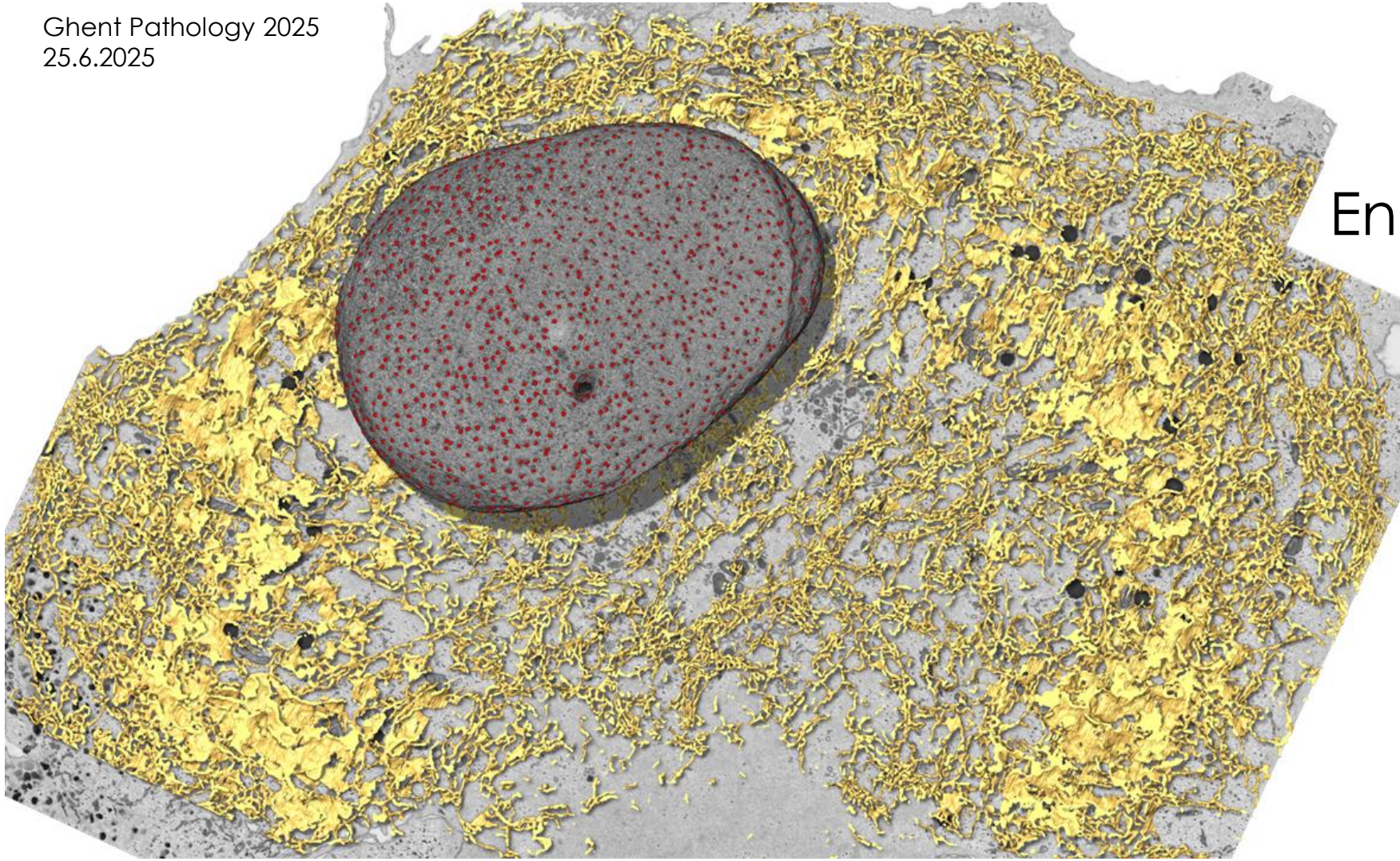


Ghent Pathology 2025
25.6.2025



Endoplasmic reticulum

Eija Jokitalo



UNIVERSITY OF HELSINKI

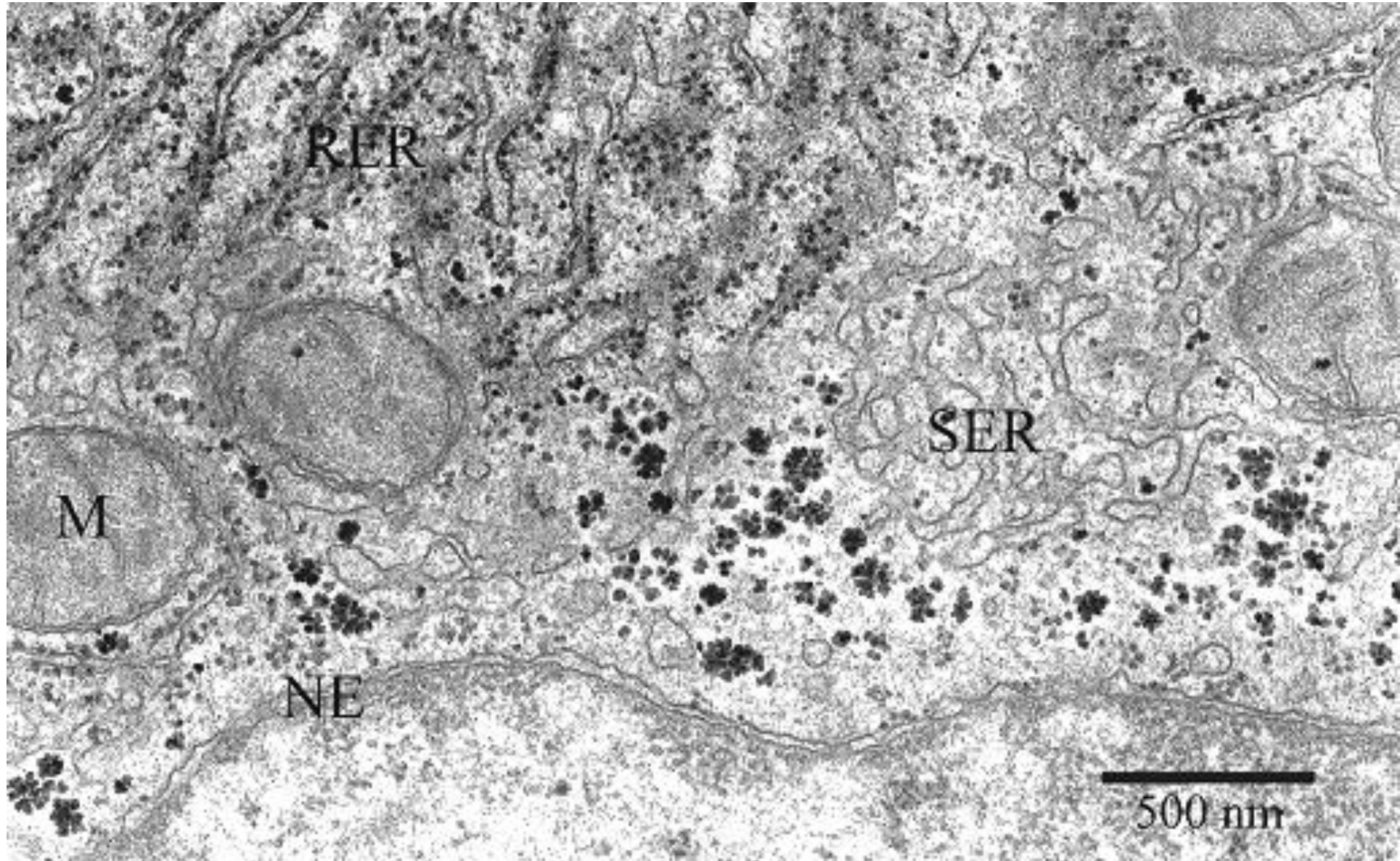
Electron Microscopy

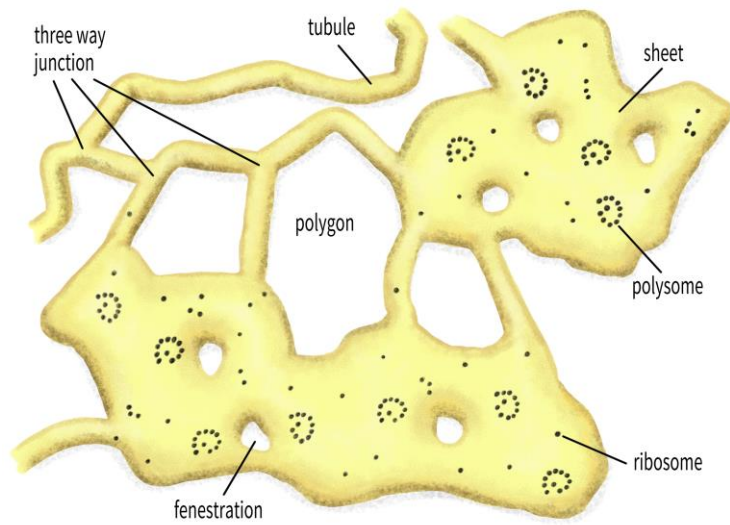
EMBI

Institute of Biotechnology

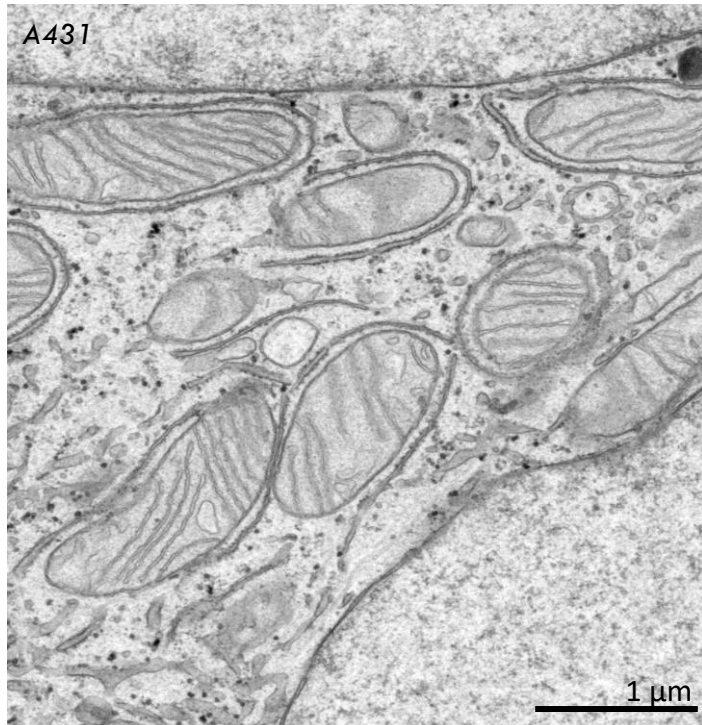
Text book view on ER:

- ER is organized into three subdomains: NE, RER & SER





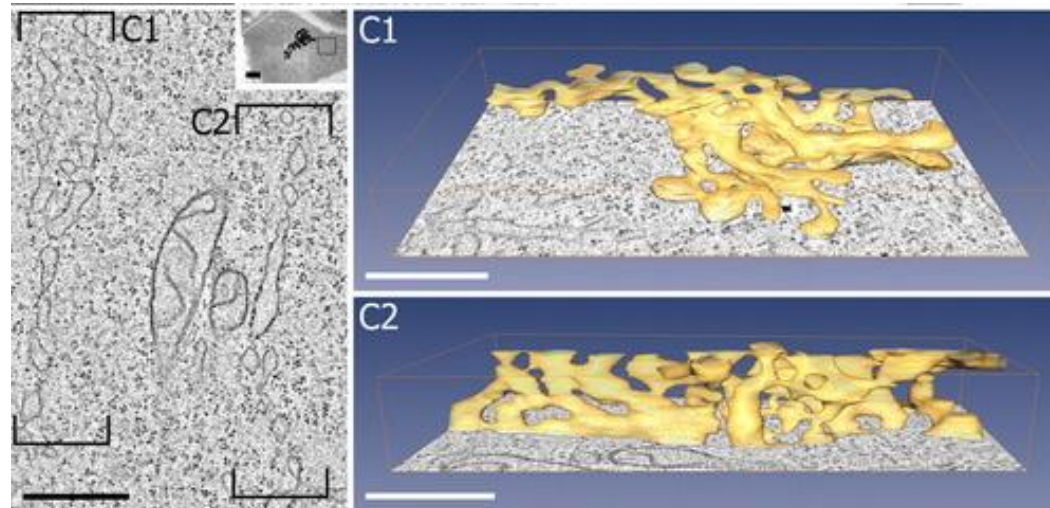
Maro Järvinen; Sree et al., 2021



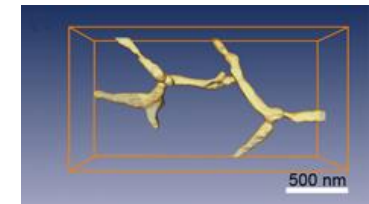
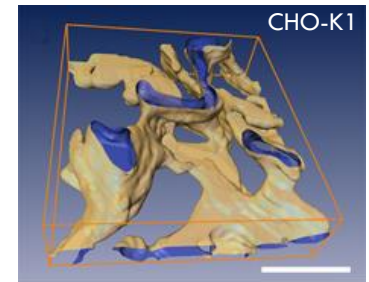
