IOCB SERVICE DAYS 2023

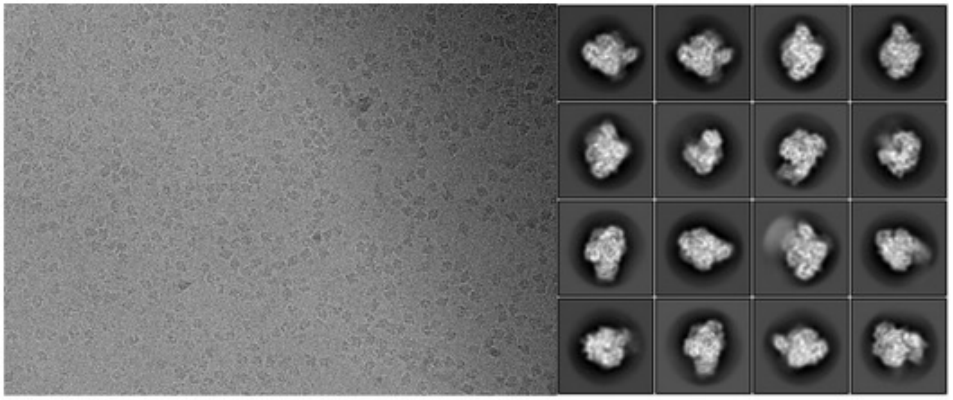
Presentation of services by:
Research-Service Groups / Service Groups / Core Facilities

Cryogenic Electron Microscopy

Tomas Kouba

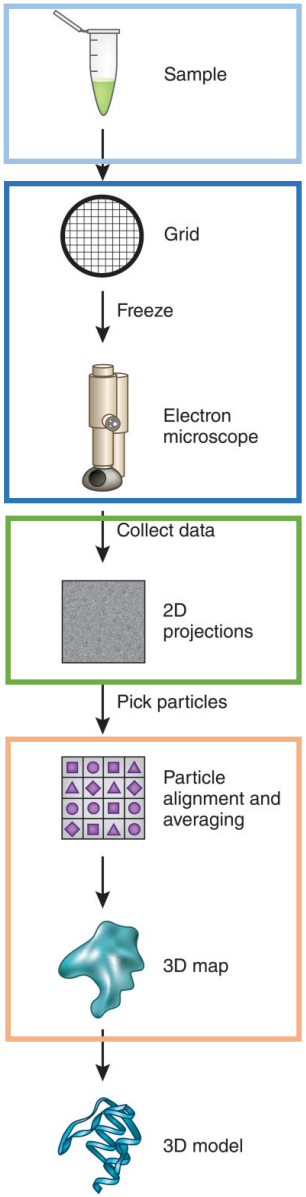
ÚOCHB AV ČR
IOCB PRAGUE

CRYO-EM at IOCB
TOWARDS 3D STRUCTURES OF BIOMOLECULES



Provisional Cryo-EM facility at the IOCB

- Basic sample characterisation
- Negative stain analysis
- Cryo-EM grid preparation
- Basic cryo-EM screening
- Advanced screening in CEITEC
- Data collection at Europe facilities
- Data processing at IOCB cluster
- **Cryo-EM 3D structure**



