



UCEM

Umeå Core Facility
Electron Microscopy



NNATIONAL
MMICROSCOPY
IINFRASTRUCTURE

BIOU

Correlative Light And Electron Microscopy (CLEM)

Workflows



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CLEM Strategies

Landmark based

- Gridded MatTek dish (labelled Protein)
- Gridded Coverslip/SEM (labelled protein)

On EM grid

- On Section fluorescence (Antibody labelling/labelled protein)
- In resin fluorescence (labelled protein)

In Cryo

- Without FIB milling (labelled protein)
- With FIB milling (labelled protein)

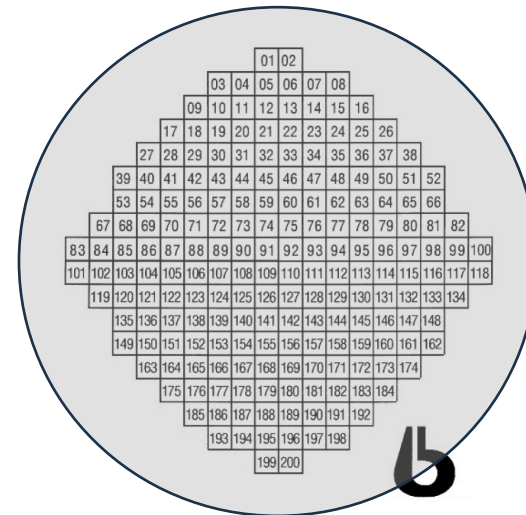


Correlation - Landmark Based

- Gridded MatTek dish

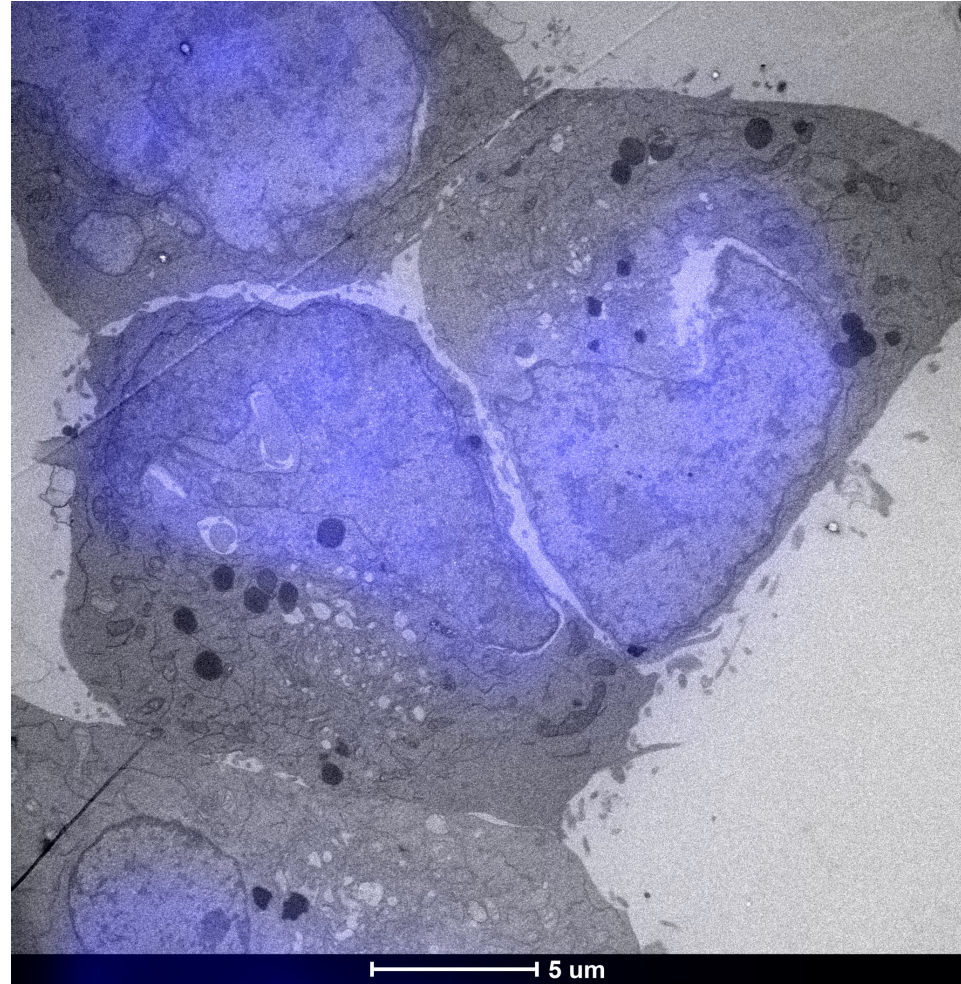
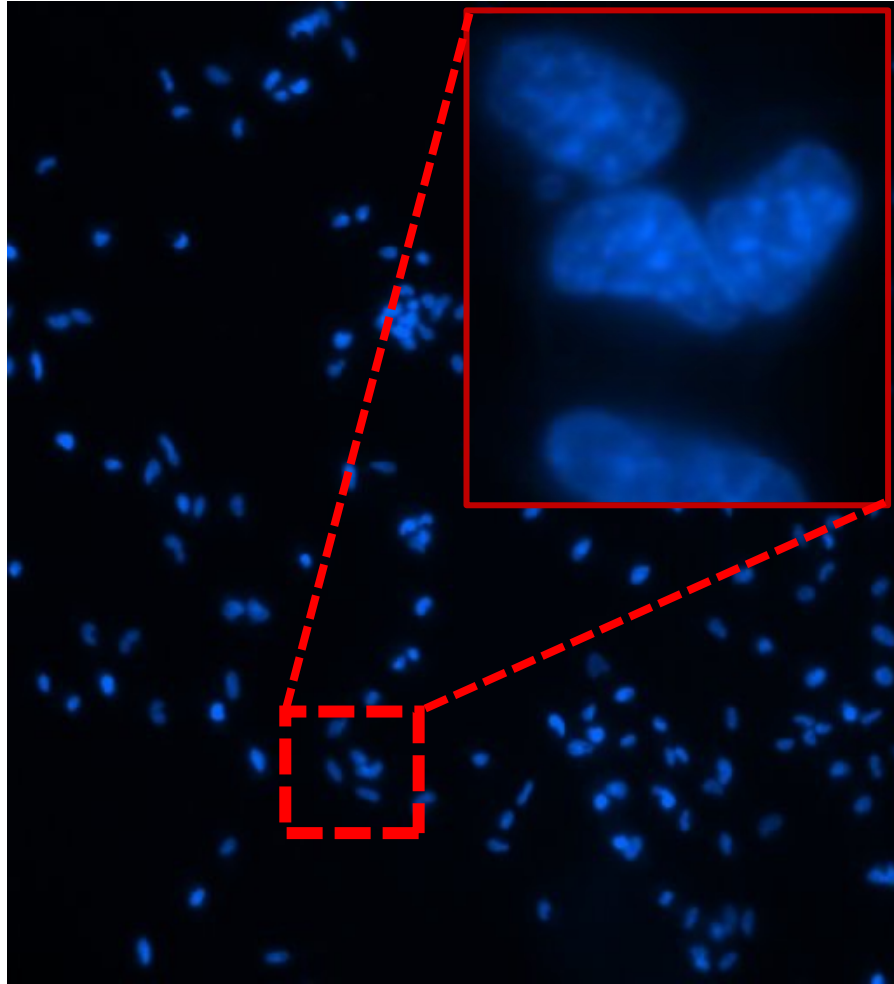