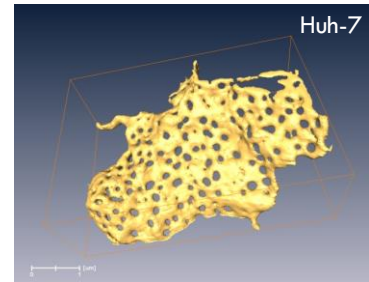
Eija Jokitalo

ER consists of sheets and tubules

- Sheets can be intact or fenestrated, and they are decorated with ribosomes
- Average size of the sheets varies between cell types
- Thickness of the sheets is quite constant and is equals to the diameter of tubules
- Diameter of the tubule equals to the diameter of COP-vesicles
- Branch points between sheets, sheets and tubules & between tubules

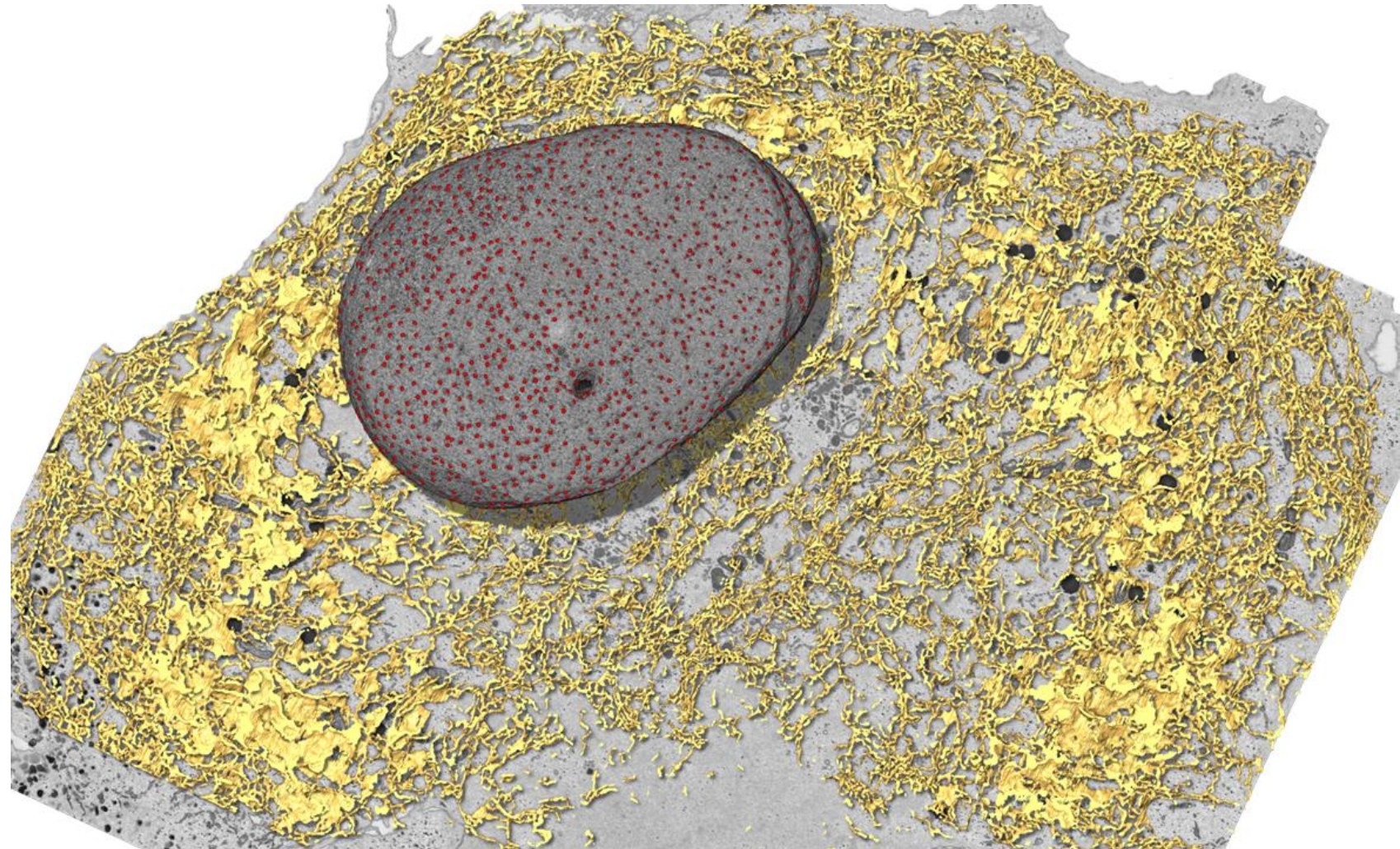
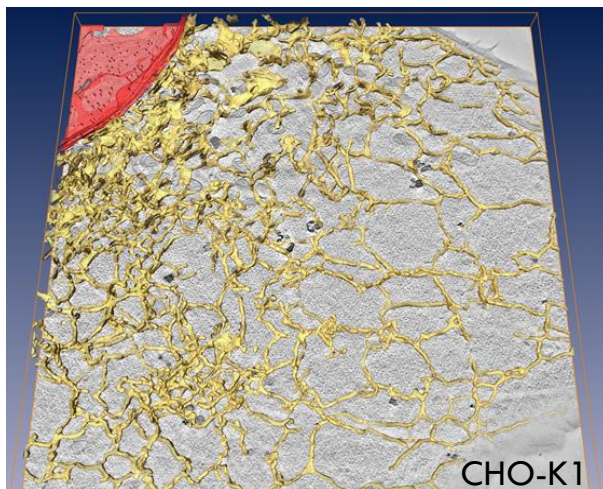
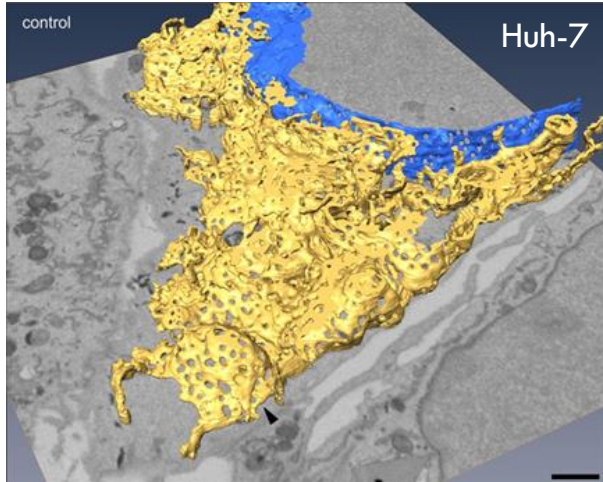


