Analysis of Archaeal Protein Sorting and Translocation

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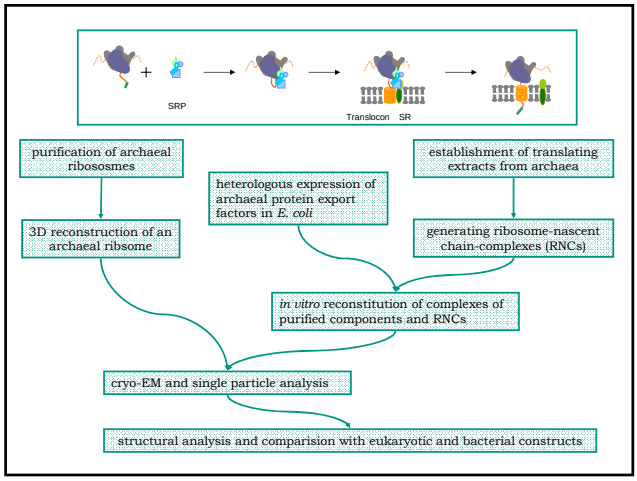
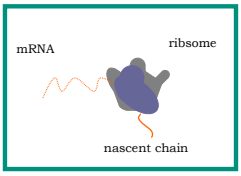
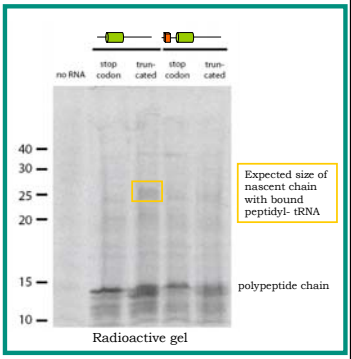


Ribosome - nascent chain complexes



RNCs consist of translating ribosomes that are programmed with certain mRNAs. The mRNA leads to stalling of the ribosome at a defined codon.



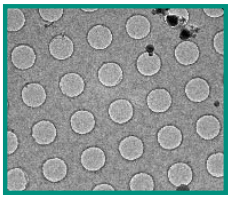
Radioactive gel

Archaeal protein export factors

- Identification of factors based on BLAST analysis and the annotated genome from *Thermococcus kodakarensis*
- Cloning into expression vectors
- Heterologous expression in *E. coli*
- Purification
- *In vitro* assembly and binding to RNCs

Cryo-sample preparation

Vitrobot Mark III, FEI

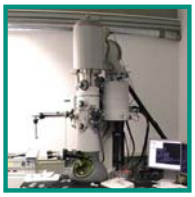


Quantifoil R 3/3 300 mesh Cu grids +2nm carbon on top

Cryo-electron microscopes

Tecnai G2 Spirit

-120 kV transmission electron microscope
- computer-controlled cryostage
- 2k x 2k CCD camera
- 3.5 Å/pixel



Tecnai G2 Polara

- 300 kV field emission gun
- helium stage
- data collection on film

Titan Krios

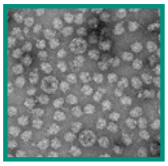
- 80 - 300 kV tunable field emission gun
- cryo-autoloader sample stage
- 4k x 4k CCD-camera
- Automated data acquisition

Digitalization of micrographs

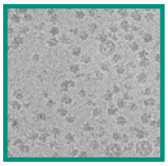
- Heidelberg Tango drum scanner
  Step size 4.3 µm ➔ 1.2 Å/pixel

Workflow

- Sample preparation
- Negative stain image: sample quality
- Data collection on Tecnai Spirit G2
  ➔ **low resolution reconstruction**
- Data collection on Tecnai Polara G2
  ➔ **high resolution reconstruction**
- (Data collection on Titan Krios
  ➔ **high resolution reconstruction)**



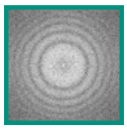
Negative stain



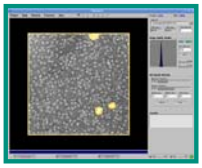
Cryo

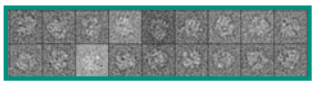
Pre-processing

- CTF determination and visual inspection of power spectra (Spider/Ctffind, Web)