- SEM gridded Coverslip



Correlation - Landmark Based

- **Gridded MatTek dish** : Example 1: Mammalian cells with nuclear staining correlated in TEM

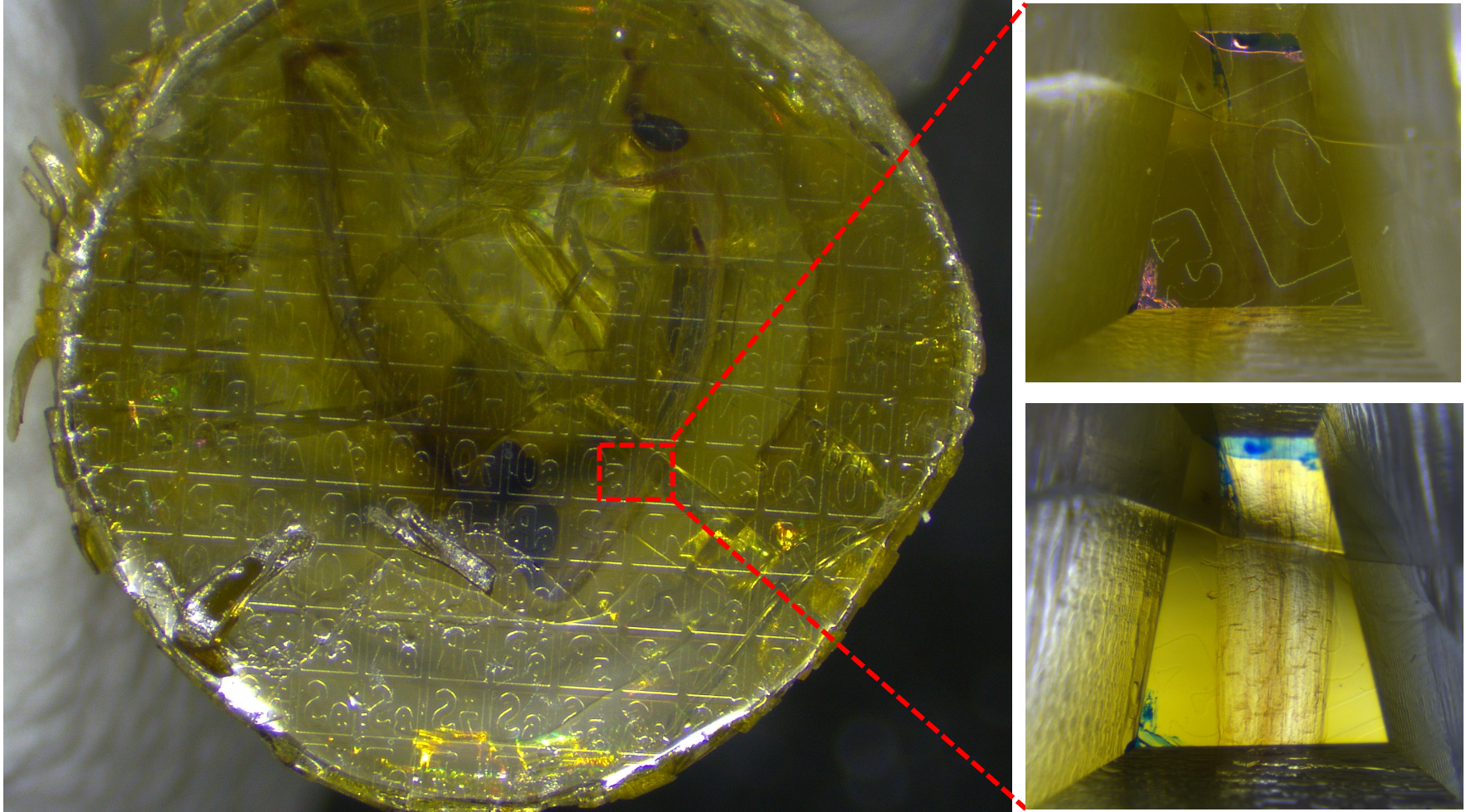


Live/Fixed imaging >> Chemical fixation >> Resin embedding >> Sectioning >> TEM



Correlation - Landmark Based

- **Gridded MatTek dish** : Example 2: Plant sample embedded in gelatin on gridded MatTek dish imaged on the Light microscope and correlated in TEM

