NOTOX

Predicting long-term toxic effects using computer models based on systems characterization of organotypic cultures

Electron cryo-tomography for observation of macromolecular changes induced by test compound within a cell

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Introduction

Structures obtained by NMR, X-ray diffraction and single particle cryo-electron microscopy lack the cellular context. In the cell, quite often chemicals that are supposed to bind a specific target show lower affinity and are not delivered to the target or they interact with unpredicted cellular components with possible toxic side effects.

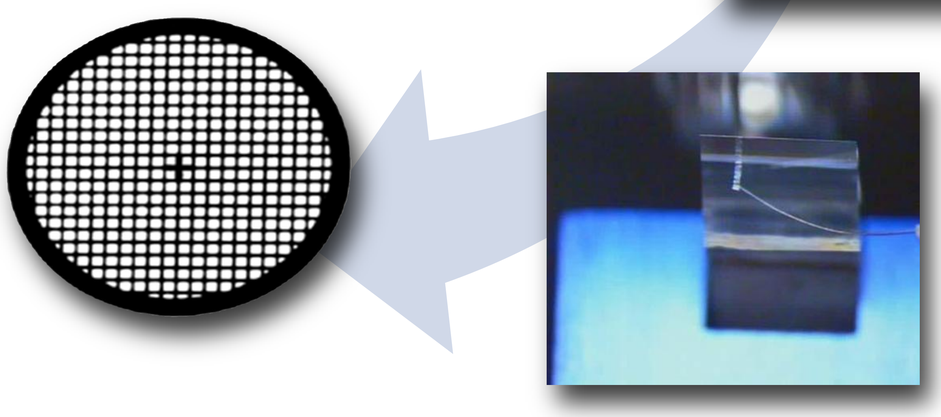
Cryo-electron tomography is an emerging tool that allows us to visualize macromolecular complexes within high pressure frozen cells (*1)* With novel methods for generating vitreous cryo-sections and iterative reconstruction techniques, developed within the project, we push forward the resolution to the point of being able to identify structural changes induced by drugs on macromolecular complexes in a native cellular context (*2*).

The implementation of this technology will bring additional dimension to our understanding of the toxic effect of chemicals in the cell.

Step 1: Sample Preparation

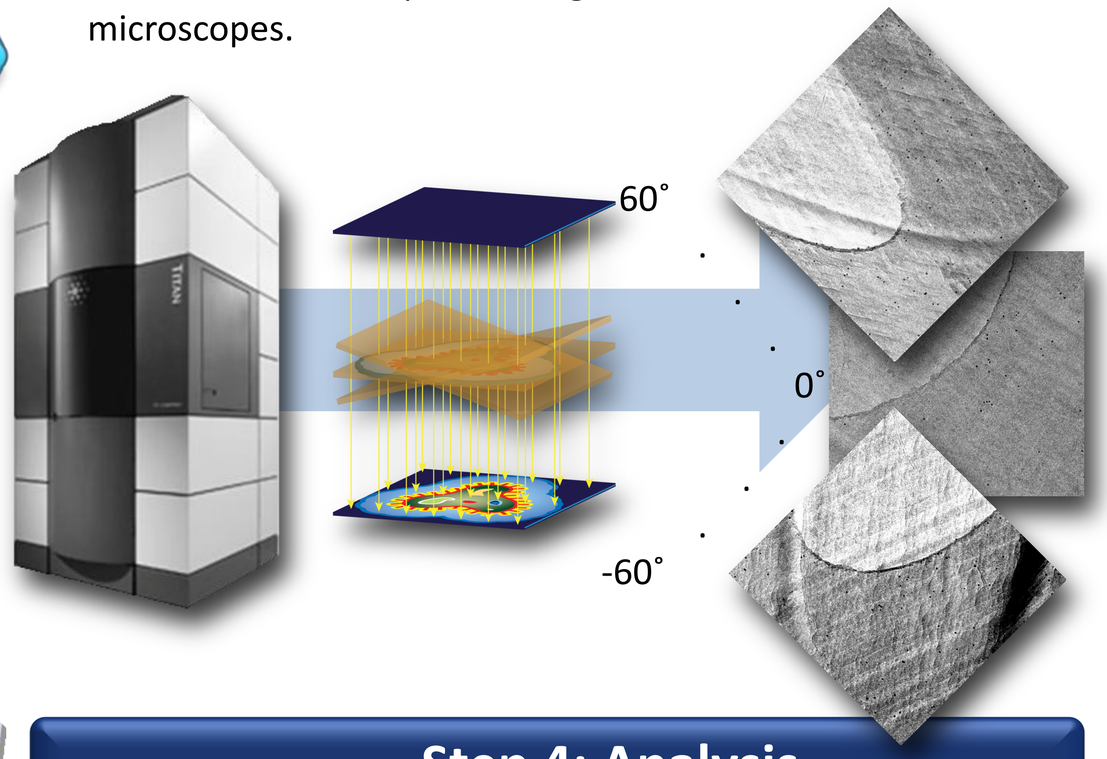
The specimen of interest is high-pressure frozen, preserving the ultra-structure of the cell in near-live conditions.

Then they are cut to thin sections and placed onto support grids with the help of electrical charge to ensure adherence.