Puhka et al., MBoC2012

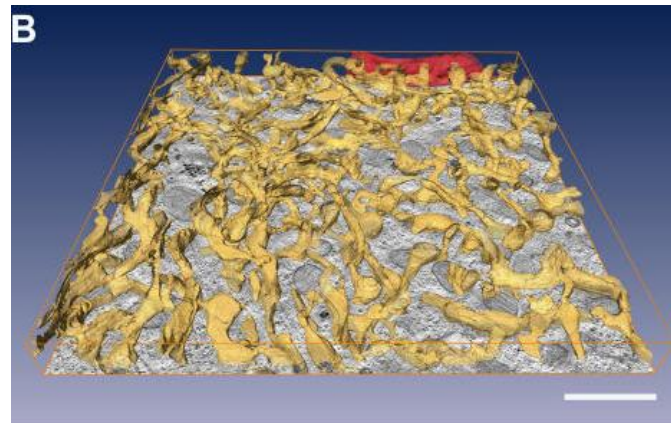
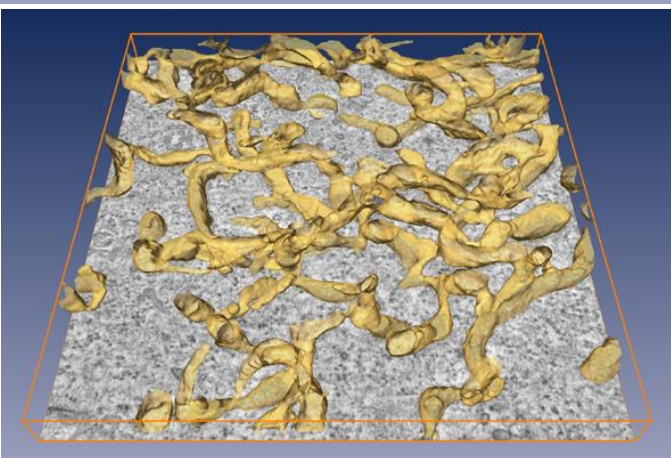
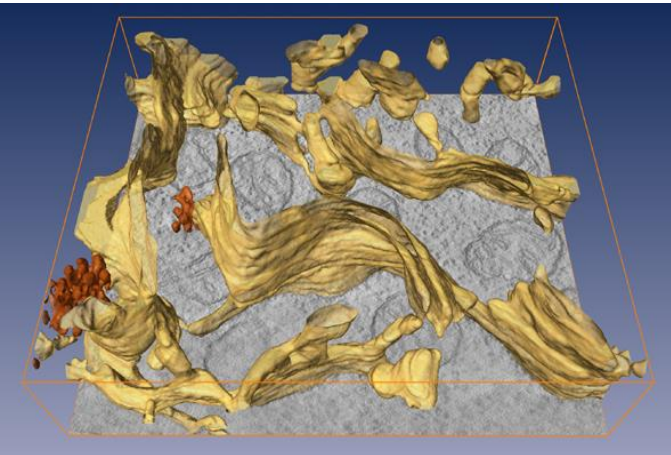


Helena Vihinen

- Live cell imaging & vEM: One continuous network
- Sheet to tubule ratio
 - varies between cell types and within a cell



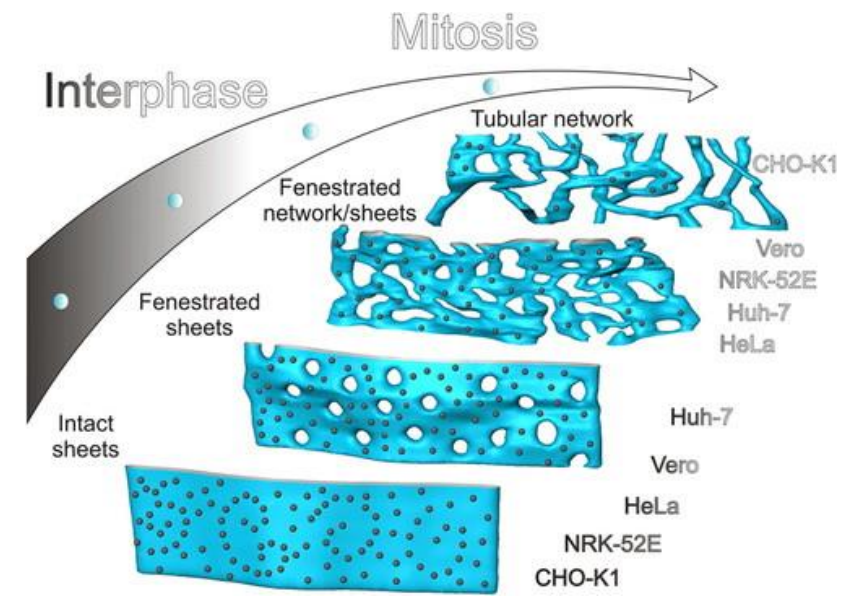
- Live cell imaging & vEM: One continuous network
 - Sheet to tubule ratio
 - varies between cell types
 - and during cell cycle
- ER converts to higher curvature during mitosis



Puhka et al., JCB 2007

Mitosis:

Translational halt: 70.4% decrease in number of ribosomes of transversely sectioned ER