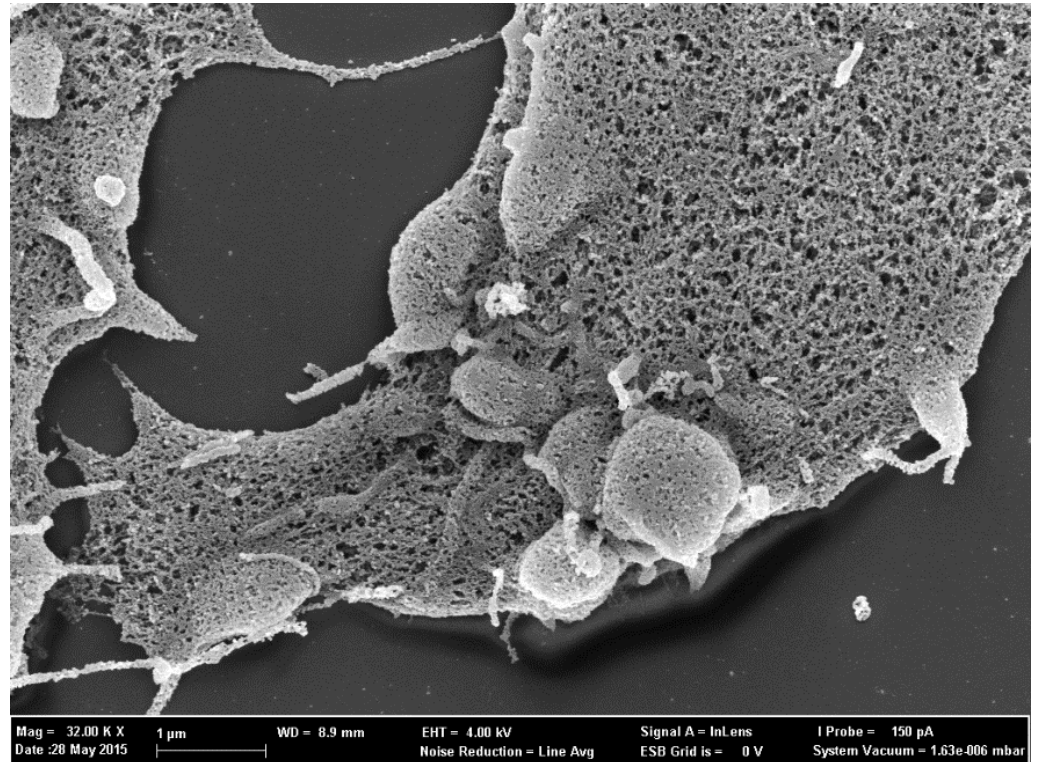
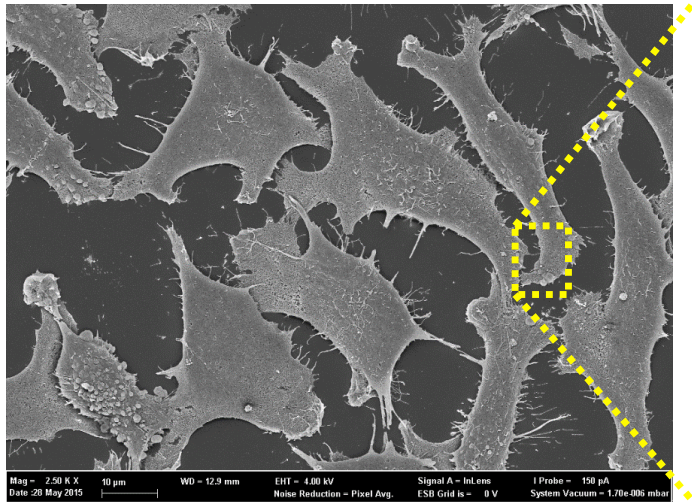
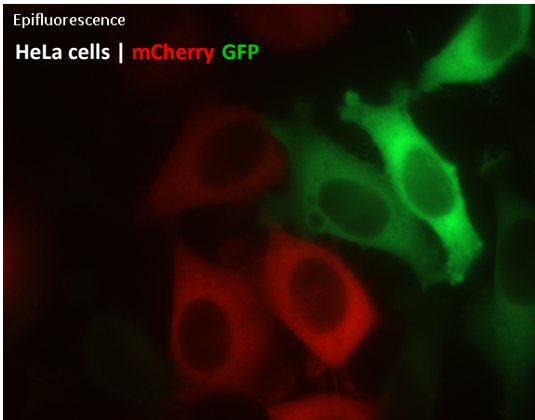


Live/Fixed imaging >> Chemical fixation >> Resin embedding >> Sectioning >> TEM



Correlation - Landmark Based

- **SEM Coverslip:** Example: Mammalian cells sample on gridded SEM coverslip imaged on the Light microscope and correlated in SEM



