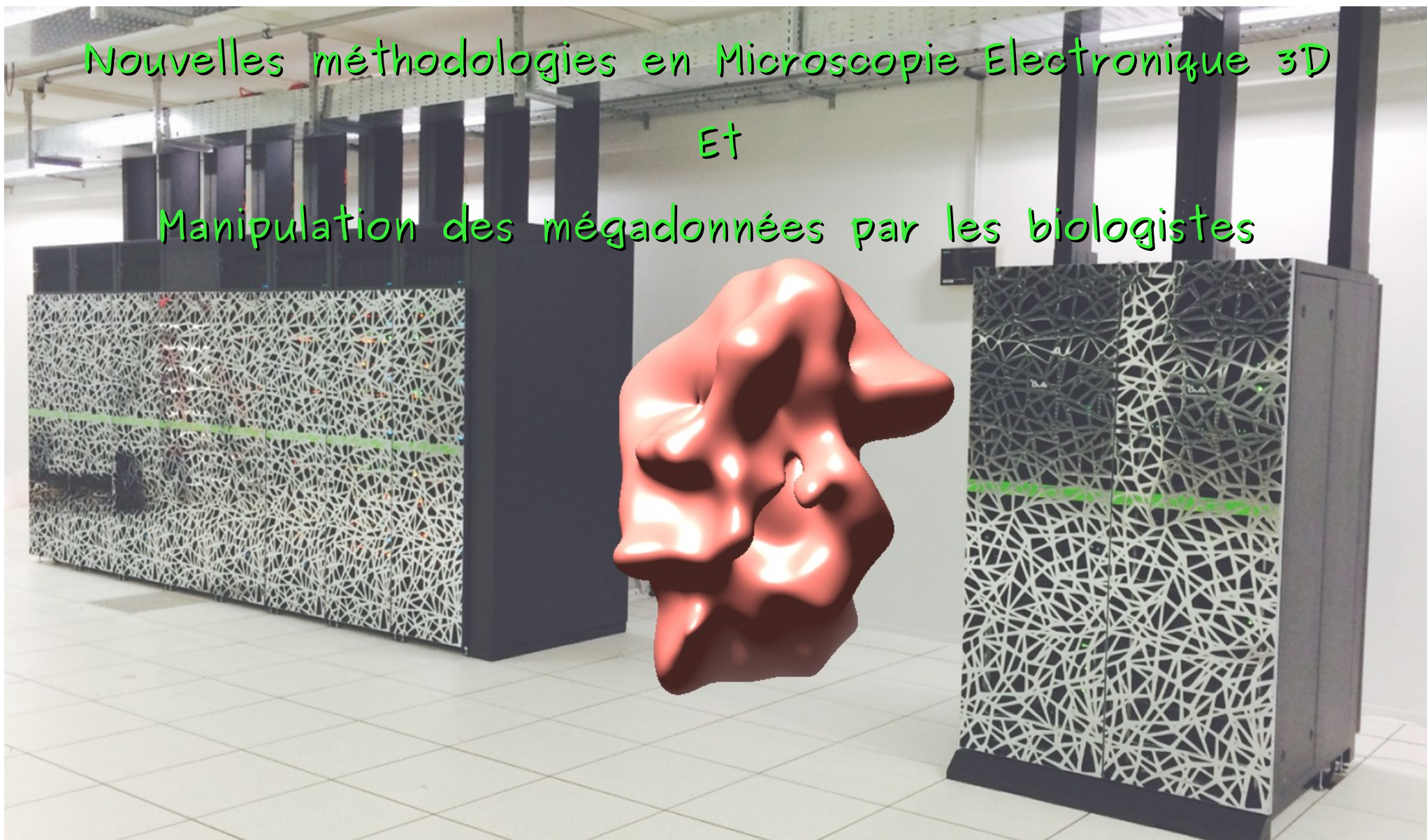
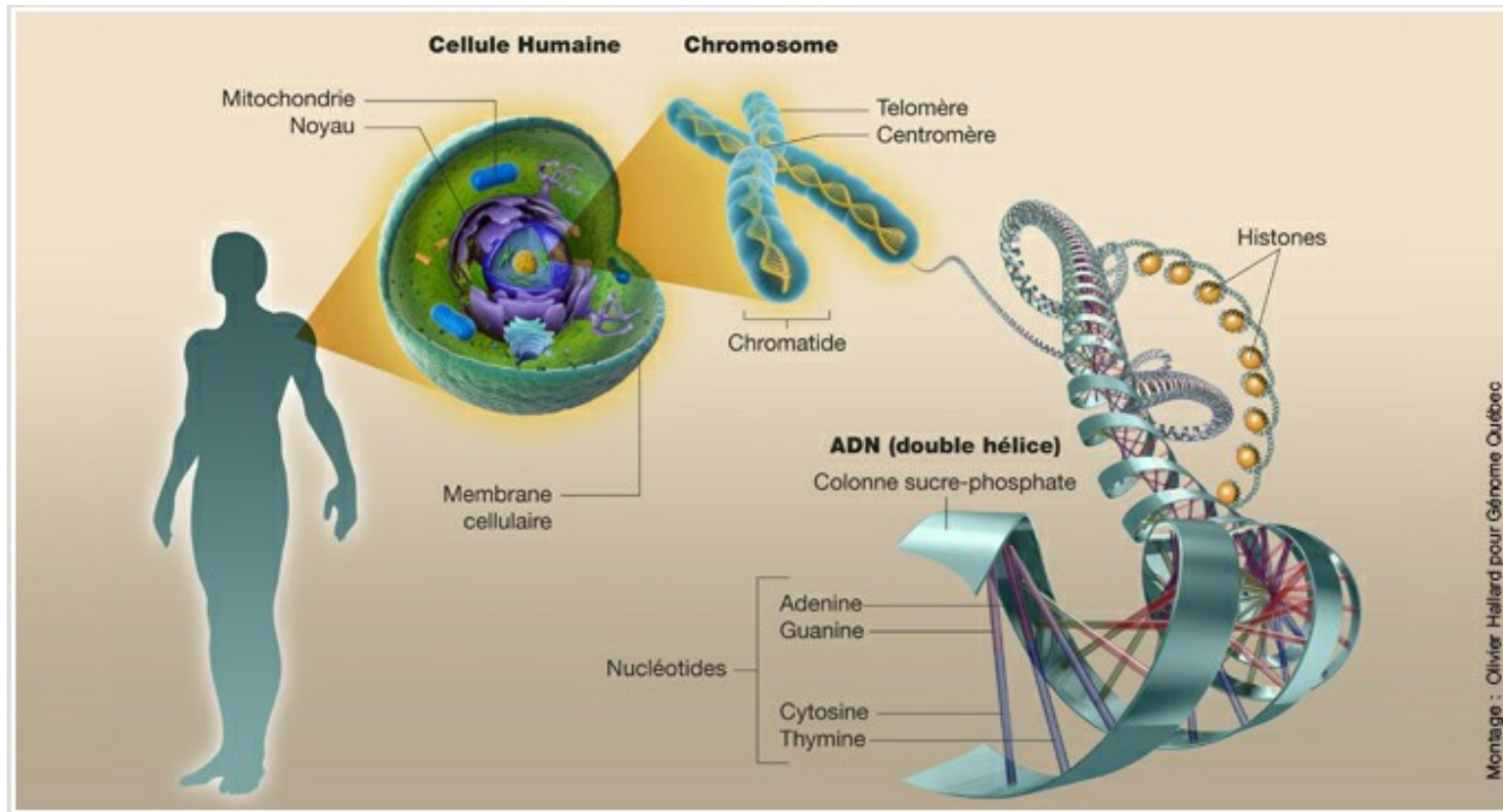