Puhka et al., MBoC 2012

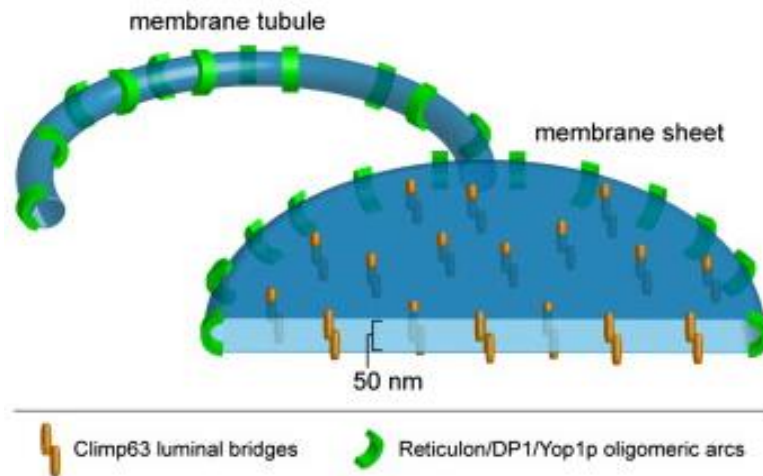
Puromycin:

Causes premature chain termination

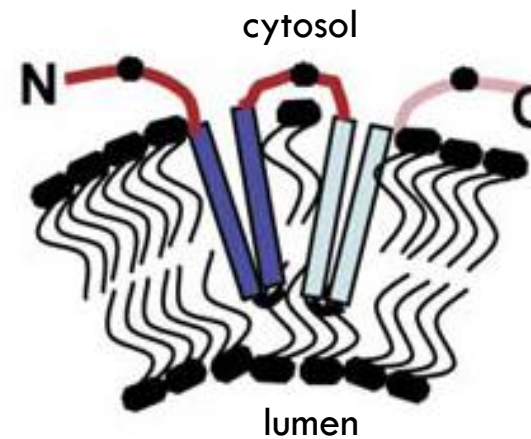
- dissociates polysomes from ER membranes
- 83% decrease in number of ribosomes of transversely sectioned ER

Mechanisms determining the morphology of the ER

- one type of membrane curvature: sheet = edge (half of a tubule) + flat part
- Several **sheet promoting factors**, **tubule promoting factors** and **homotypic fusion factors** play a role in defining the ER network morphology at any given moment
- Membrane bound polysomes in complex with translocon and associated proteins: trigger sheet formation, stabilize sheets
- P180: ribosome receptor, interacts with microtubules & limits lateral mobility of polysomes
- Climp-63: maintains luminal width of ER sheets by forming bridges through their coiled coil domains
- Reticulons and other RHD proteins: Provide curvature: Promote tubules and generates sheets by stabilizing high curvature edges of sheets



Shibata et al., Cell 2010

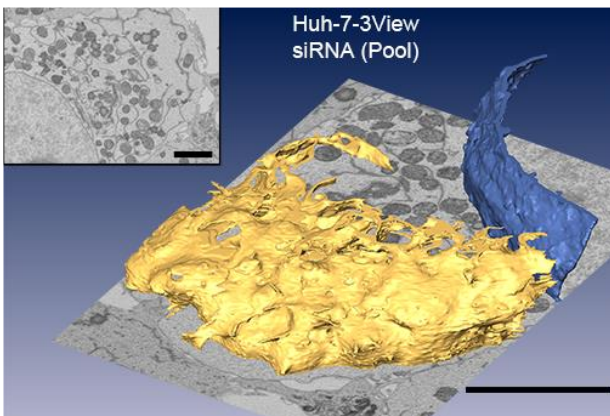
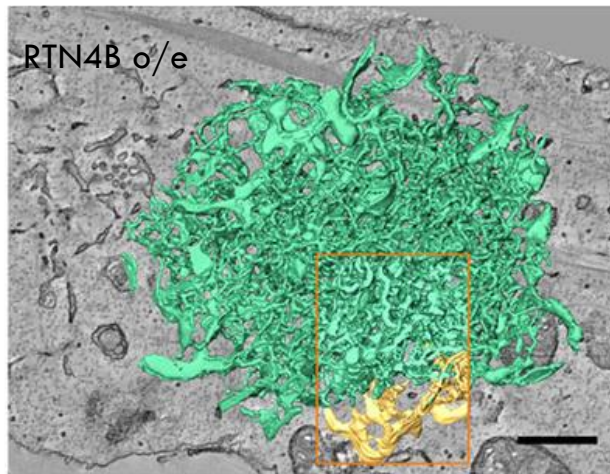


RHD:
unsymmetrical
membrane insertion

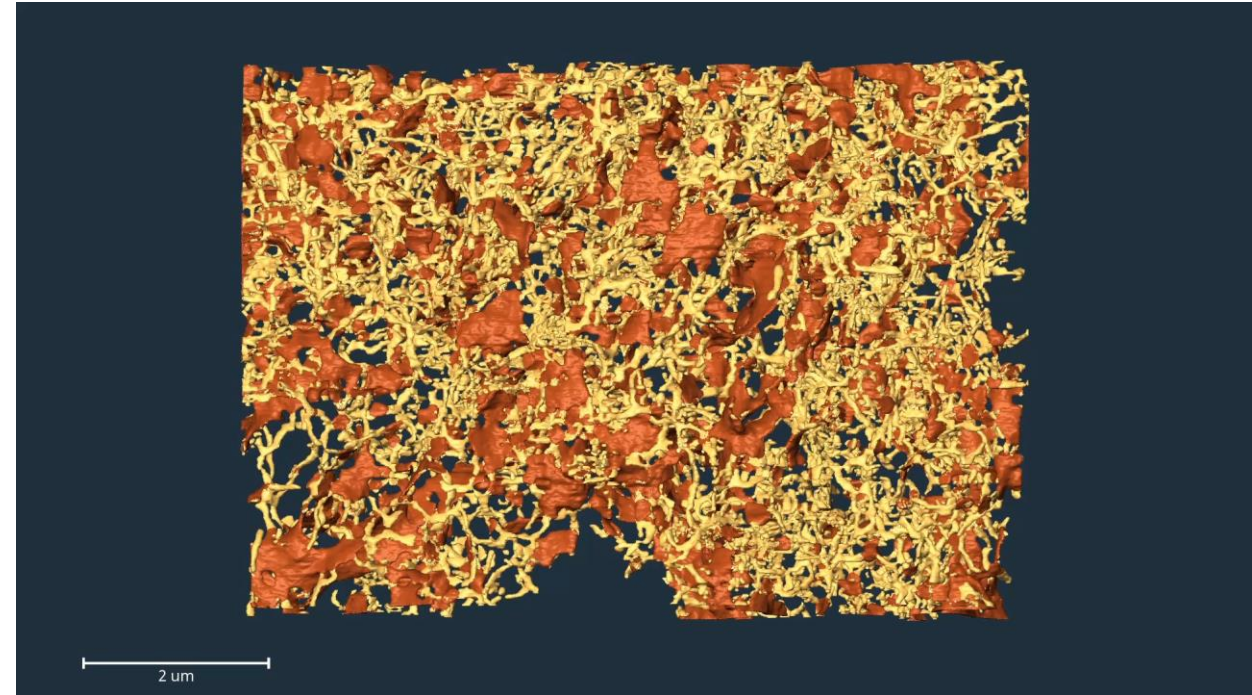
Voeltz et al., Cell 2006

Sheet to tubule ratio

- is sensitive to changes in the ER proteome:
generation of long tubules and tubular clusters,
proliferation of sheets, formation of OSER,
invagination of NE