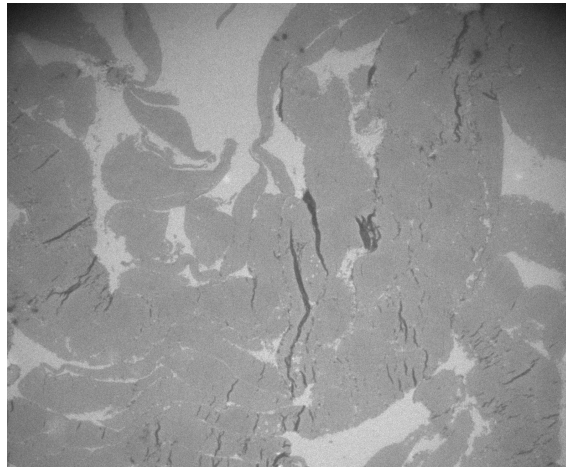
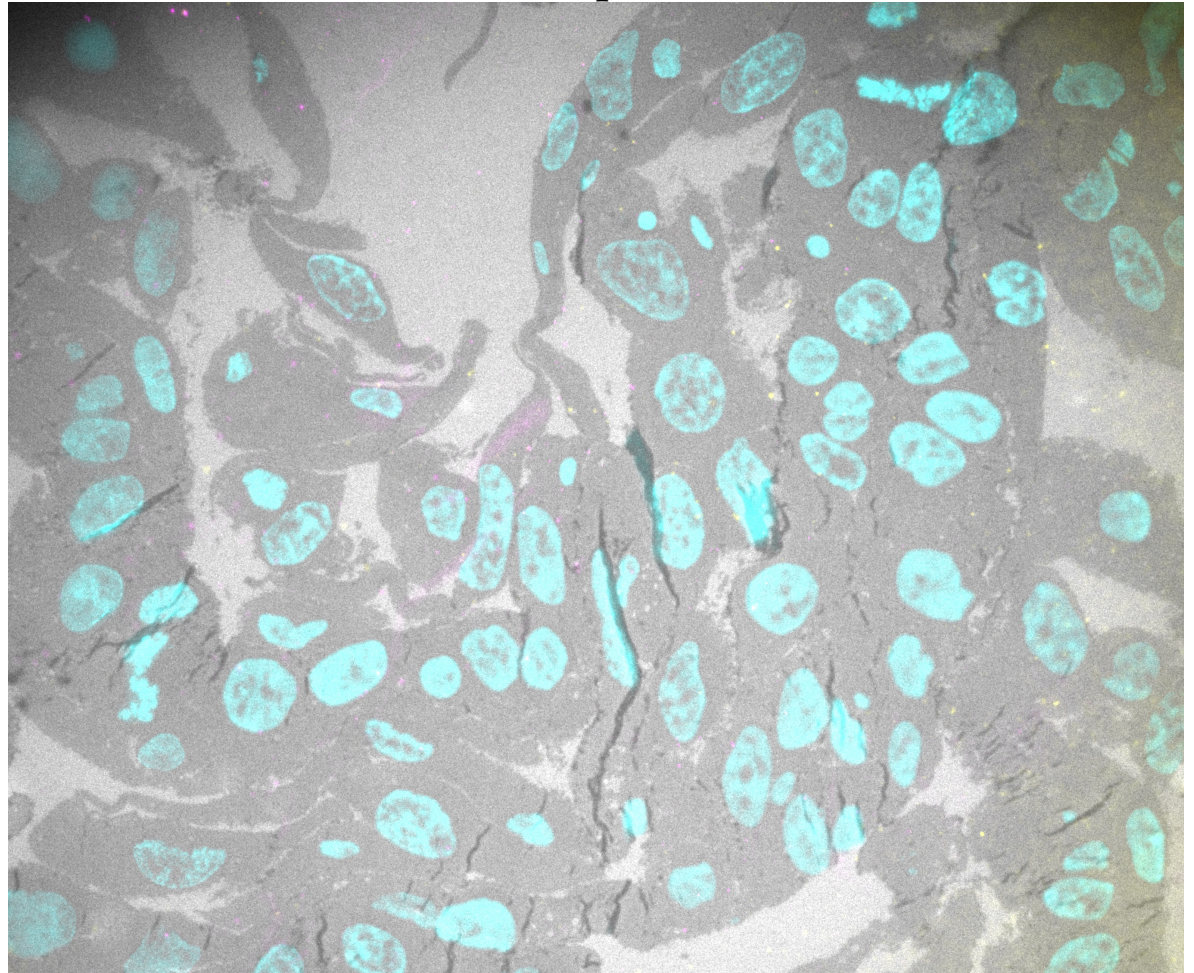
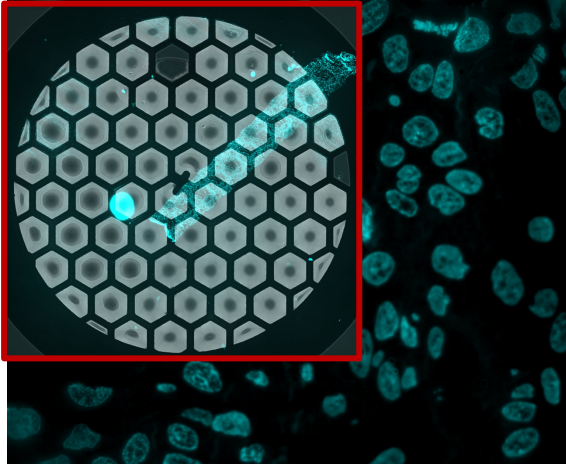
Live/Fixed imaging >> Chemical fixation >> Dehydration, CPD >> SEM



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Correlation - On EM Grid

- **On section fluorescence** – Example 1: Mammalian cells with fluorescently tagged proteins and nuclear stain correlated based on the shape of the cells on the section