Nouvelles méthodologies en Microscopie Electronique 3D Et

Manipulation des mégadonnées par les biologistes

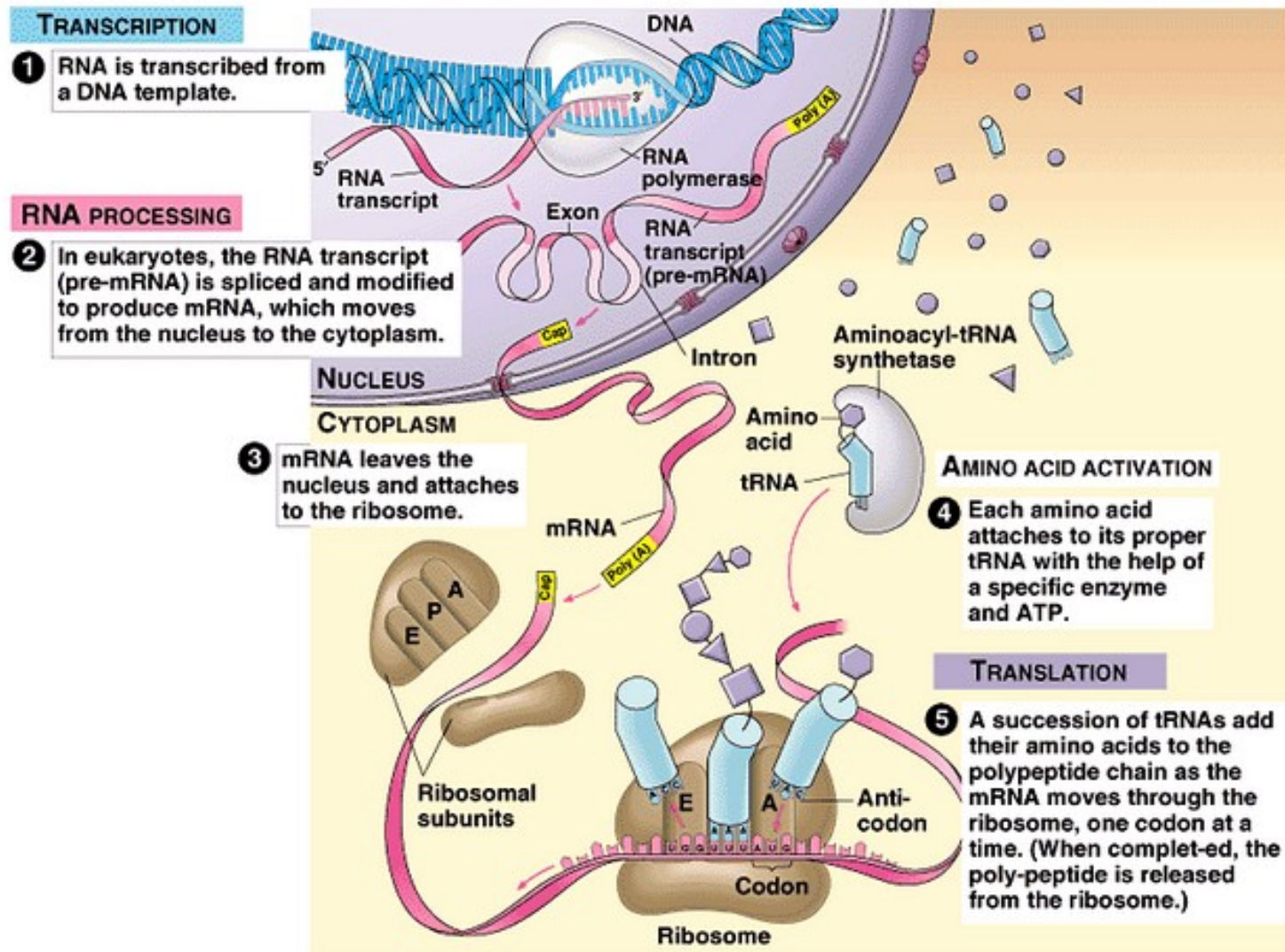


Le corps humain, la cellule, l'ADN



Le ribosome, maître d'oeuvre de la synthèse protéique

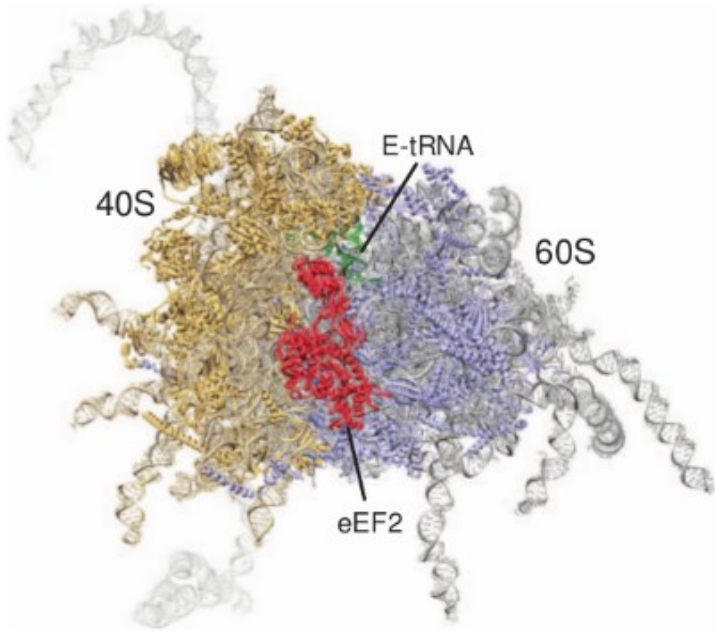
Traduit le code génétique en protéines



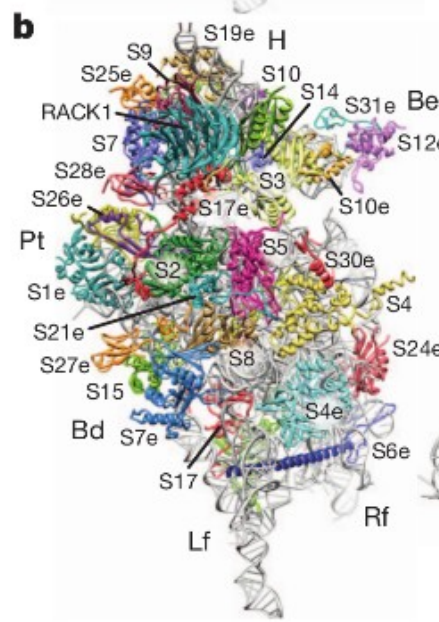
Structure du ribosome mature

Huge (3.2 to 4.2 MDa in eukaryotes) molecular machines responsible for protein synthesis

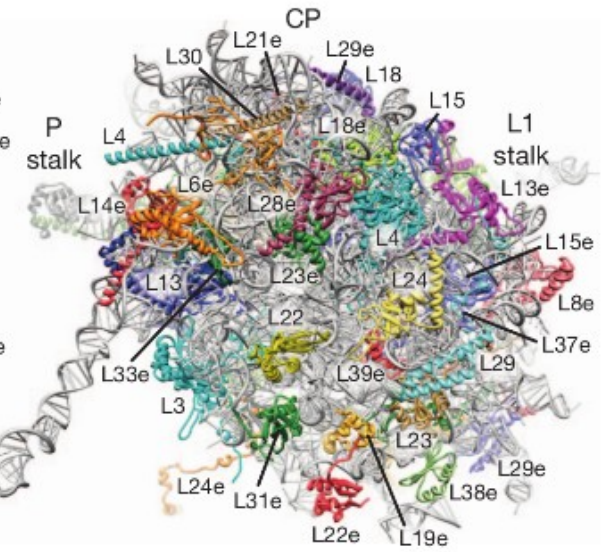
Human mature 80S ribosome, 6 Å resolution (3D EM + X-ray structure fitting), Anger et al., 2013



80S ribosome



40S ribosomal subunit

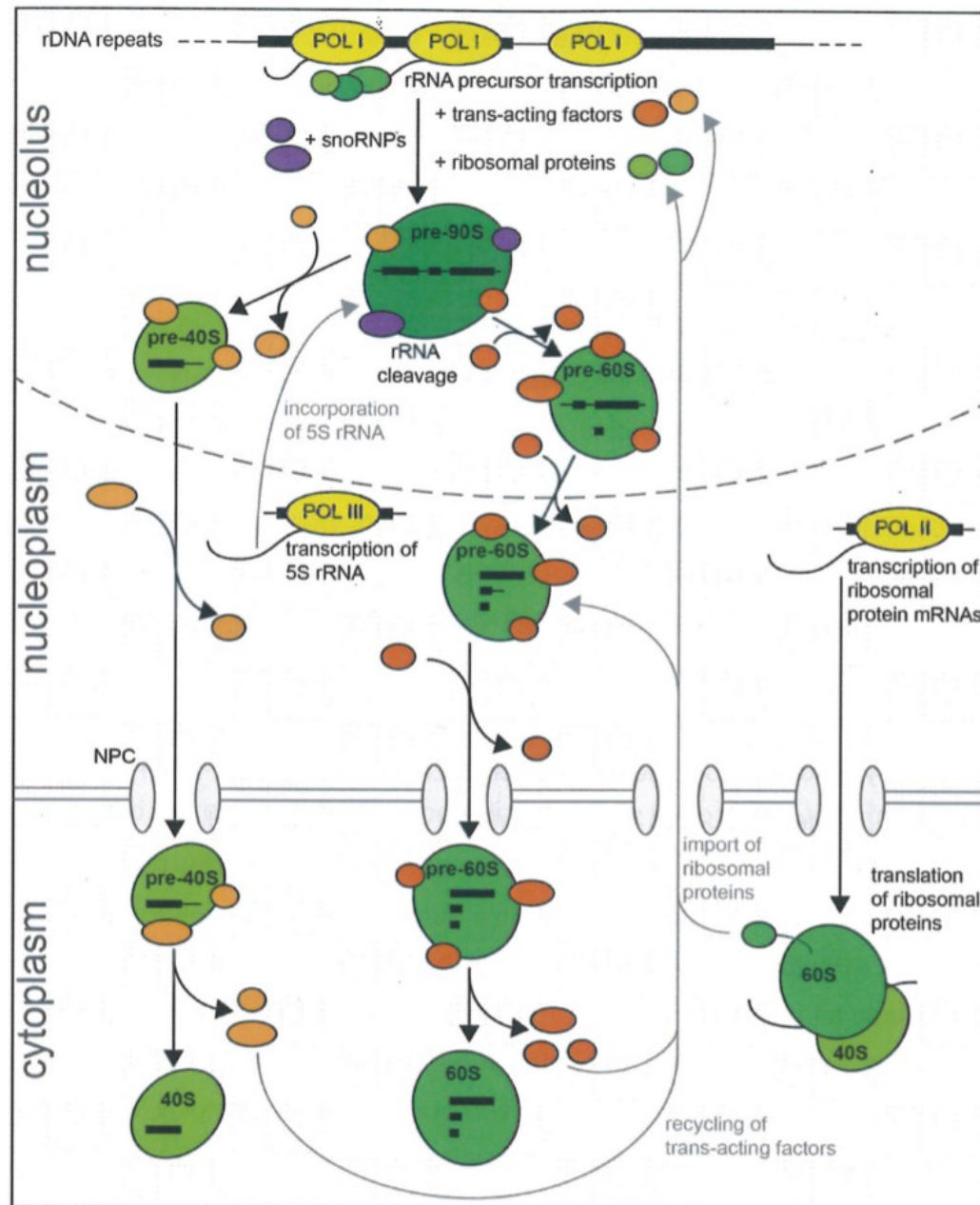


60S ribosomal subunit

Small Ribosomal subunit (40S) : 1 rRNA (18S) + 33 proteins RPS

Large Ribosomal subunit (60S) : 3 rRNA (5,8S, 25S/28S and 5S) + 46 proteins RPL

Eukaryotic ribosome assembly : a complicated process



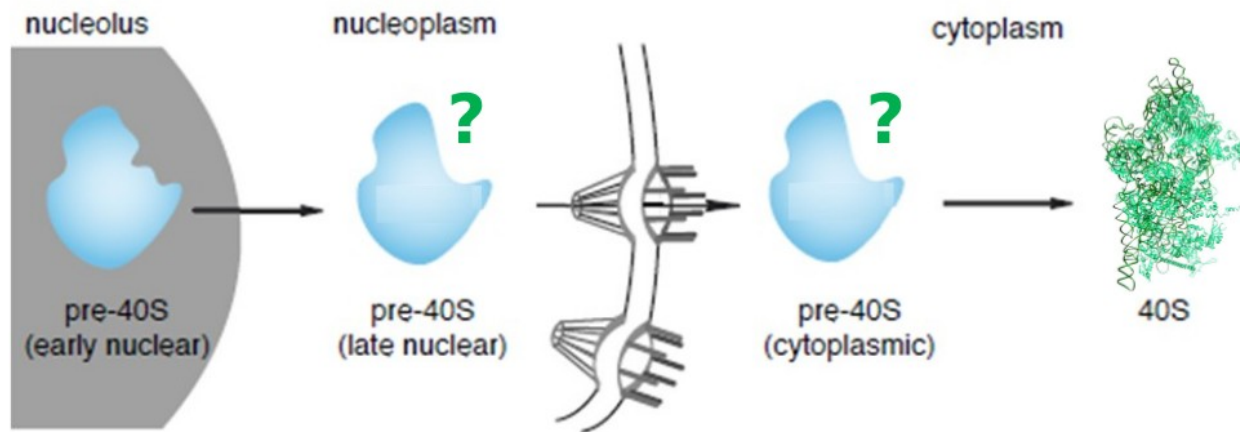
Ribosome synthesis in eukaryotes (Human)