IOCB groups

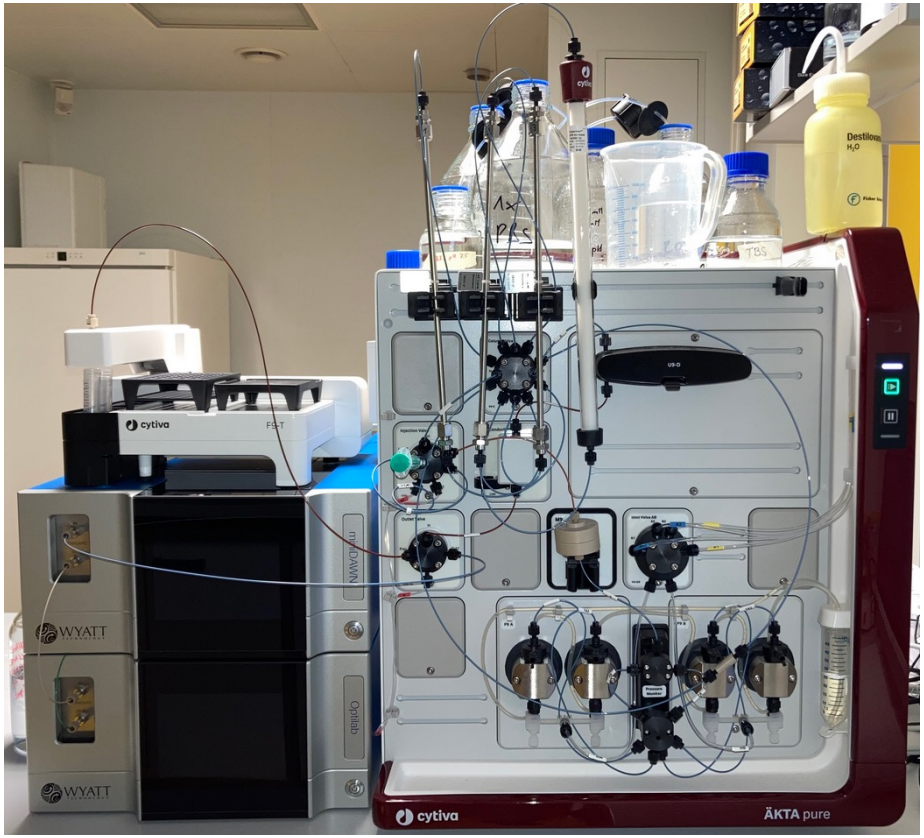
IOCB cryo-EM

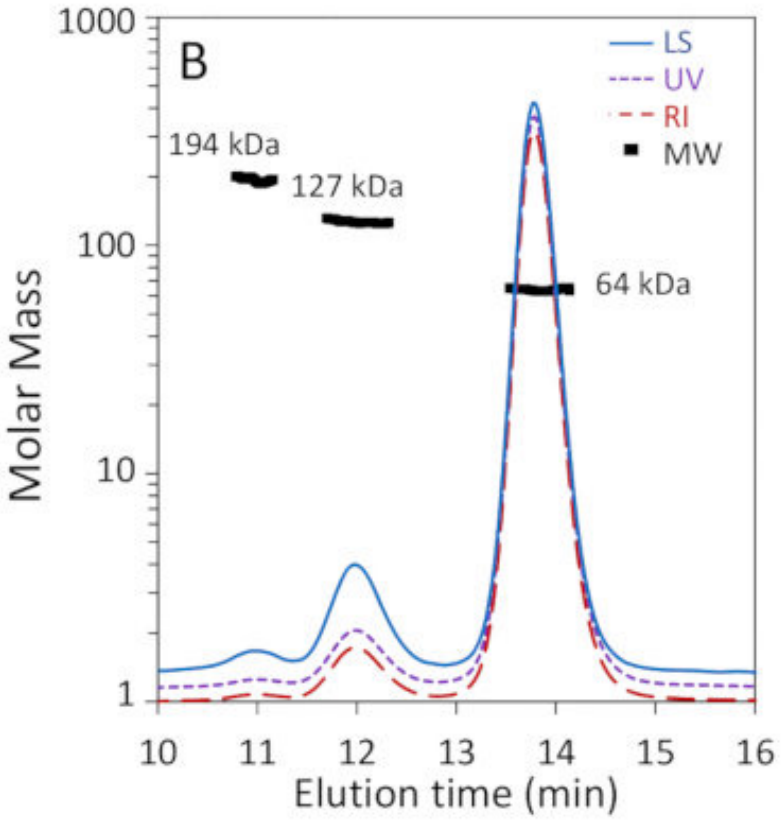
CEITEC or other facilities

IOCB cryo-EM data processing cluster

Cryo-EM sample preparation

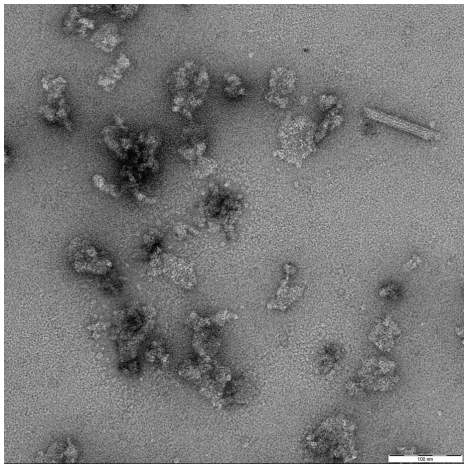
- Collaboration with Zoll group
- SEC on micro-column (AKTA pure micro), 20 ul injection volumes
- SEC-MALS to characterise molecular weigh, oligomer state
- Shodex PROTEIN KW40(3-5)-4F silica based columns – 15 min analysis