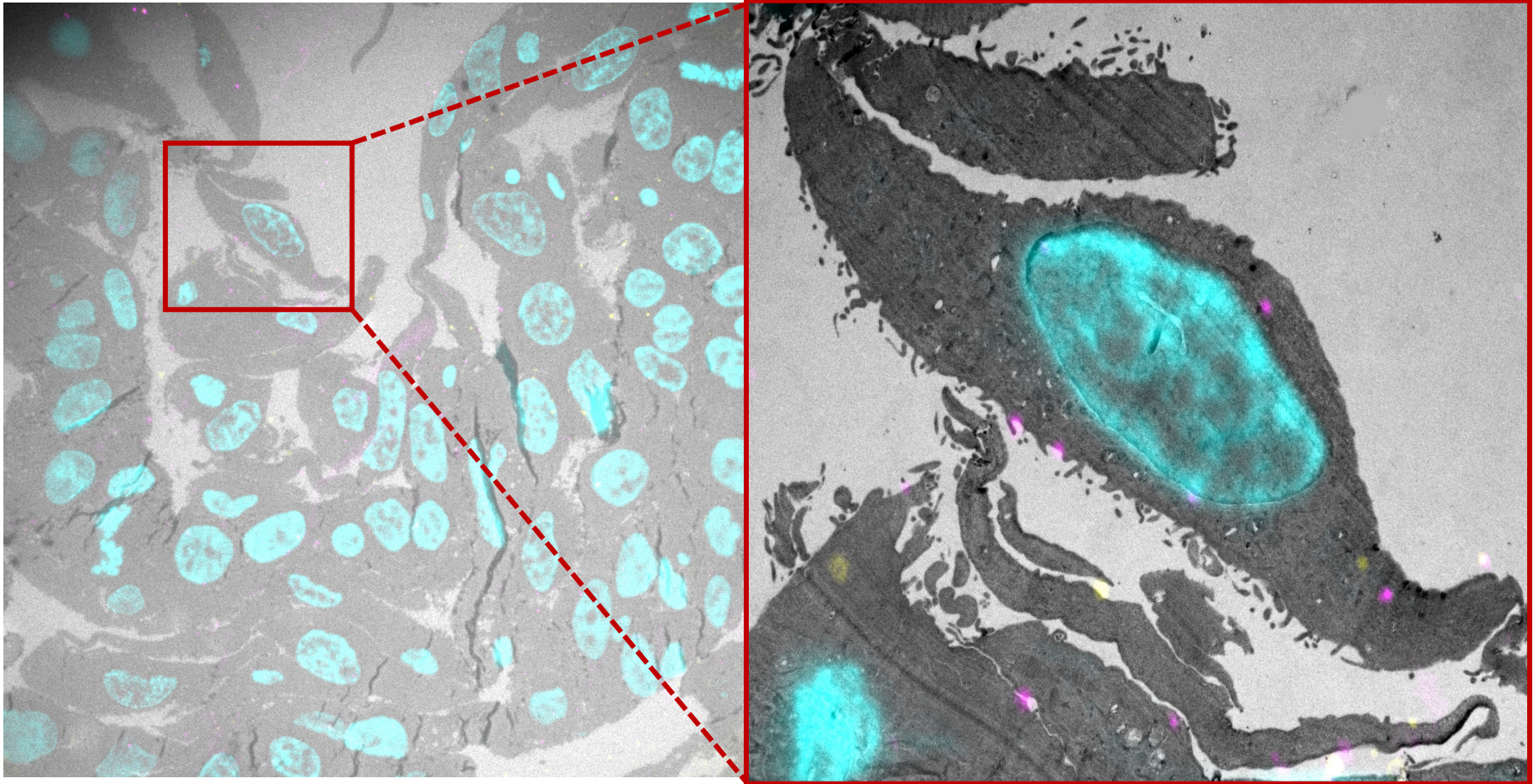


Chemical fixation >> Tokuyasu(cryo) >> cryo-sectioning >> Immuno-label >>
Light microscopy >> TEM