RNA maturation steps well characterized

Maturation factors : exhaustive list ; precise role(s) starting to be deciphered

Increasing number of genetic diseases and cancers linked to ribosome maturation defects

No structure of Human pre-ribosome solved yet

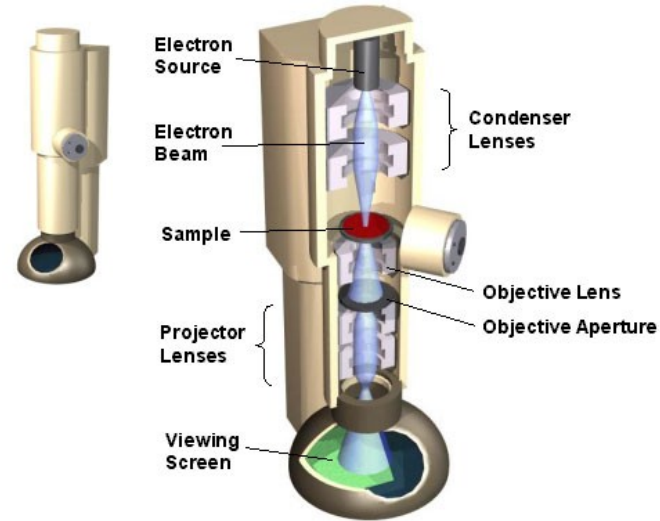


- What is the 3D structure of the cytoplasmic pre-40S ribosome ?
- How does it relate to the mature 40S subunit ?
- Where are maturation factors on this pre-40S structure ?
- Are the cytoplasmic pre-40S particles structures different in yeast and human ?

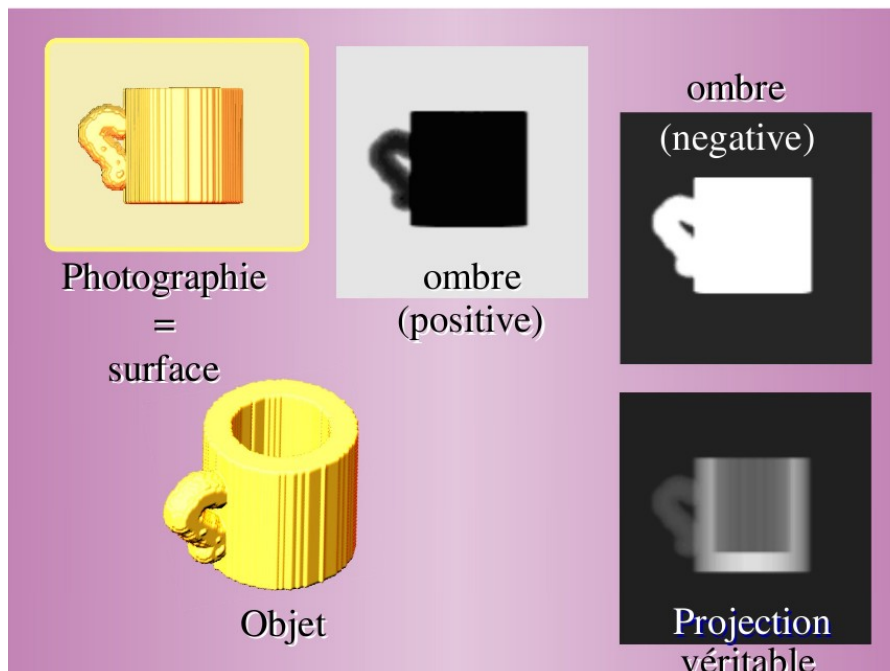
3D EM



Principles of 3D (T) EM

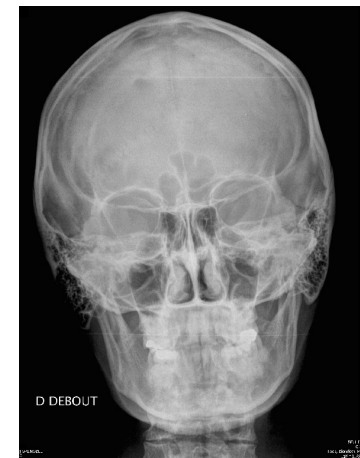


- Signal from electrons transmitted throughout the object
- Images = 2D projections of a 3D object



© Elena Orlova

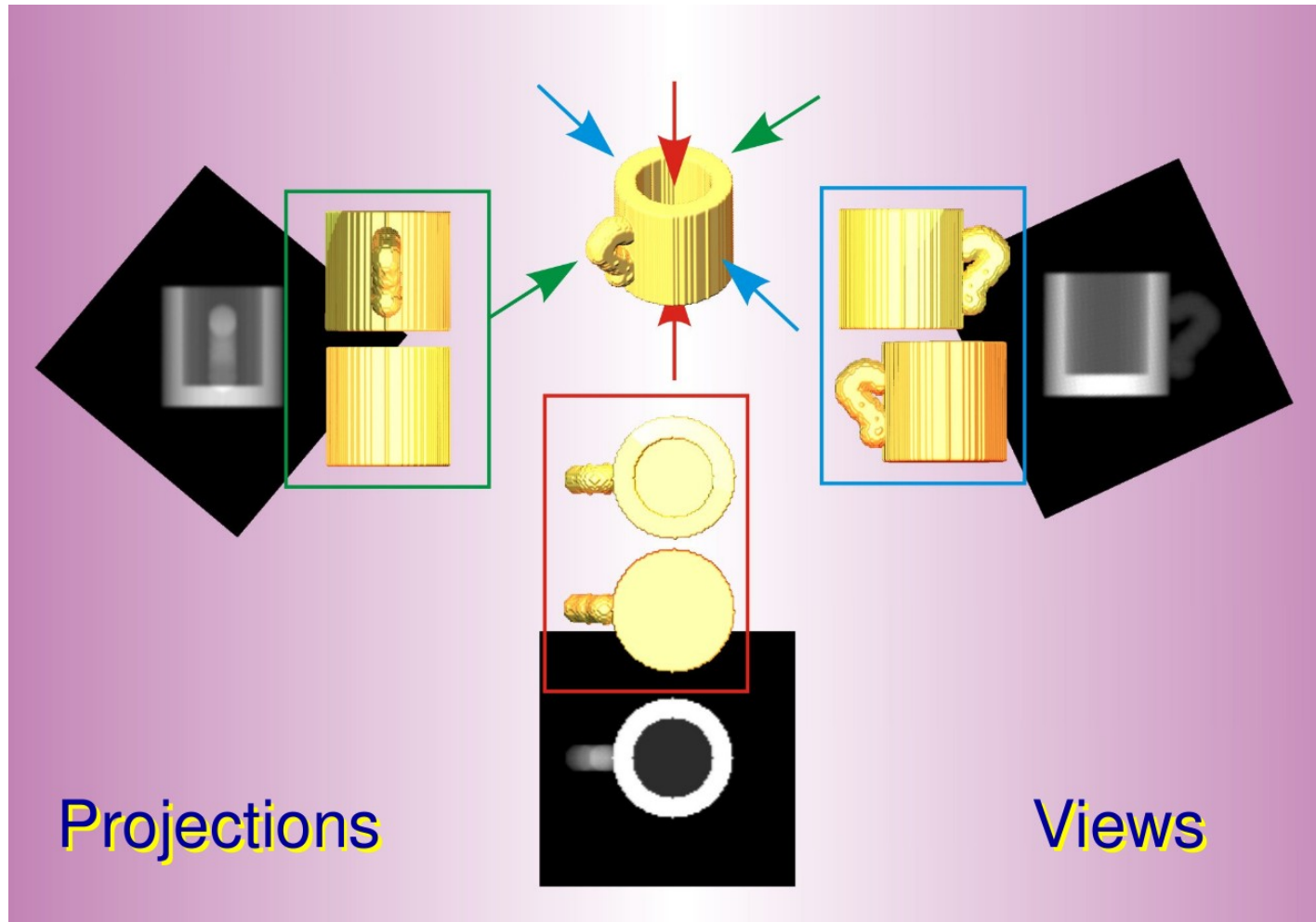
Another 2D projection :
X-Ray radiograph