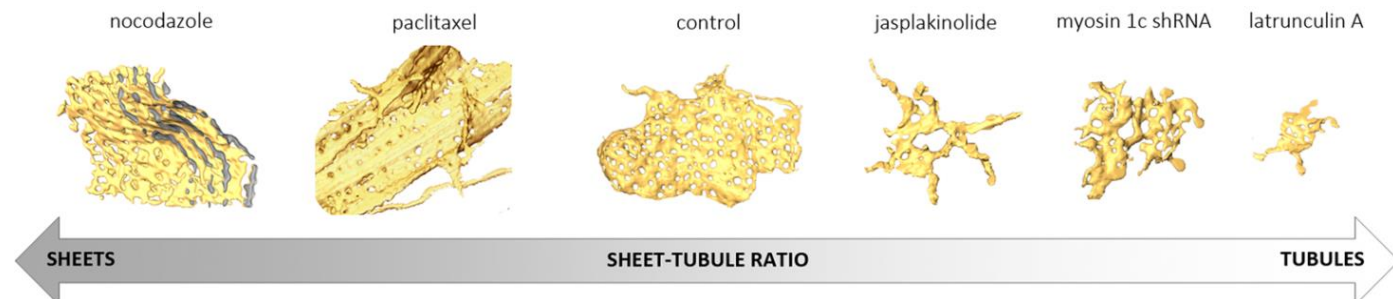


Rämö, Kumar et al., Sci Rep. 2016



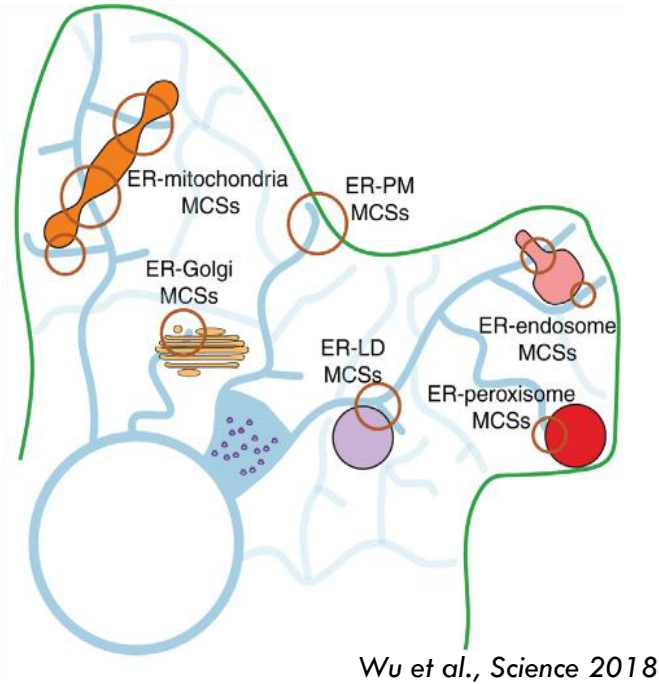
U2-OS, FIB-SEM; dataset: EMPIAR-11746; video: 10.5281/zenodo.10024014

- and to the manipulation of cytoskeleton

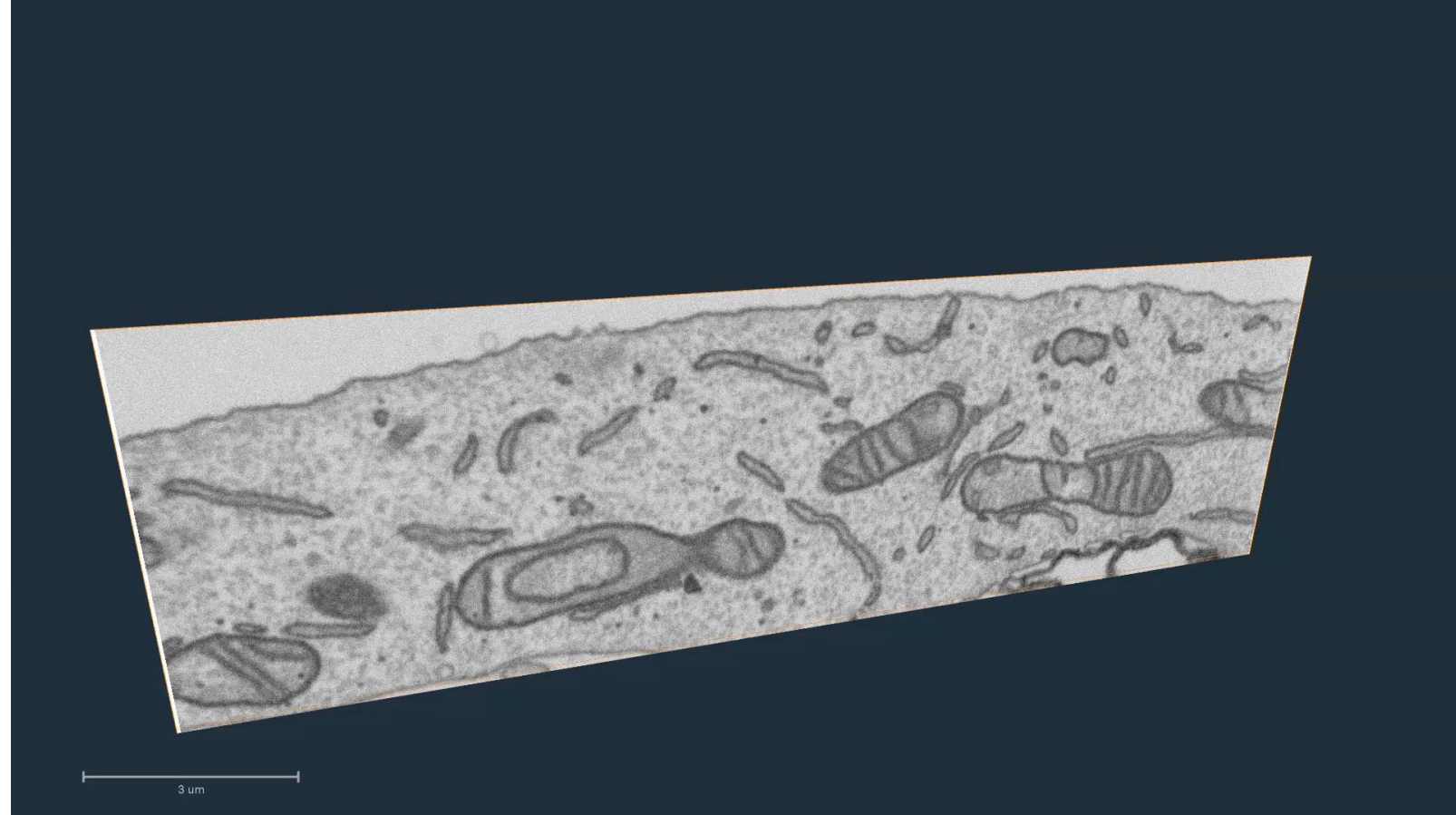


Joensuu et al., MBoC 2014; Joensuu and Jokitalo, Exp. Cell Res. 2015

Interplay between organelles at membrane contact sites



- Transmit exterior signals
- Transmit signals between organelles
- Shared functions

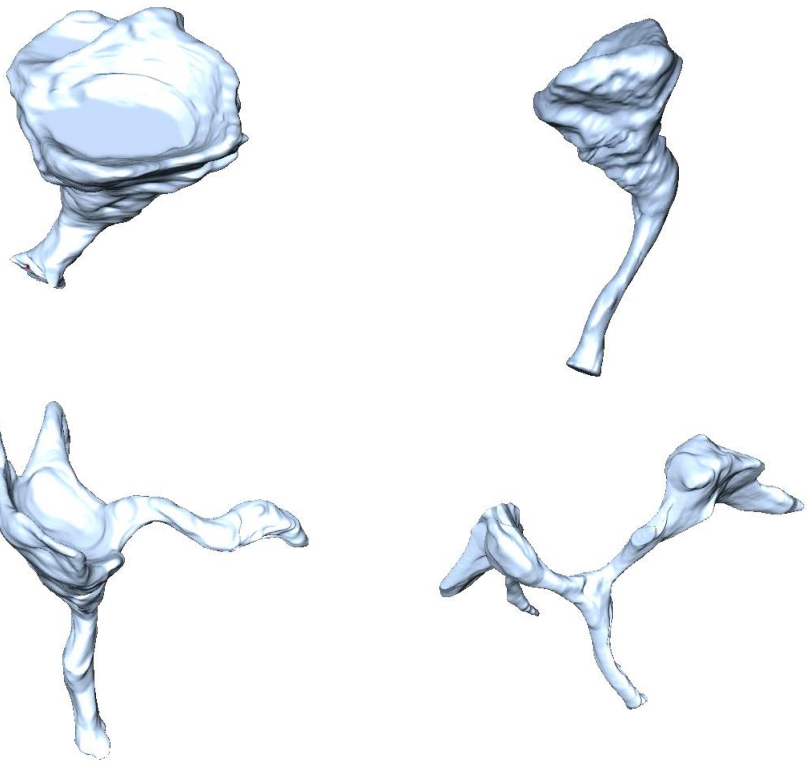


U2-OS, FIB-SEM; Imaging and modelling by Ilya Belevich, 2023

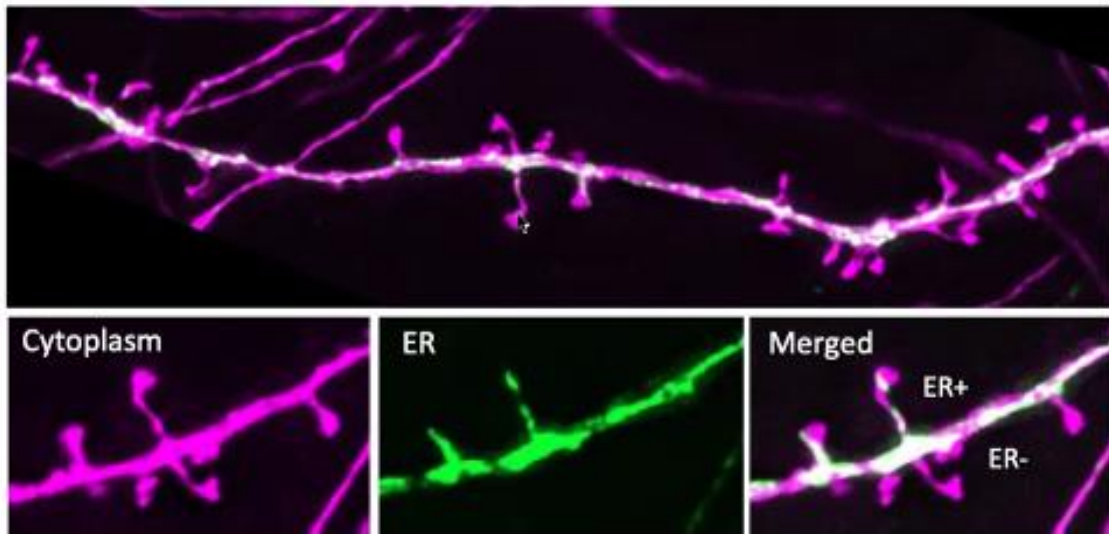
Dynamics and structure of ER in dendritic spines

Does the ER have a role in spine individuality?