Correlation - On EM Grid

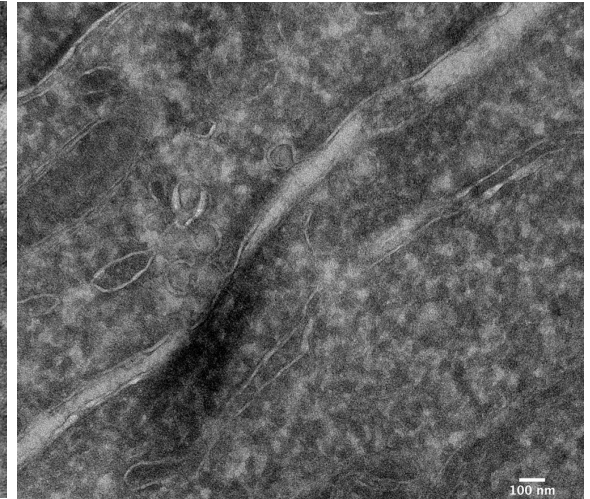
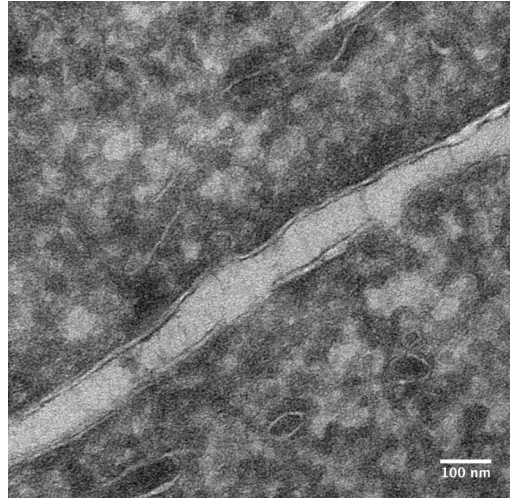
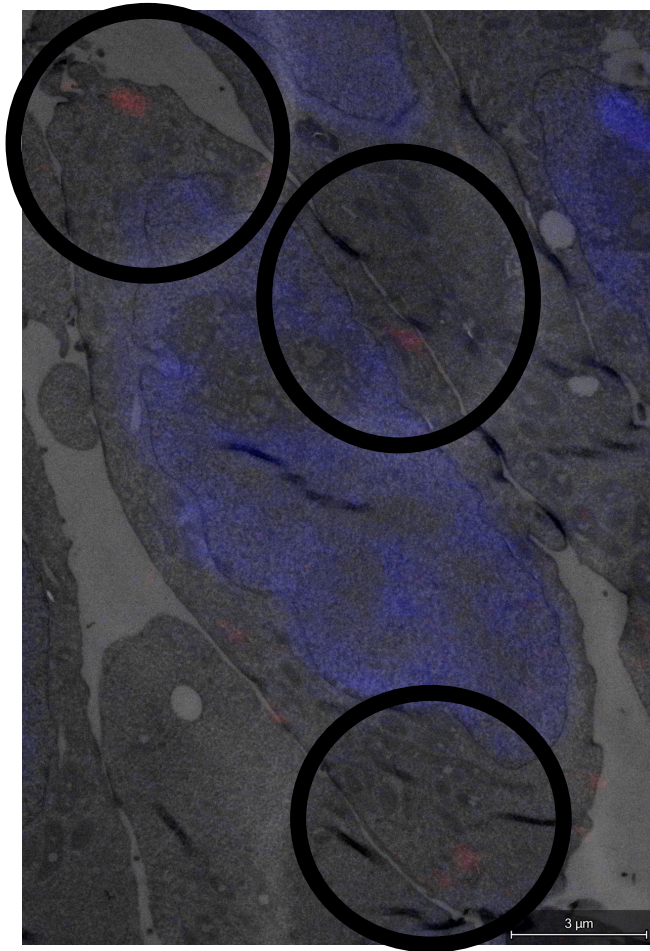
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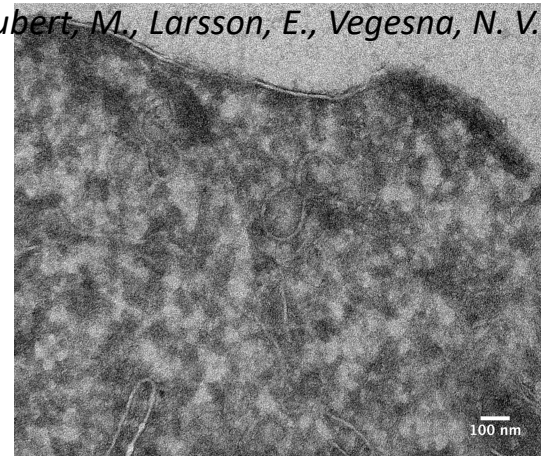
Chemical fixation >> Tokuyasu(cryo) >> cryo-sectioning >> Immuno-label >>
Light microscopy >> TEM

Correlation - On EM Grid

- **On section fluorescence** – Example 2: Mammalian cells expressing fluorescently tagged proteins on caveolae and post stained for nucleus correlated in LM and TEM



Hubert, M., Larsson, E., Vegesna, N. V. G., et al ; Elife 2020



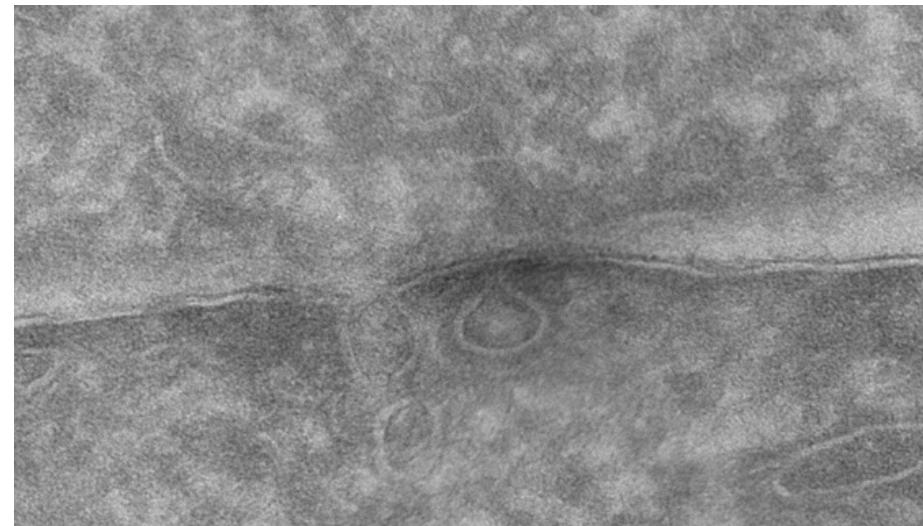
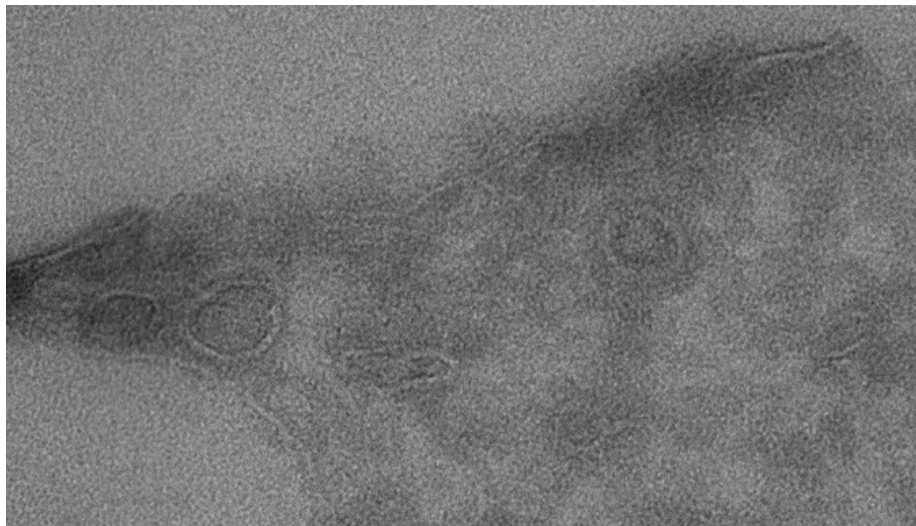
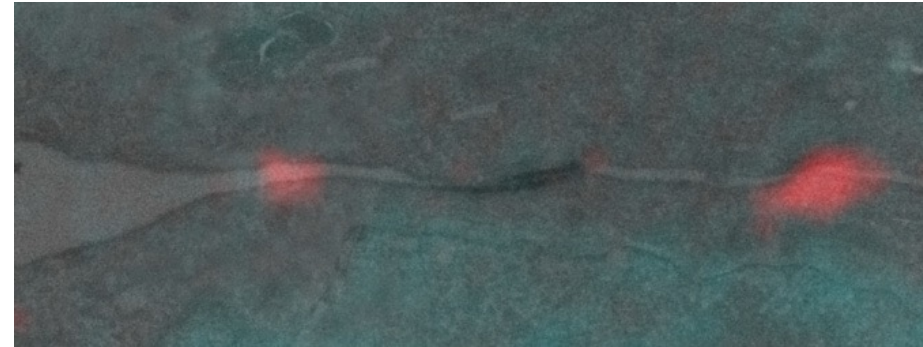
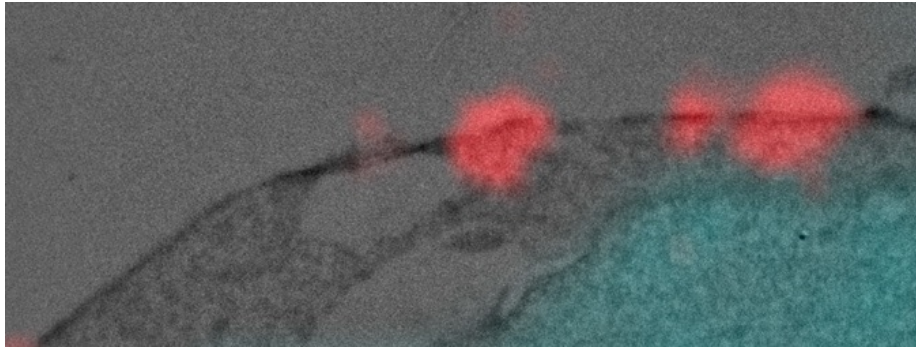
Chemical fixation >> Tokuyasu(cryo) >> cryo-sectioning >> Immuno-label >>
Light microscopy >> TEM