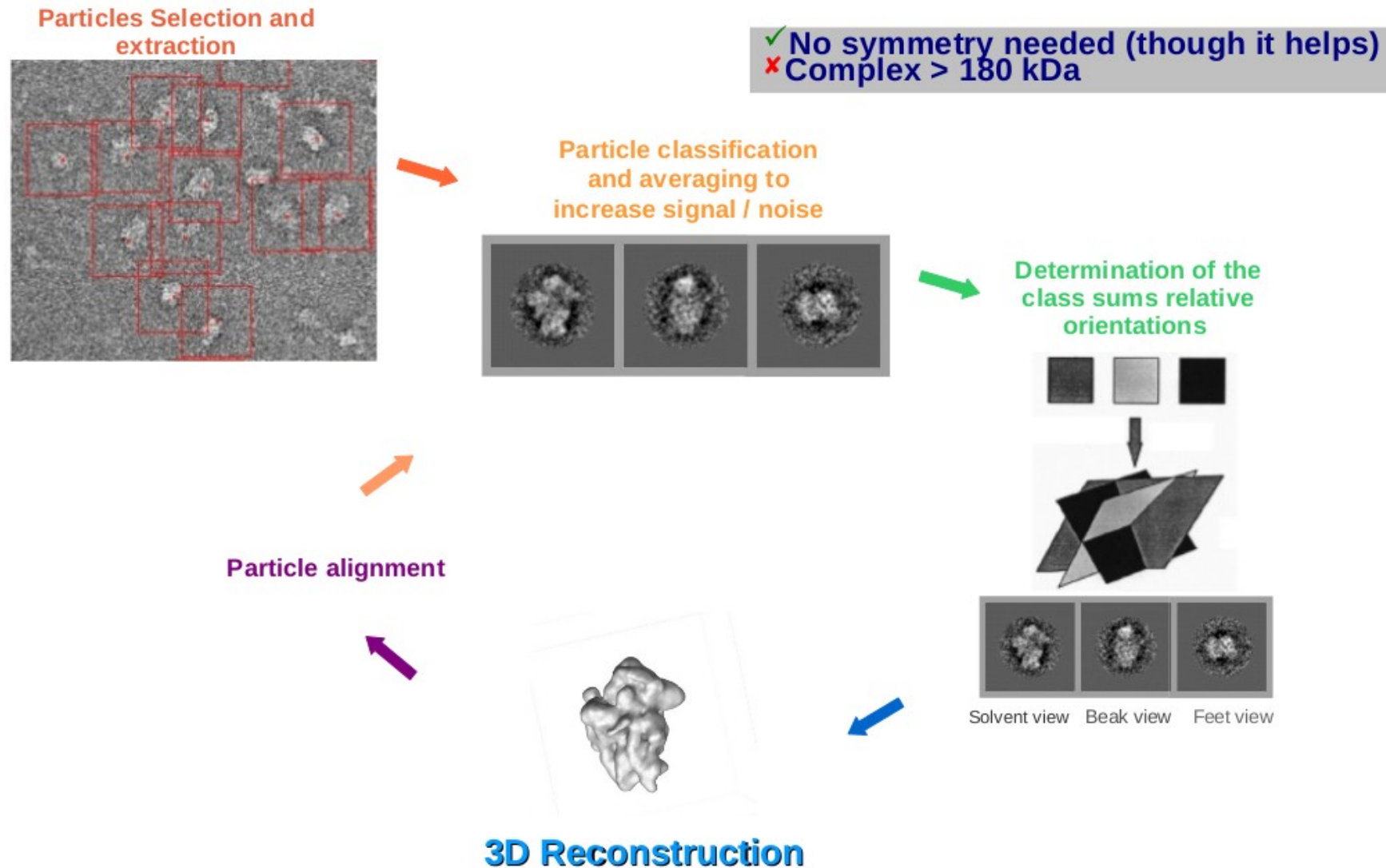
© F. Bing, CHU Grenoble

MET 3D comment faire?

- Images = projections 2D d'objets 3D
- En théorie, combinaison de 3 vues différentes => structure 3D

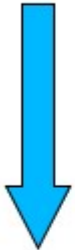


3D reconstruction using single particle analysis



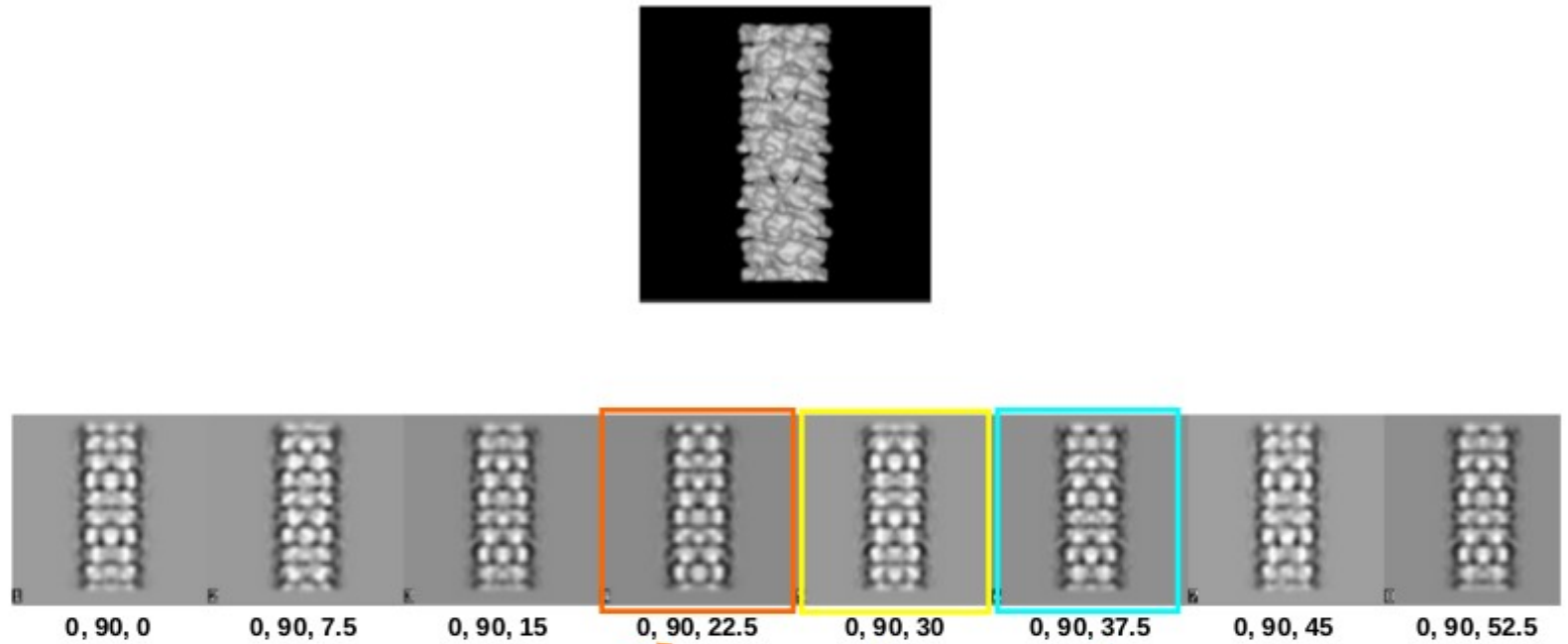
Détermination de l'orientation des images

Modèle 3D



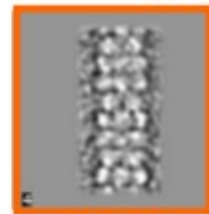
Projections
2D
du modèle

(Orientations connues)

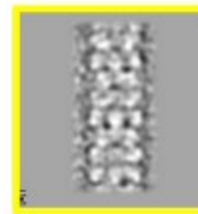


Projection
matching

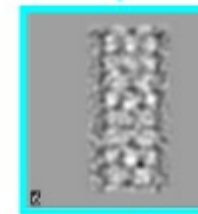
Images



0, 90, 22.5



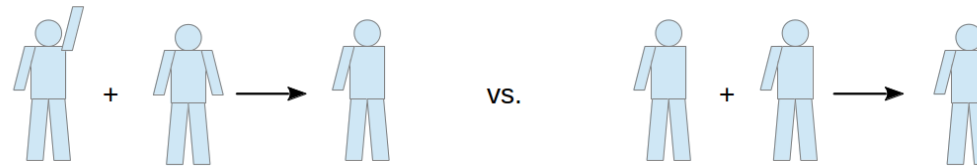
0, 90, 30



0, 90, 37.5

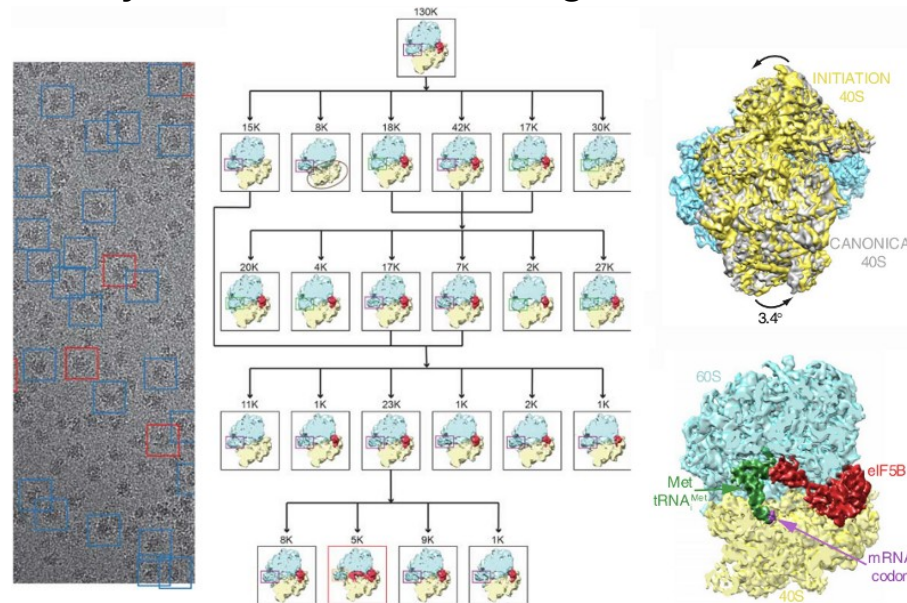
Nouvelles méthodes d'analyse d'images : The « resolution revolution » (1)

The trouble with (classical) image analysis methods based on averaging data...



Averaging all particles into a single 3D structure limits the resolution

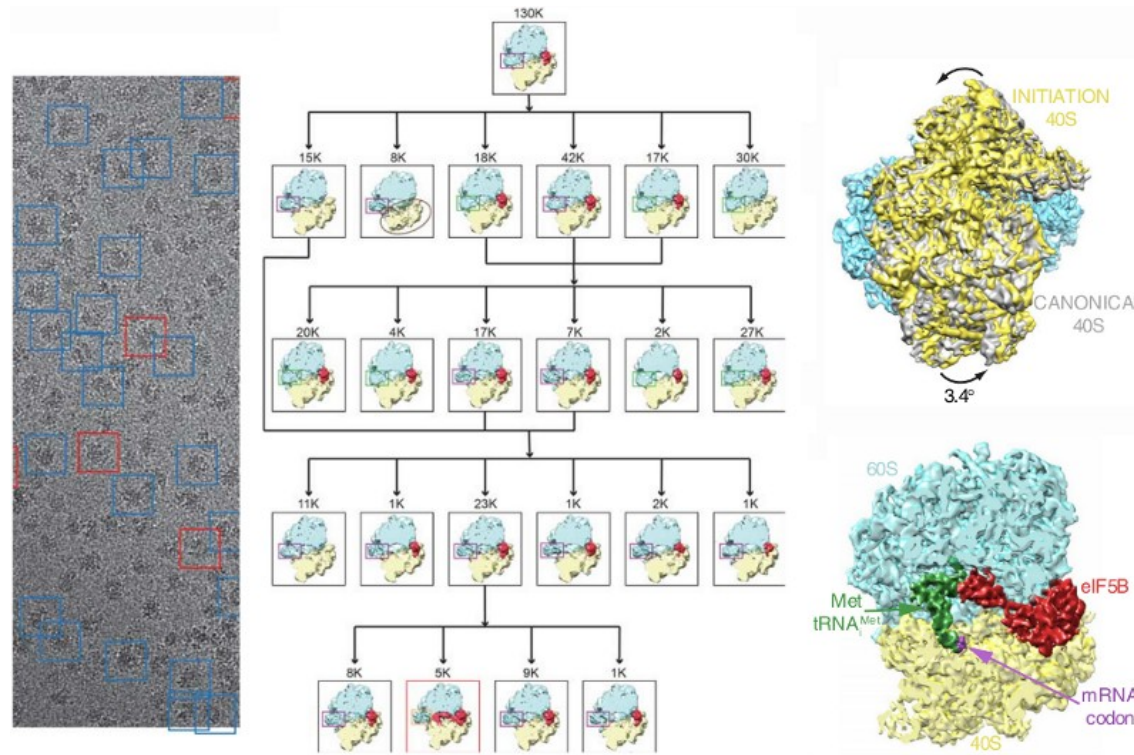
Improved image analysis methods : sorting out 3D structural heterogeneity