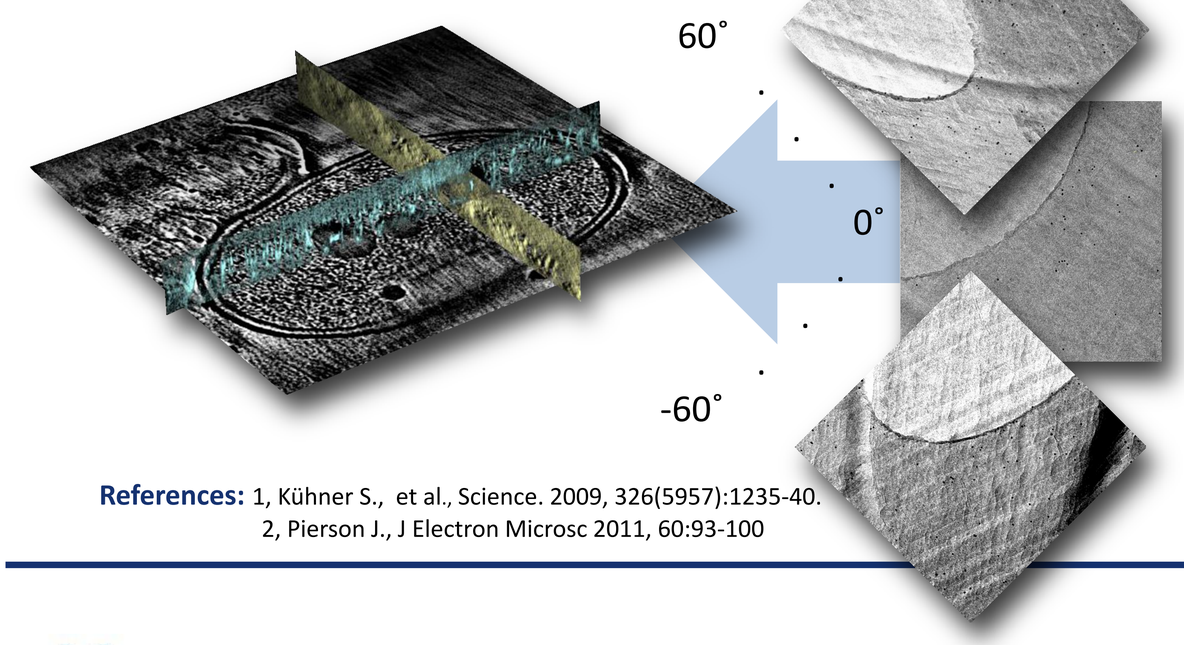
Step 2: Image Acquisition

A set of low-dose images of the samples from multiple angles – a tilt series – is acquired using state-of-art electron microscopes.



Step 3: Tomographic reconstruction

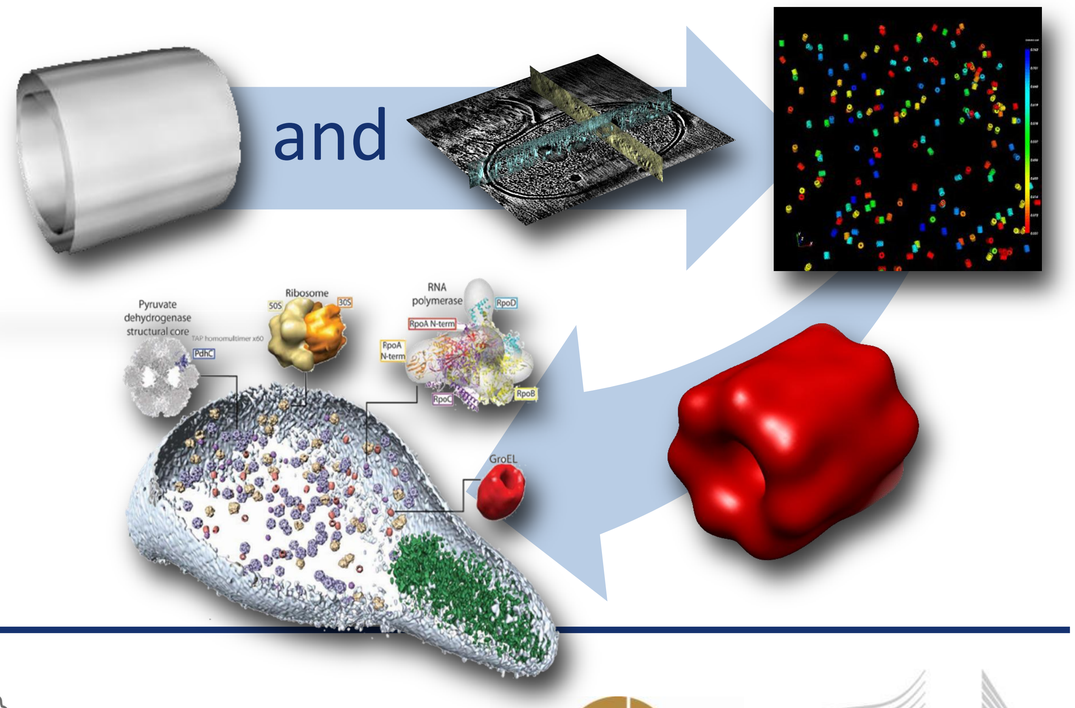
The 2D images from tilt-series are computationally combined into a 3D density map – a 3D view right through the cell – using tomographic reconstruction techniques.



Step 4: Analysis

Using sub-tomogram averaging, template matching, and cross-correlation scores, an average structure of a protein is obtained from the 3D density map.

By extracting and spatially localizing multiple complexes in the cell, a **cellular macromolecular atlas** can be built.



References: 1, Kühner S., et al., Science. 2009, 326(5957):1235-40.
2, Pierson J., J Electron Microsc 2011, 60:93-100

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