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Correlation - On EM Grid

- **On section fluorescence** – Example 2: Mammalian cells expressing fluorescently tagged proteins on caveolae and post stained for nucleus correlated in LM and TEM



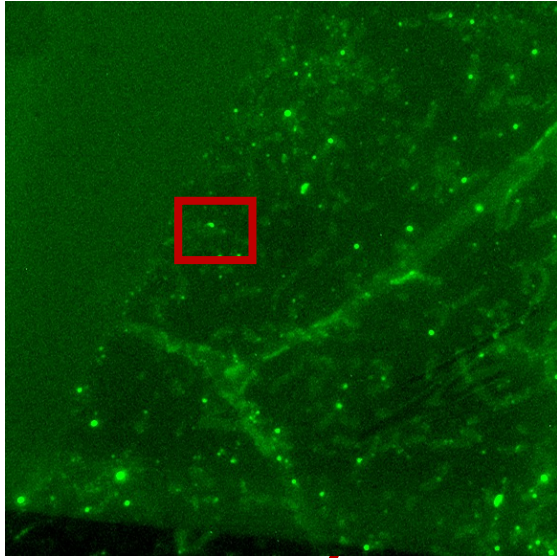
Hubert, M., Larsson, E., Vegesna, N. V. G., et al ; Elife 2020