Structure of yeast translation initiation complex, 6.6Å resolution (Fernandez *et al.*, Science 2013)

RELION 1.3 (Sjors Scheres, 2012)

Statistique Bayésienne



Projection matching + prise en compte du rapport signal / bruit de chacune des images

Calcul d'1 structure 3D consensus et / ou de n variants structuraux

« memory-intensive calculations »

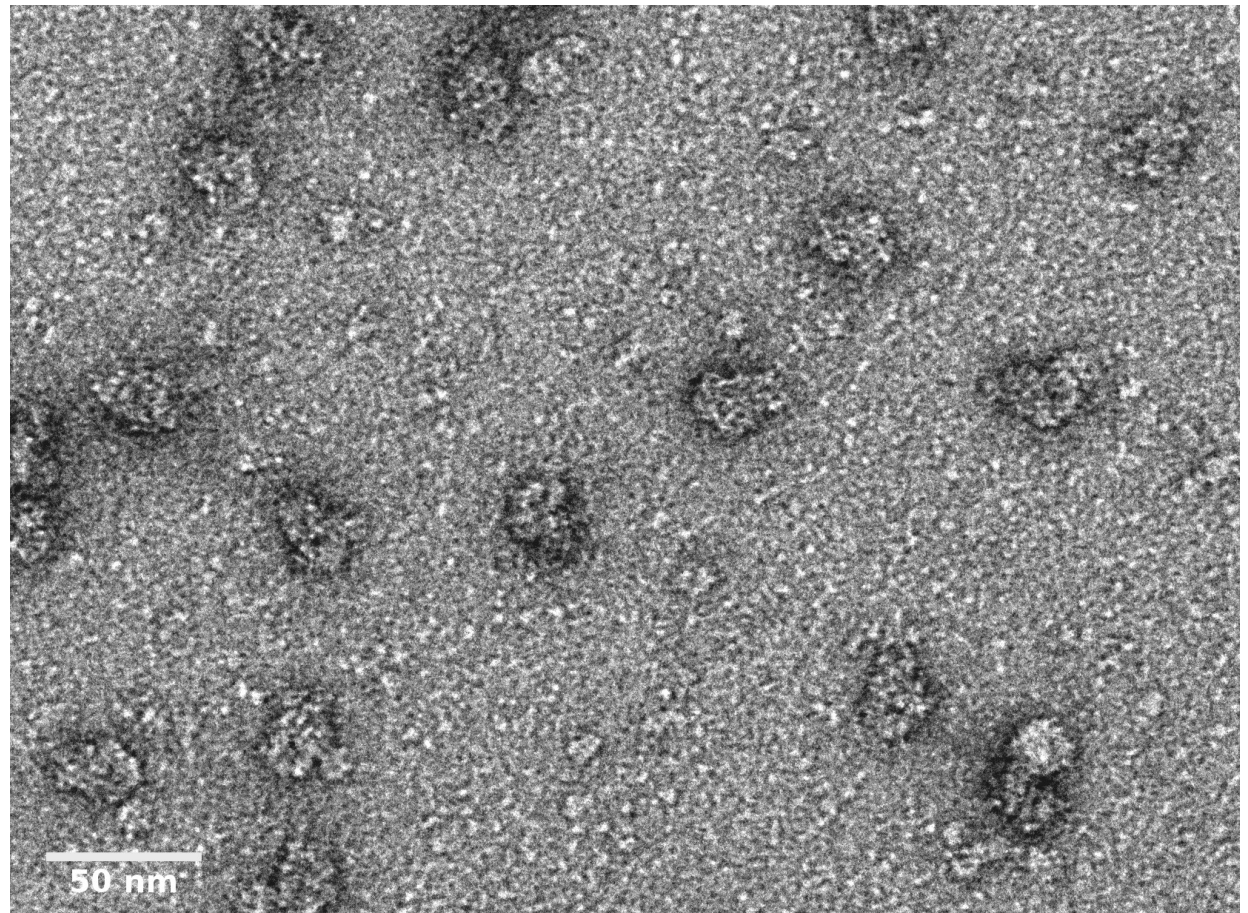
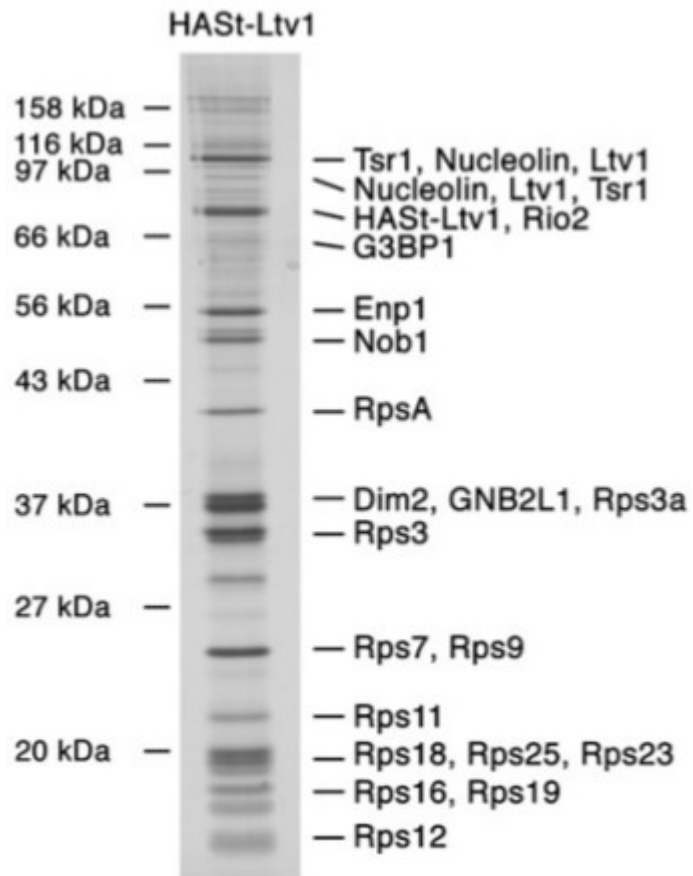
Parallélisation hybride :

calcul distribué sur plusieurs nœuds (mpirun/BULLxMPI) + hyperthreading (pthread)

Structural study of Human pre-40S ribosomal subunits

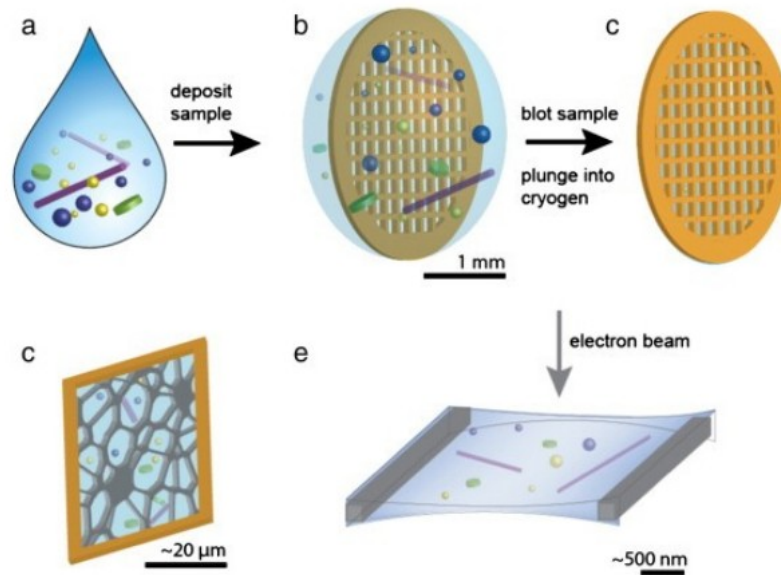
1. Purification of cytoplasmic pre-40S particles

(C. Montellese, Ulrike Kutay's group, ETH Zürich)



Human pre-40S particles suitable for a structural analysis by 3D EM

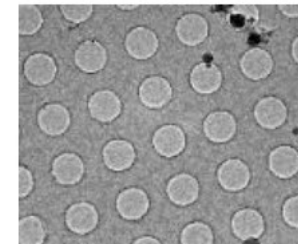
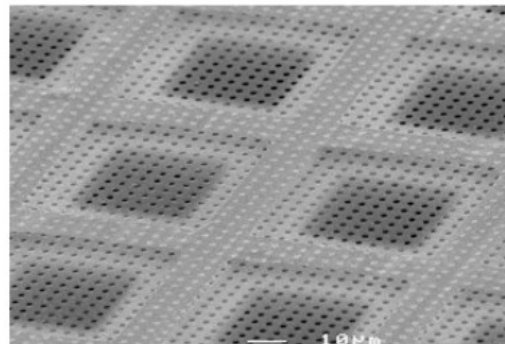
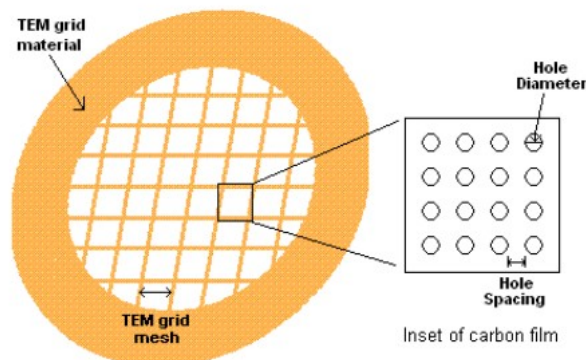
Cryo-EM general principles