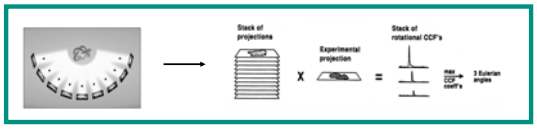
- Automated particle picking (Signature)
- Manual particle selection (Web)





Processing

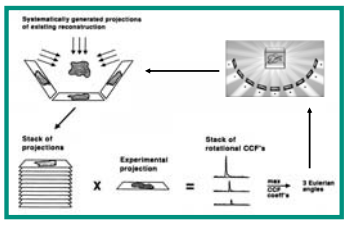
- Alignment (Spider): Projection matching



- Initial back projection (Spider)

Processing

- Resolution determination (Spider): fourier shell correlation, cutoff 0.5
- Refinement (Spider): Projection matching

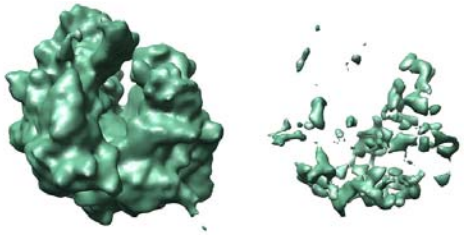


Dealing with inhomogenous datasets

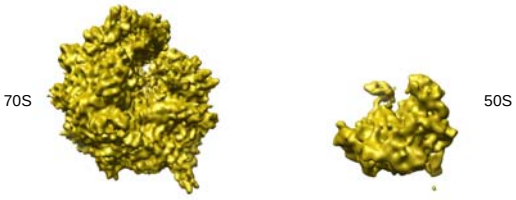
Semi-supervised sorting

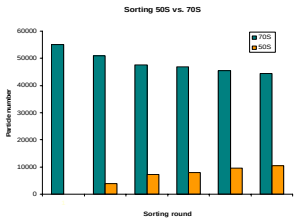
- Two different volumes are used as initial reference for all particles
- The dataset is segregated into two subpopulations depending on higher degree of similarity
- The split datasets are individually used for back projection
- Results are used as new references for the next round of sorting
- The process continues until the particle number gets stable

Example I

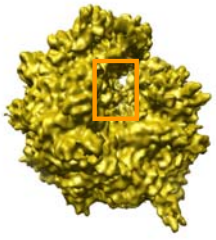


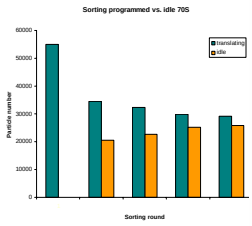
Different intensities of electron density of 50S and 30S subunits at lower contour level

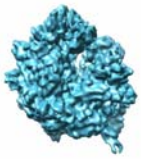




Example II

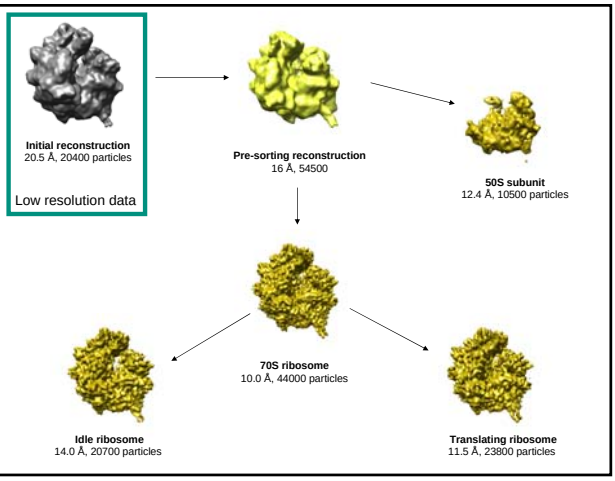






Validation of Sorting

- Convergence of particle numbers
- Improving resolution (large datasets required)
- Projection with select files from one subpopulation and sorting-independent angle files shows the same features like the sorted volume



Outlook

- Sample preparation:
  - RNCs/ribosomes and ligands

- Data interpretation:
  - Identification of conformational changes
  - tRNA positions (A/P/E-site)
  - Ligand occupancy and conformational heterogeneity

Gene Center Munich

Thank you

- Beckmann lab
  - Dr. Thomas Becker
- Wilson lab
- Prof. Dr. Michael Thomm, University of Regensburg

FCI FONDS DER CHEMISCHEN INDUSTRIE

IMPRS From Biology to Medicine

Early Cryo-EM