Chemical fixation >> Tokuyasu(cryo) >> cryo-sectioning >> Immuno-label >>
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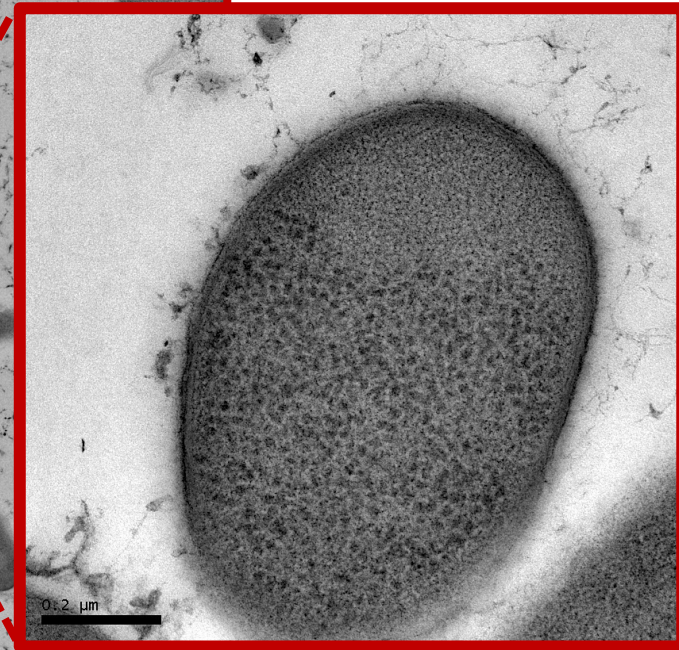
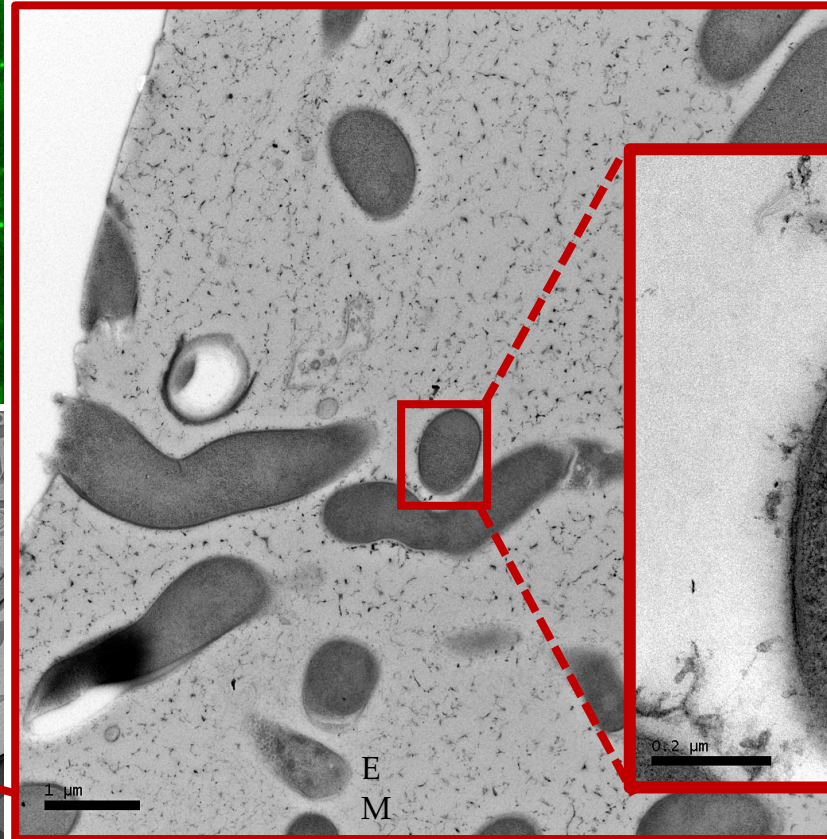
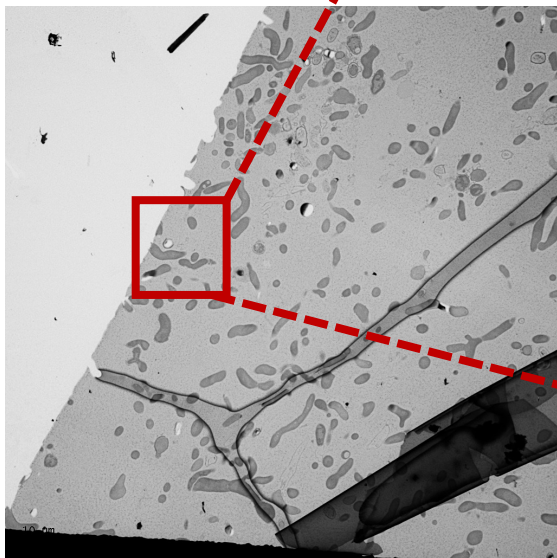
Correlation - On EM Grid

- **In resin fluorescence** – Example: Bacterial sample with a tagged protein imaged with LM on resin section and correlated in EM based on the shape of cells on the section

Light Microscopy



Electron Microscopy



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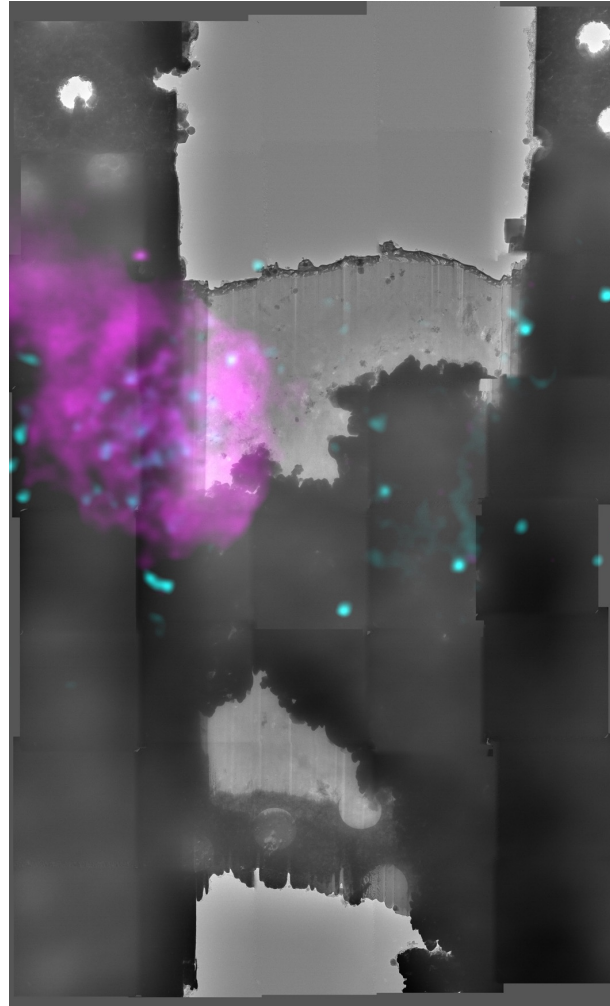
Streptomyces colicolor

Linda Sandblad, Sara Henriksson

High-pressure freezing >> HM20 Resin embedding >> Sectioning >> Light microscopy >> TEM

Correlation - In Cryo

- **With FIB** : Example - Mammalian cells labelled for lipid droplets and ER post correlated in LM and TEM



Cryo fixation >> Cryo-light microscopy (LM) >> FIB milling (SEM) >> Cryo-TEM >> Cryo-LM



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