Sample embedded into vitreous ice :
+ Quasi native state
- Very low contrast

Grids with circular, regular holes for automated acquisition

Quantifoil Cu 200 Mesh, 2/2

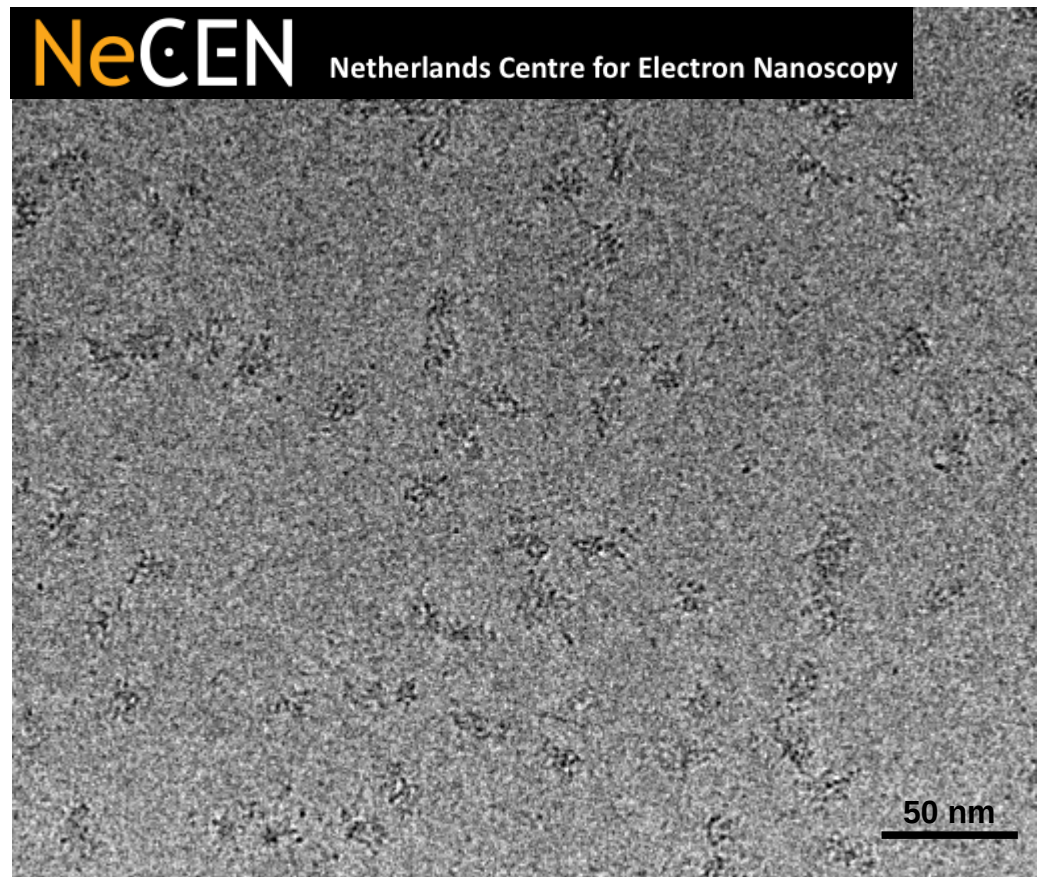
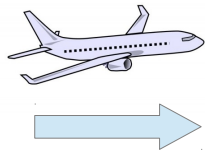
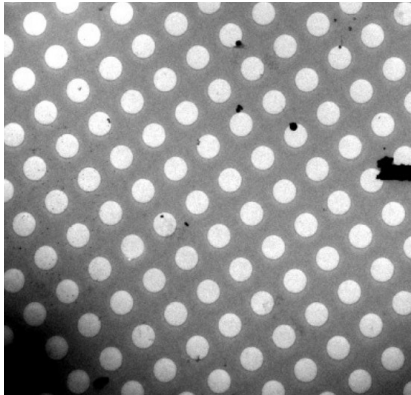


Structural study of Human pre-40S ribosomal subunits

cryo-TEM images acquisition on a last generation microscope



Cryo-EM grids
preparation and checking



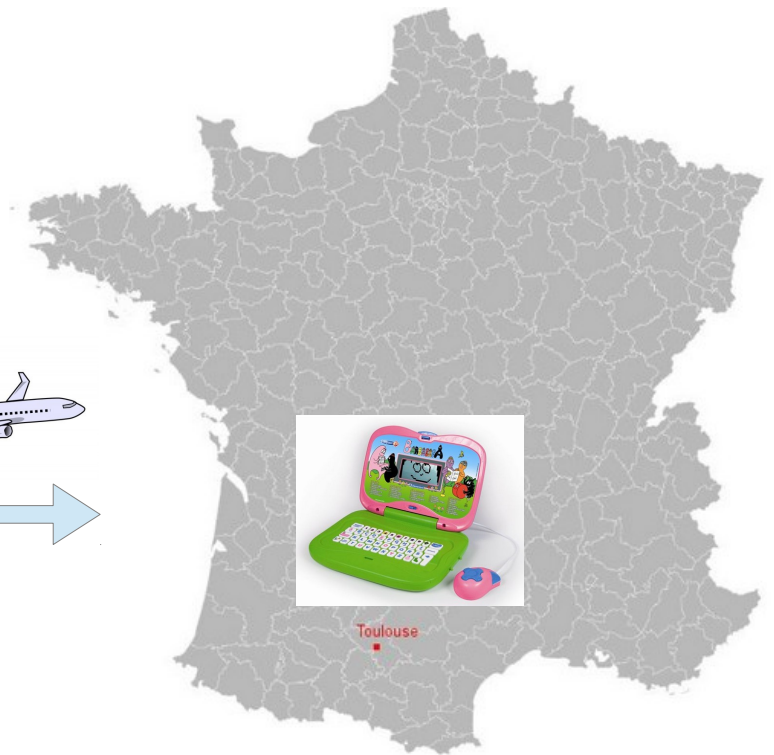
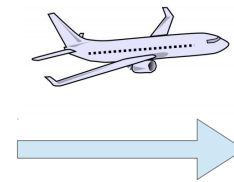
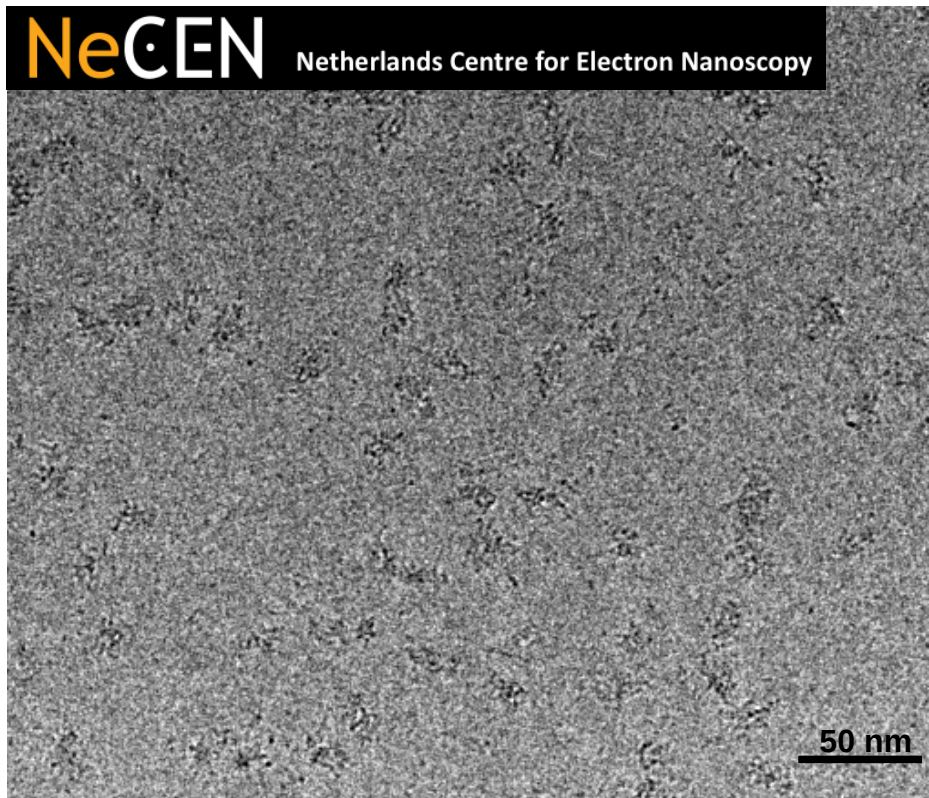
Acquisition des images de cryo-microscopie électronique