- Many shapes (mushroom, thin, stubby, branched)
 - Size correlates with the magnitude of synaptic strength (larger the head, stronger the synaptic current)
 - Dynamic: change shape, size and number in response to neuronal activity
 - Small spines can disappear in a day, large can persist months/years
-
- Only portion of the spines have the ER
 - Spine ER is motile: enter and leave continuously or form more stable structures



Dendritic spines of mouse primary hippocampal neurons; *Helena Vihinen*

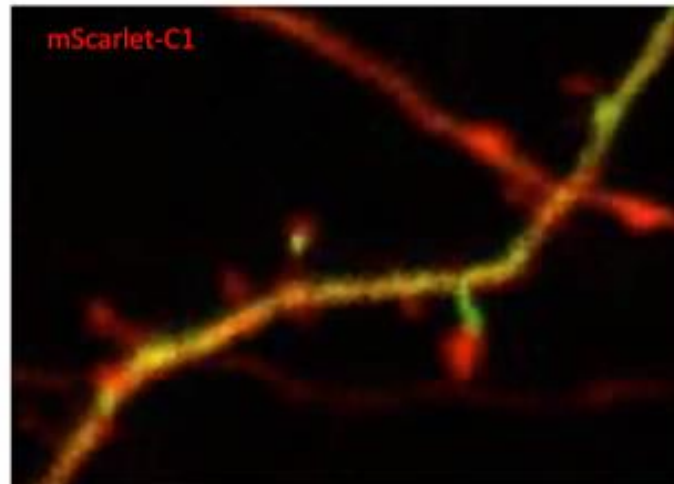
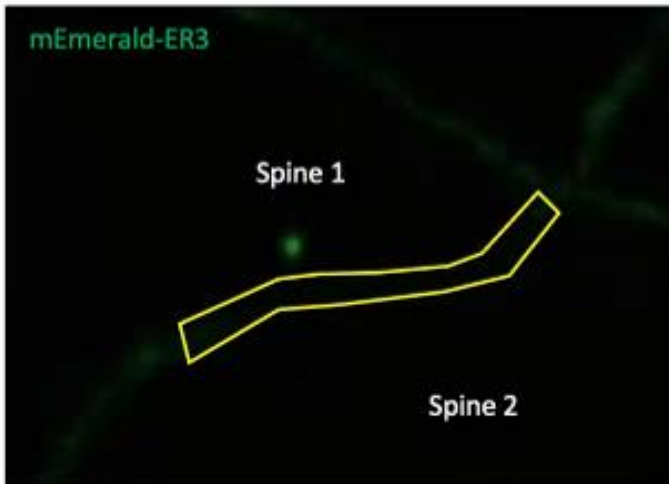
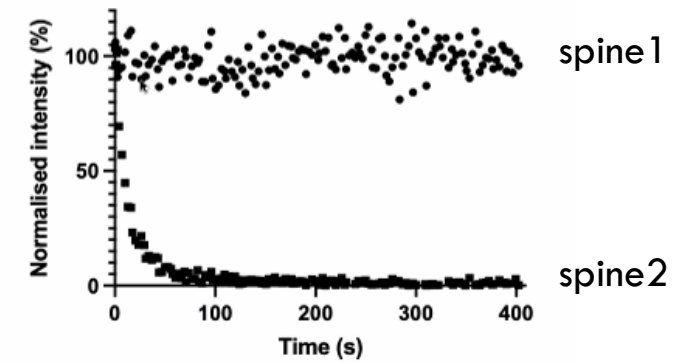


Dendrite of primary hippocampal neuron; *Image courtesy of Leena Kontturi*

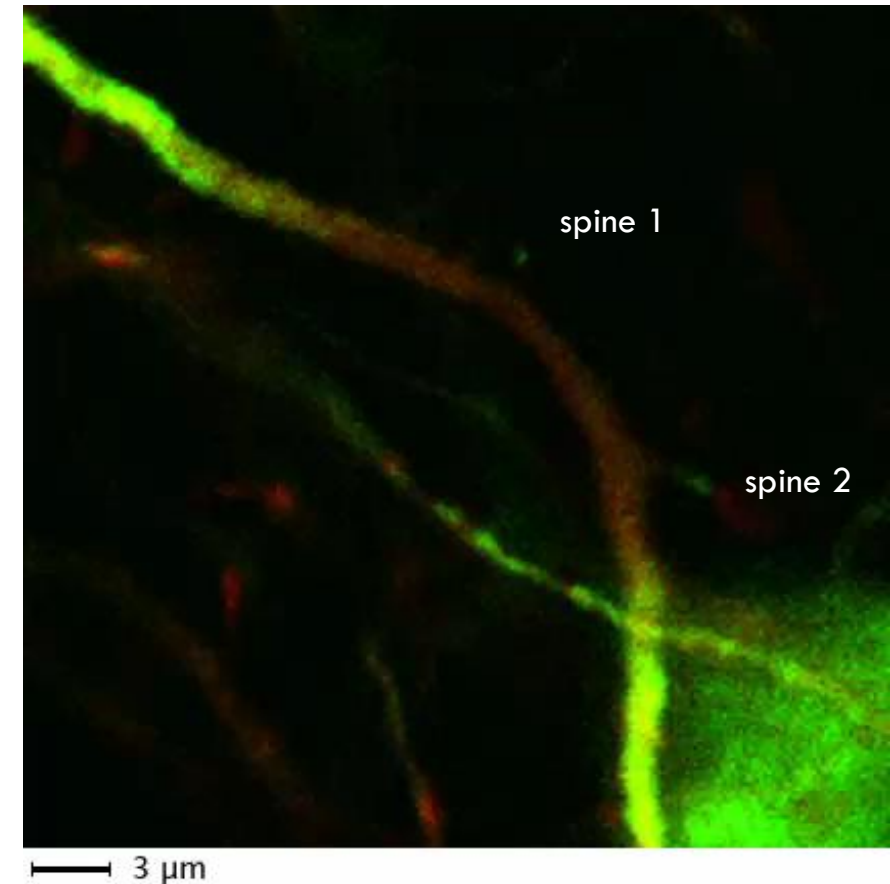
In collaboration with Leena Kontturi and Juha Saarikangas, University of Helsinki

Dynamics of the ER in dendritic spines

- Fluorescence Loss in Photobleaching (FLIP)
 - the region of interest is just outside the region that is being photobleached
 - Complete loss of fluorescence = organelles are continuous
 - Incomplete loss of fluorescence = organelles are compartmentalized and therefore closed off to the transfer of any photobleached fluorophores



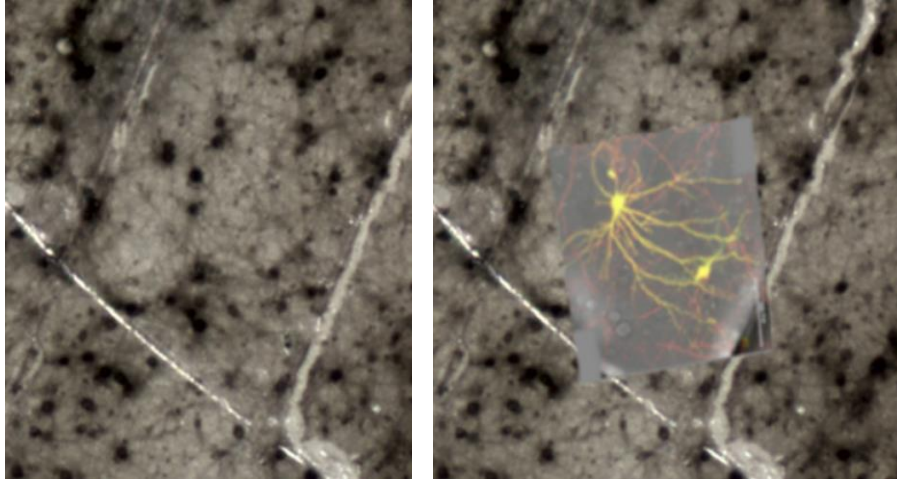
Primary hippocampal neurons transfected with ER-marker mEmerald-ER3 and cytosolic marker mScarlet-C1 cultured for 14 days
Repetitive bleaching 0.5 -1 sec depending on the size of the dendrite and the distance from soma