96h temps de faisceau

10 422 images

4096 x 4096 pixels ; 33 M / image + fichiers annexes

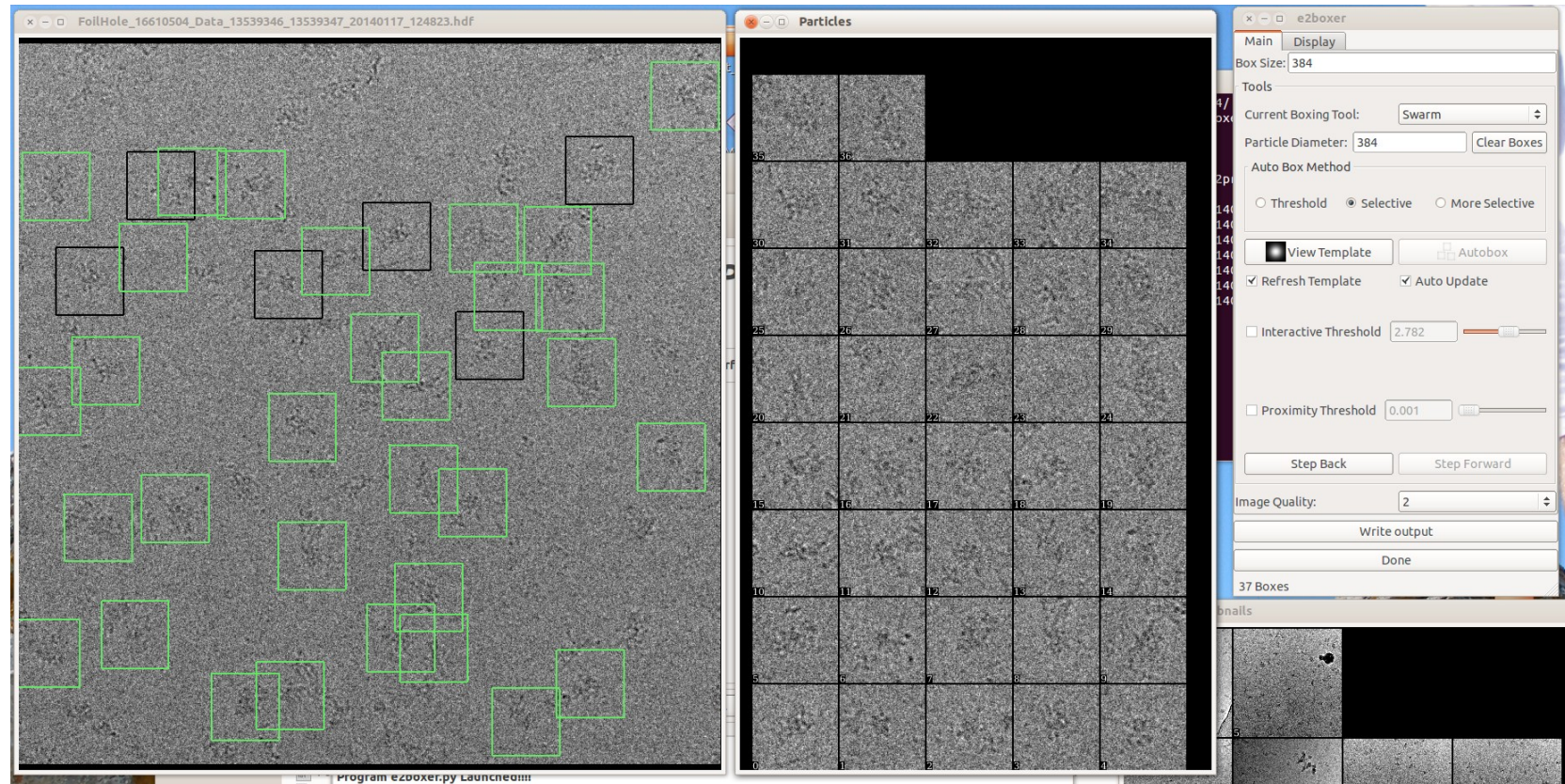
~ 0,4-0.5 T pour le dataset total



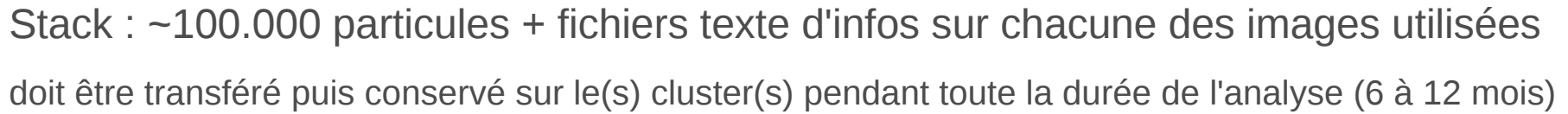
Transfert / archivage du dataset brut ???

Pre-processing

- Sélection des images :
 - (1) visuelle
 - (2) estimation de la ctf des images
- Picking & Extraction des particules (1 à 3 mois de travail!)



Dataset initial exploité (images sélectionnées) : 252 G



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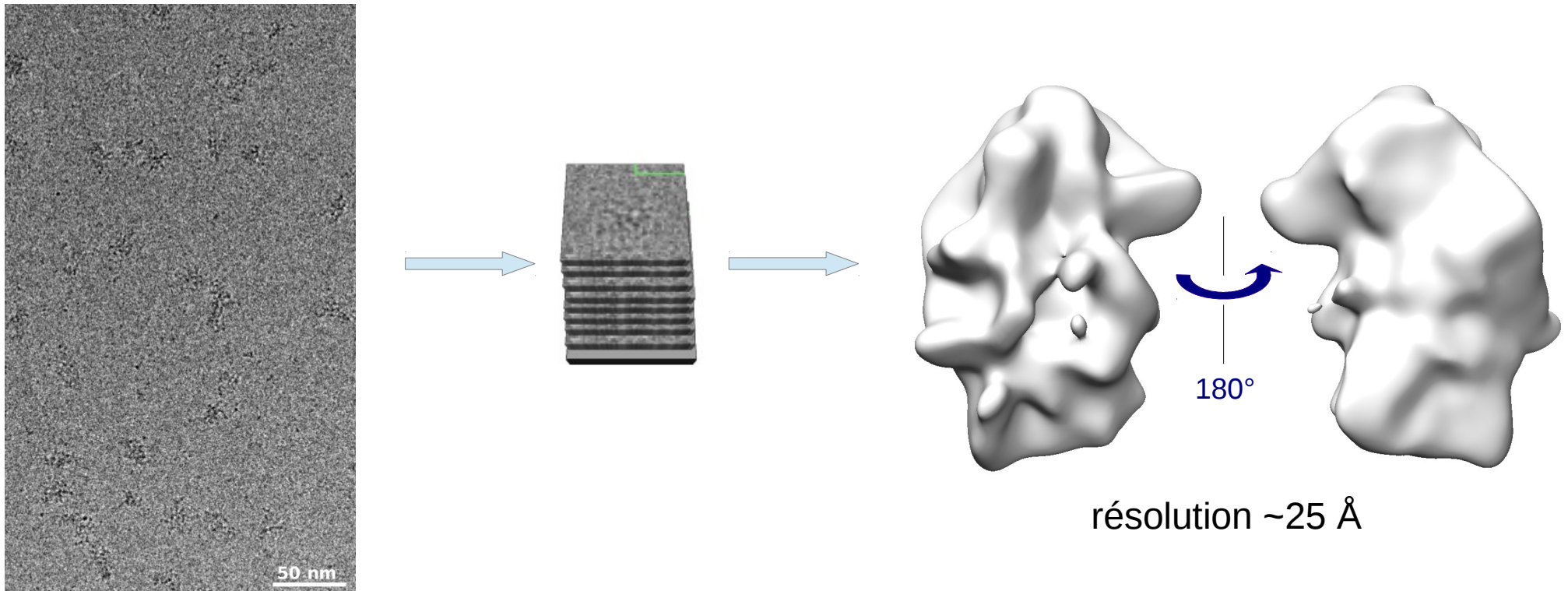
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Processing - Refine

Programme Relion 1.3



Obtention d'un modèle 3D consensus, à la plus haute résolution possible



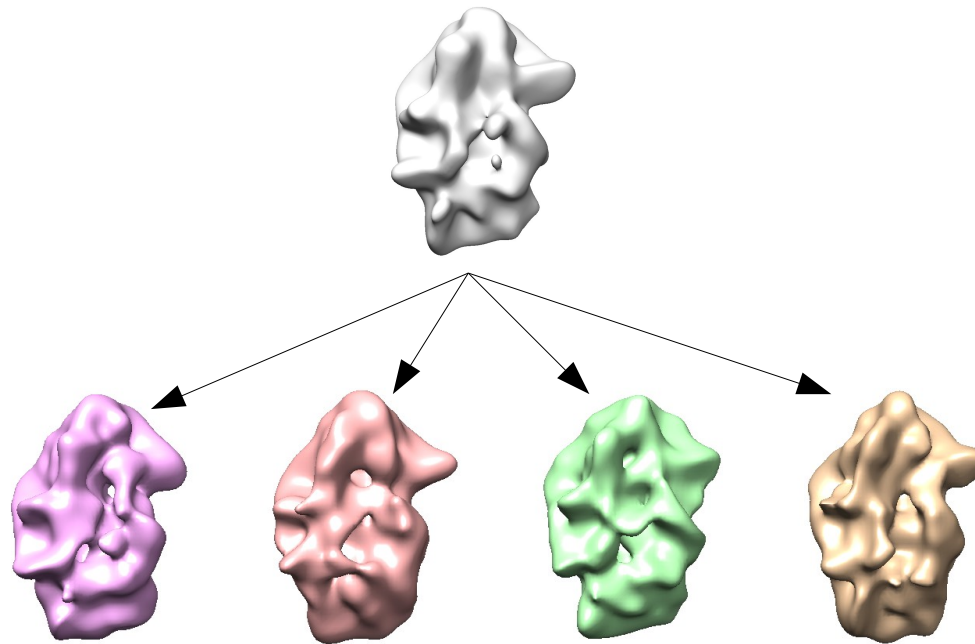
RAM requise (89 000 particules dans des boites de taille 384x384 pxl) : 15 Gb /noeud ;
12 noeuds (8 cœurs / 36 Gb /noeud),
1 mpi process / noeud, pthreads = 8 ;
Walltime = 96 h

AUGMENTE AVEC LA RESOLUTION ET LE Nb PARTICULES

Processing – Classification 3D

Programme Relion 1.3

Obtention de n variants structuraux, attribution de chacune des particules à l'un des variants



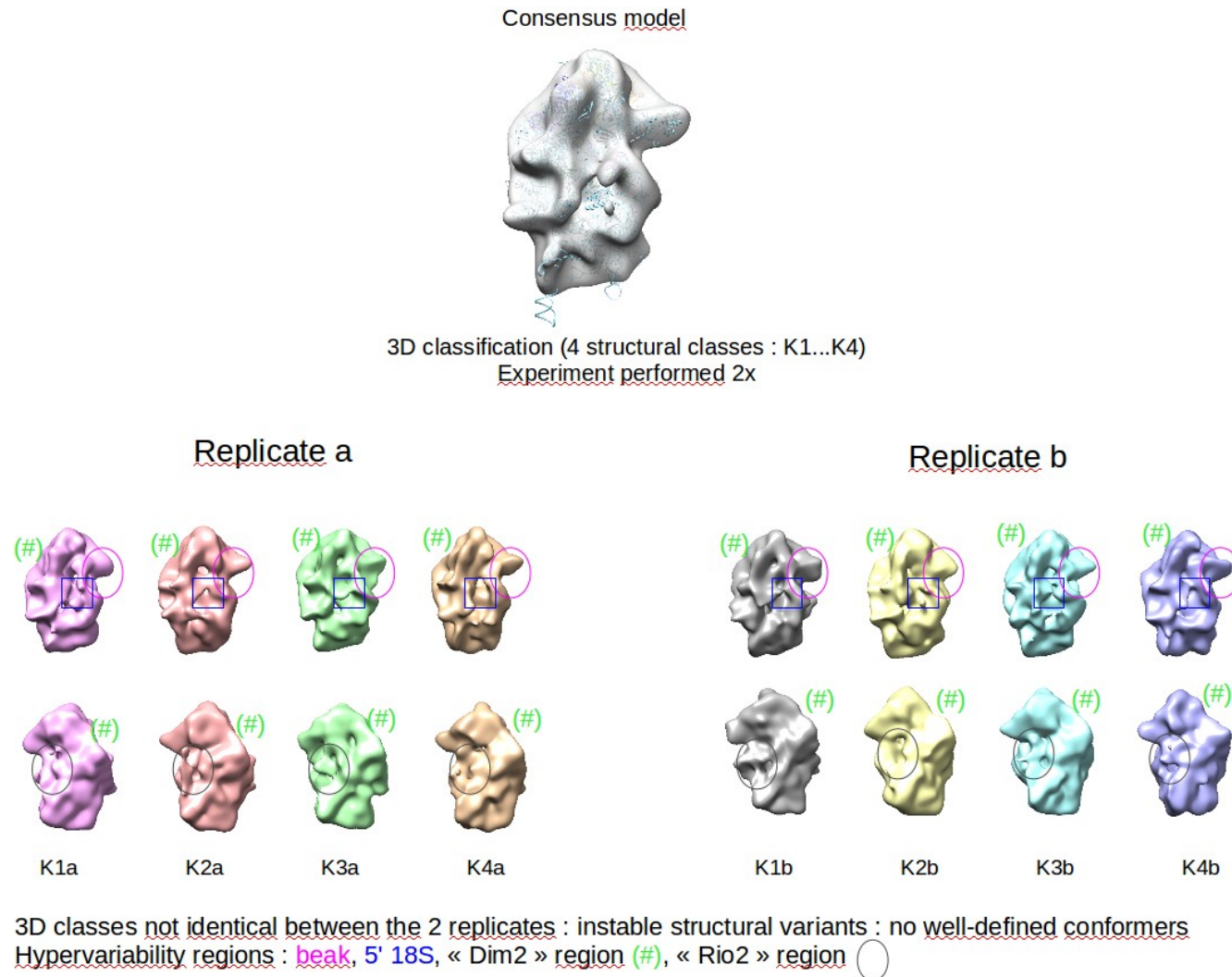
RAM requise (89 000 particules dans des boites de taille 384 x 384 pxl) : 4 Gb /noeud ;
10 nœuds (8 cœurs / 36 Gb /noeud),
1 mpi process / nœud, pthreads = 8 ;
Walltime = ~ 80 h AUGMENTE AVEC LA RESOLUTION ET LE Nb PARTICULES