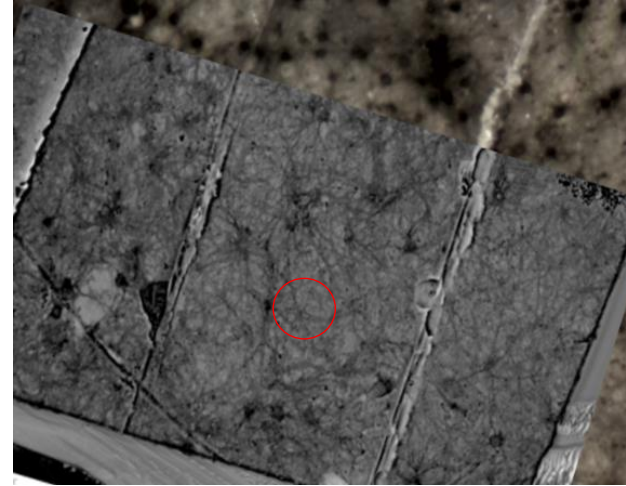
- FLIP revealed differences in ER dynamics between dendritic spines

Correlation of LM and FIB-SEM

LM

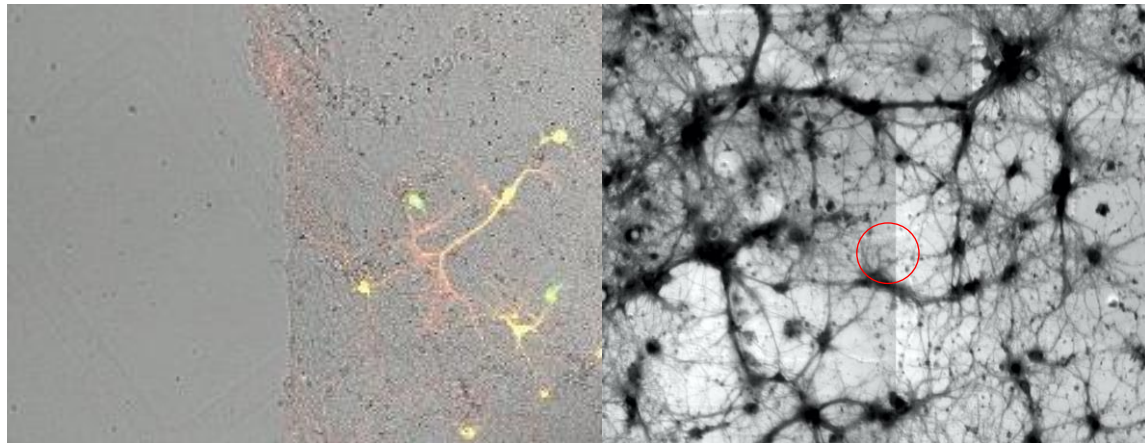


BSED @15 kV

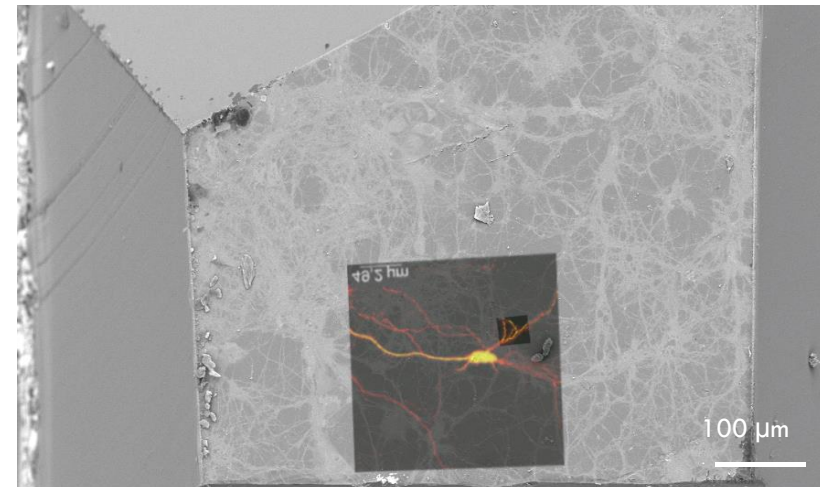


MatTek dish with
gridded cover slip

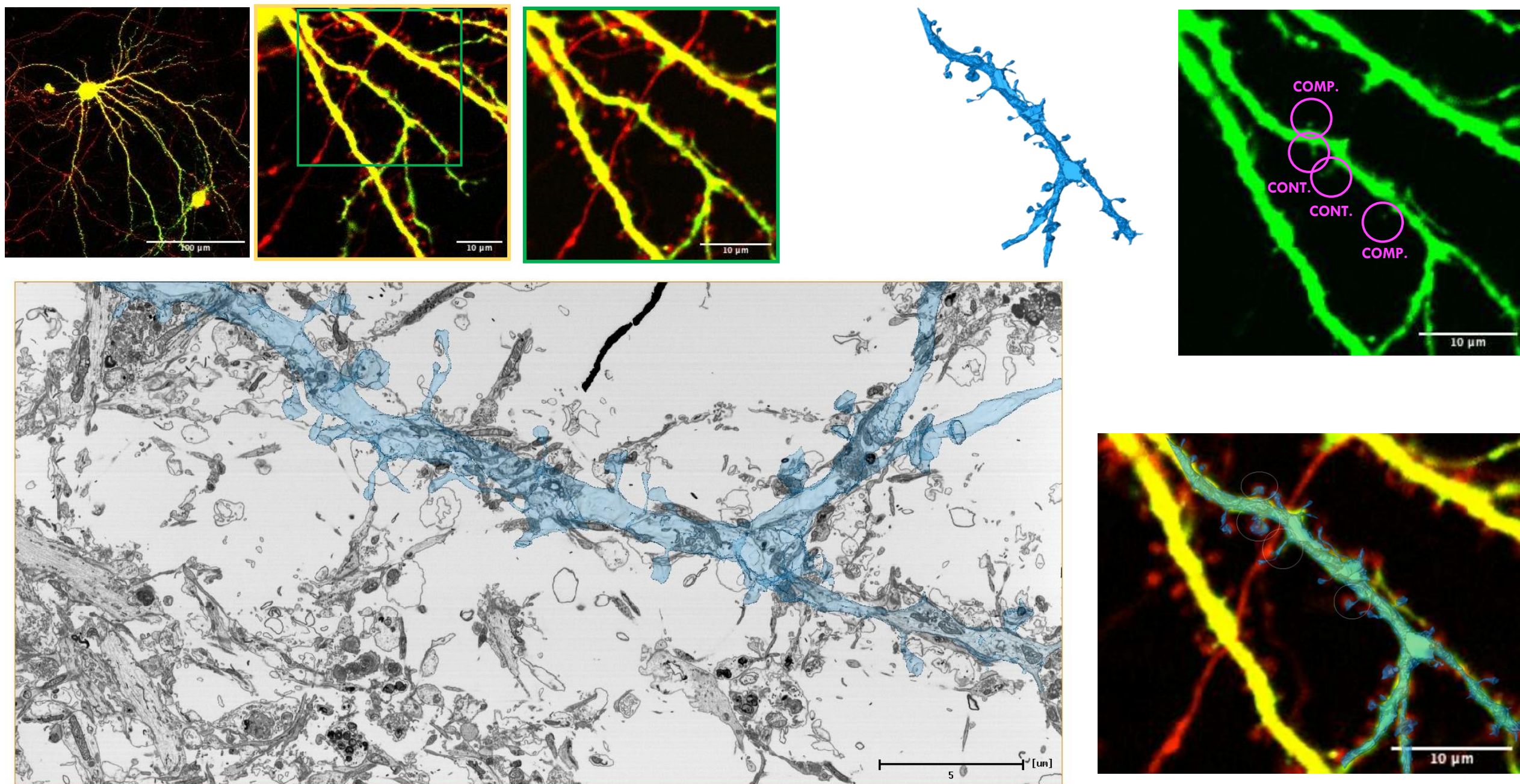
Diamond pen markings



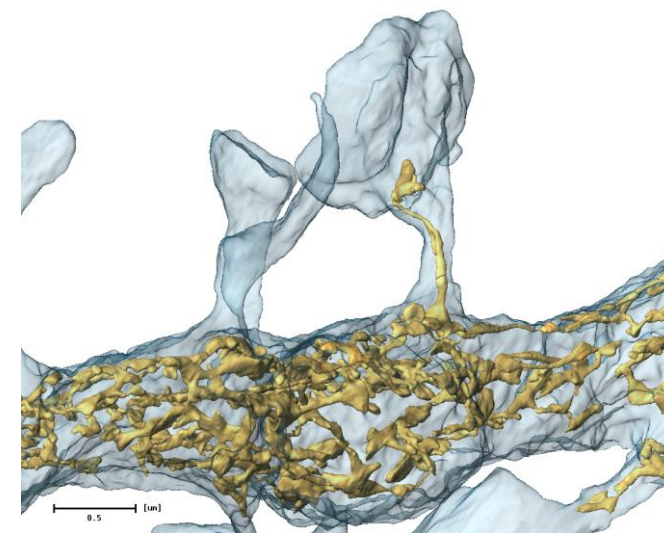
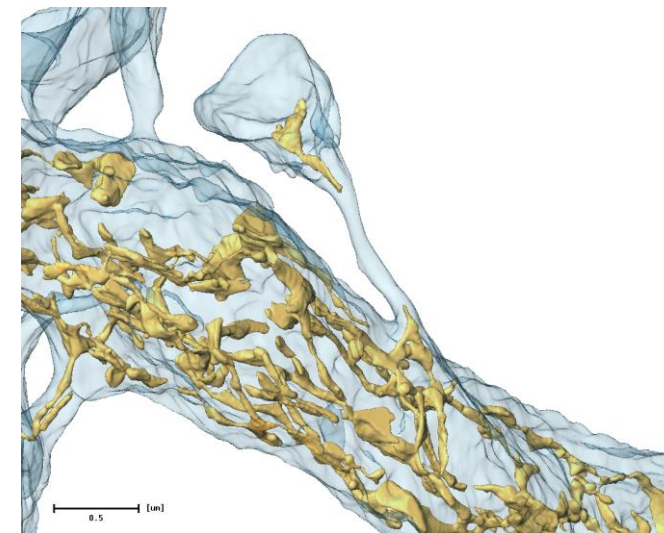
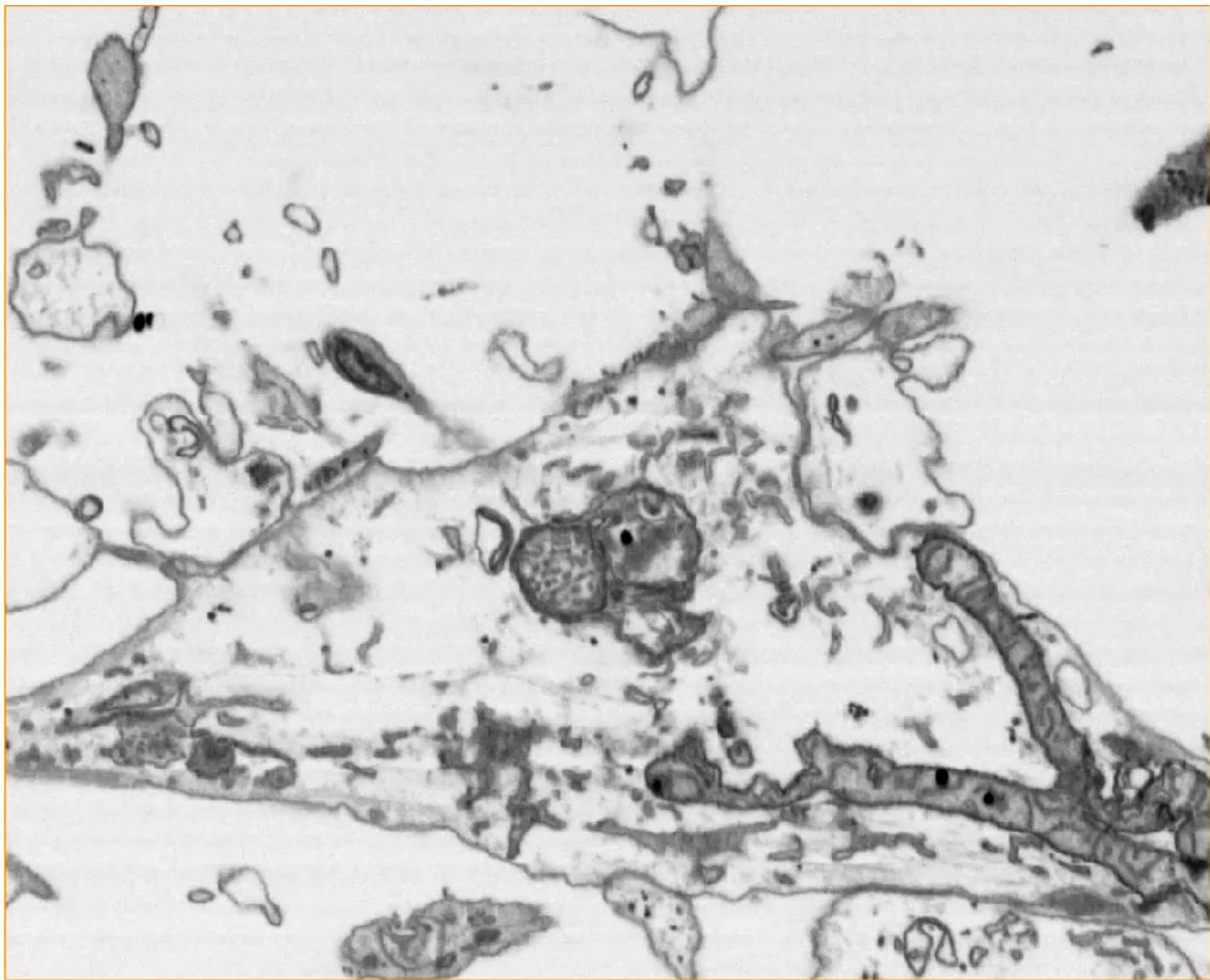
Primary hippocampal neurons transfected with ER-marker mEmerald-ER3 and cytosolic marker mScarlet-C1 cultured for 14 days



Leena Kontturi and Helena Vihinen



Primary hippocampal neurons cultured for 14 days.
 Imaging with Zeiss Crossbeam 550 FIB-SEM (6 nm voxel; ROI 40x26x20 μm; 35 h)
 Modeling with Microscopy Image Browser, rendered with Amira





Mouse hippocampal coronal section; Imaging with Zeiss Crossbeam 550 FIB-SEM (6 nm voxel)

Modeling with Microscopy Image Browser, rendered with Amira.

Helena Vihinen (imaging) and Marta Serrano López (modeling); In collaboration with Leena Kontturi and Juha Saarikangas

Human diseases with associated ER defects

- ER dysfunction is a common pathogenic mechanism in a variety of human diseases, especially those involving protein misfolding, chronic metabolic stress, or genetic mutations affecting secretory proteins:
 - ER's quality control systems are overwhelmed by the accumulation of disease-specific misfolded proteins, leading to chronic ER stress and activation of the UPR. If unresolved, this stress can trigger neuronal dysfunction and cell death.
- The convergence of ER dysfunction and protein misfolding is a central pathogenic feature, making these pathways attractive therapeutic targets.

Disease/Disorder	ER Defect Mechanism	Example/Notes
Alzheimer's, Parkinson's, ALS	Protein misfolding, ER stress, UPR	Neuronal death, aggregation of misfolded proteins
Cystic fibrosis, Robinow syndrome	ER retention of mutant proteins	Loss of function, monogenic inheritance
Type 2 diabetes, NAFLD, atherosclerosis	ER stress in metabolic tissues	Beta cell death, lipid accumulation
Alpha-1 antitrypsin deficiency	ER retention of misfolded protein	Liver/lung pathology
Cancer	Chronic ER stress, UPR activation	Tumor survival, therapy resistance
Prion diseases, NCLs	ER stress due to mutant/aggregated proteins	Neurodegeneration
Viral hepatitis (HCV, HBV)	Manipulation of ER stress pathways	Viral persistence, cell death



60° 10 1.2 N, 24° 57 18 E

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