Perspectives / Besoins



- Formations
 - Initiation informatique
 - Calcul parallélisé
- Migration sur eos => Optimisation des calculs
- Gestion des fichiers intermédiaires produits lors de l'analyse
(Dataset 15G => n Structure(s) 3D 28 M + 120 G fichiers temporaires)
- Augmentation du volume de données
 - nombre de particules : > 150 000 / dataset
 - nombre de structures étudiées (> 2-3 datasets en parallèle)

Hétérogénéité structurale continue (flexibilité) vs. discontinue (changements conformationnels)



=> Outils mathématiques et statistiques pour exploiter la flexibilité des molécules biologiques

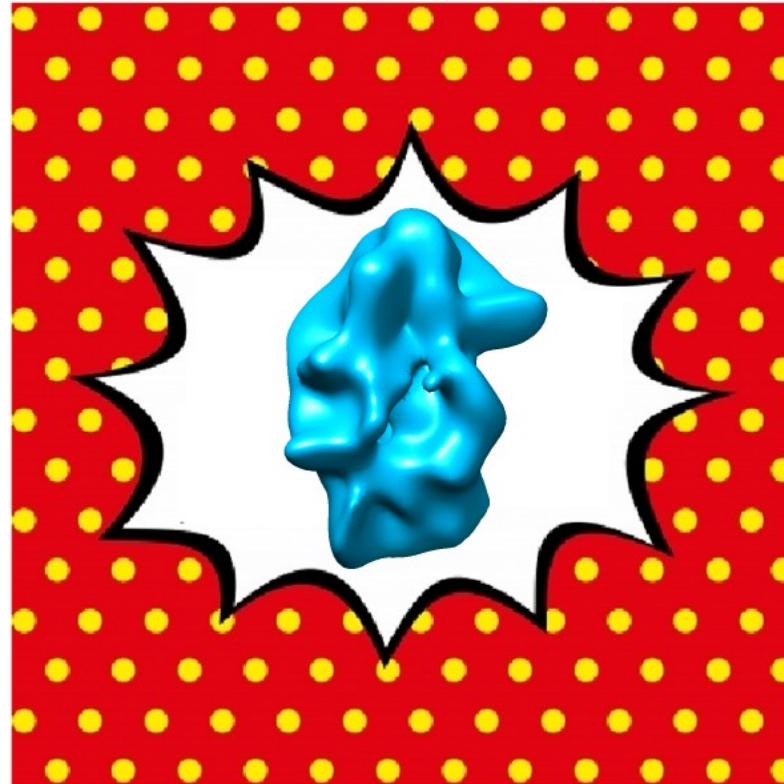
**Group : Dynamics and disorders
of ribosome biogenesis**

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Ribosomopathies

Rare, inherited diseases

Mutations in genes encoding r-proteins or ribosomal assembly machinery components

Variable clinical manifestations ; tissue specificity

Small stature, predisposition to cancer, craniofacial abnormalities

Table 1
The ribosomopathies, including putative mechanisms causing tissue specificity.

Disease	Clinical manifestations	Gene	Function in ribosome biogenesis	Occurrence	Putative mechanism of specificity	References
Autosomal dominant						
Diamond-Blackfan anemia	Anemia, bone marrow failure, craniofacial abnormalities, cardiac defects, cancer predisposition, pre- and postnatal growth retardation, thumb abnormalities, heart defects	<i>RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPL5, RPL11, RPL26, RPL35A</i>	Ribosomal proteins	1 in 100 000-1 in 200 000 live births	Translation of IRES-containing <i>BAG1</i> and <i>CSDE1</i> mRNAs in erythroid progenitors	[5–9,38]
Treacher Collins syndrome	Craniofacial abnormalities, occasional microcephaly, mental retardation and psychomotor delay	<i>TCOF1, POLR1D, POLR1C</i>	Transcription of rRNA genes	1 in 40 000-1 in 70 000	Treacle strongly expressed in neural crest cells; Treacle interaction with UBF, fibrillarin, NOP56, Plk1	[10,12–14,39,40]
Isolated congenital asplenia	Agenesis or hypoplasia of spleen leading to immunodeficiency	<i>RPSA</i>	Small subunit ribosomal protein	73 cases reported	Unknown	[15,41,42]
Aplasia cutis congenita	Agenesis of skin, usually on scalp vertex	<i>BMS1</i>	Ribosomal GTPase	>500 cases	Unknown	[16,43,44]
Autosomal recessive						
Shwachman–Diamond syndrome	Exocrine pancreas insufficiency, growth retardation, hematologic defects, skeletal abnormalities, cancer predisposition	<i>SBDS</i>	Removal of eIF6 from 60S in final maturation step, allowing binding of 40S and 60S subunits	1 in 76 000 live births	SBDS strongly expressed in developing pancreas	[17,18,45–48]
Bowen–Conradi syndrome	Severe pre- and postnatal growth retardation, psychomotor retardation, microcephaly, micrognathia, joint contractures, rockerbottom feet	<i>EMG1</i>	Pseudouridine-N1-specific methyltransferase	1 in 355 live births in Hutterite population	Unknown	[19,49–52]
Cartilage hair hypoplasia	Short stature, sparse hair, immunologic defects, hematological defects, malabsorption, cancer predisposition	<i>RMRP</i>	Pre-rRNA cleavage	Amish: 1 in 500-1 in 1000; Finnish: 1 in 23 000	Short stature related to rRNA cleavage defect; cancer predisposition putatively caused by defective cyclin B cleavage	[20,21,53,54]
Anaxetic dysplasia	Severe short stature, hypodontia, mental retardation	<i>RMRP</i>	Pre-rRNA cleavage	7 cases reported	Short stature related to rRNA cleavage defect; cyclin B cleavage unaffected thus no cancer predisposition	[20,21,53,54]
Alopecia, neurological defects and endocrinopathy syndrome	Hypoplastic hair, microcephaly, mental retardation, progressive motor retardation, adrenal insufficiency	<i>RBM28</i>	Nucleolar component of the spliceosomal small nucleolar ribonucleoprotein, necessary for 60S biogenesis	5 cases reported	Unknown	[23,55,56]
North American Indian childhood cirrhosis	Transient neonatal jaundice progressing to biliary cirrhosis	<i>CIRH1A</i>	Pre-rRNA processing	Carrier status: 1 in 10 in Quebec Ojibway-Cree population	Cirhin strongly expressed in developing liver	[24,25,57–59]
X-linked recessive						
X-linked dyskeratosis congenita and Hoyeraal–Hreidarsson syndrome	Abnormal skin pigmentation, nail dystrophy, leukoplakia, bone marrow failure, cancer predisposition, short stature, microcephaly, immunodeficiency	<i>DKC1</i>	Pseudouridine synthase	1 in 1000 000	Translation of IRES-containing mRNAs including <i>p27</i> , <i>XIAP</i> , <i>Bcl-xL</i>	[26,28,60–62]
Sporadic						
5q [−] syndrome	Macrocytic anemia, predisposition to acute myeloid leukemia	<i>RPS14</i>	Small subunit ribosomal protein	Unknown	Unknown	[31]
T cell acute lymphoblastic leukemia	Leukemia affecting the T-cell lineage	<i>RPL5, RPL10, RPL22</i>	Large subunit ribosomal proteins	In T-ALL: <i>RPL5</i> mutations 5.2%, <i>RPL10</i> mutations 1.9%, <i>RPL22</i> deletions 10%	<i>RPL22</i> deficiency blocks $\alpha\beta$ T cell development, unknown	[32,33,63]

Particules pré-40S humaines : Analyse structurale à grande échelle par MET 3D



Purification des particules pré-40S
(ETH, Zürich)



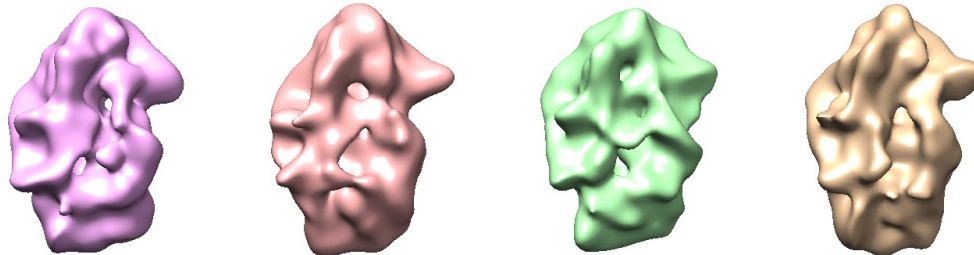
Préparation et vérification des grilles de cryo-MET (plateforme MeTi)



Acquisition des images de cryo-MET (NeCEN, Leiden, Pays-Bas)



Analyse des images => Structure(s) 3D (IBCG, Calmip)



The « Resolution Revolution » (2)

Microscopes dernière génération comparables à des synchrotrons

Correction des aberrations des lentilles => augmentation de la résolution des images

Acquisition des images entièrement automatisée => augmentation du nombre d'images

Caméras à détecteurs directs d'électrons => Augmentation du rapport signal / bruit des images