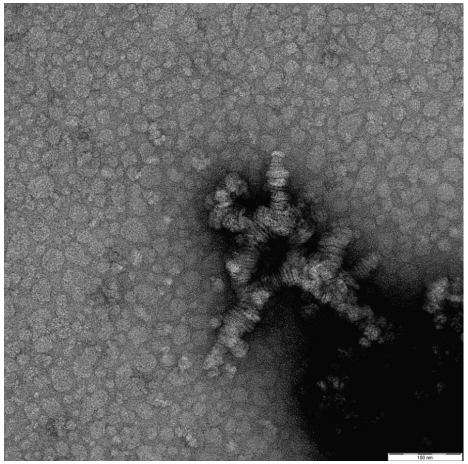


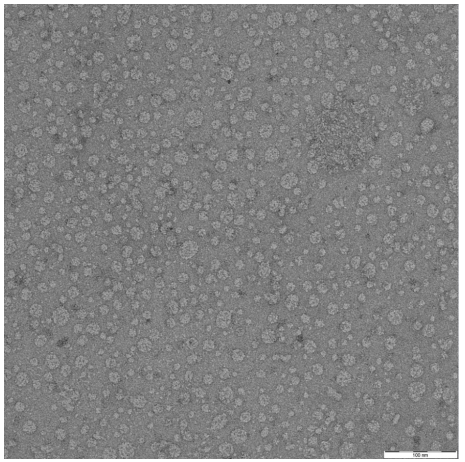
Negative stain analysis

- Quick (half-day) sample quality check

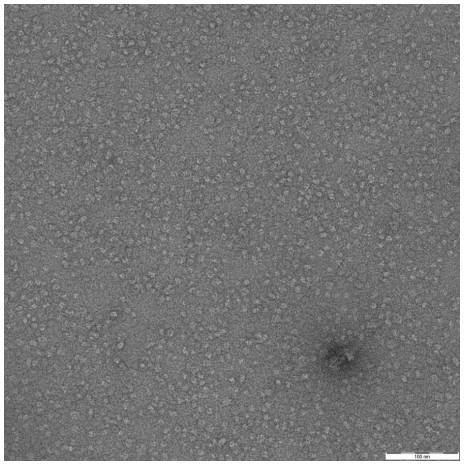


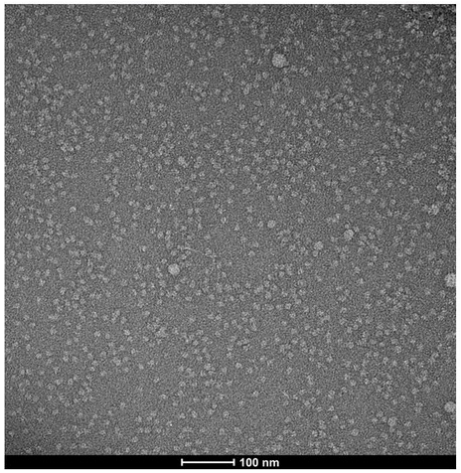
Aggregates/contamination

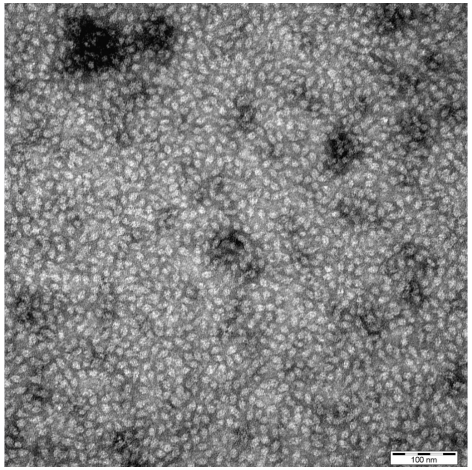




detergent artefacts/heterogeneous sample



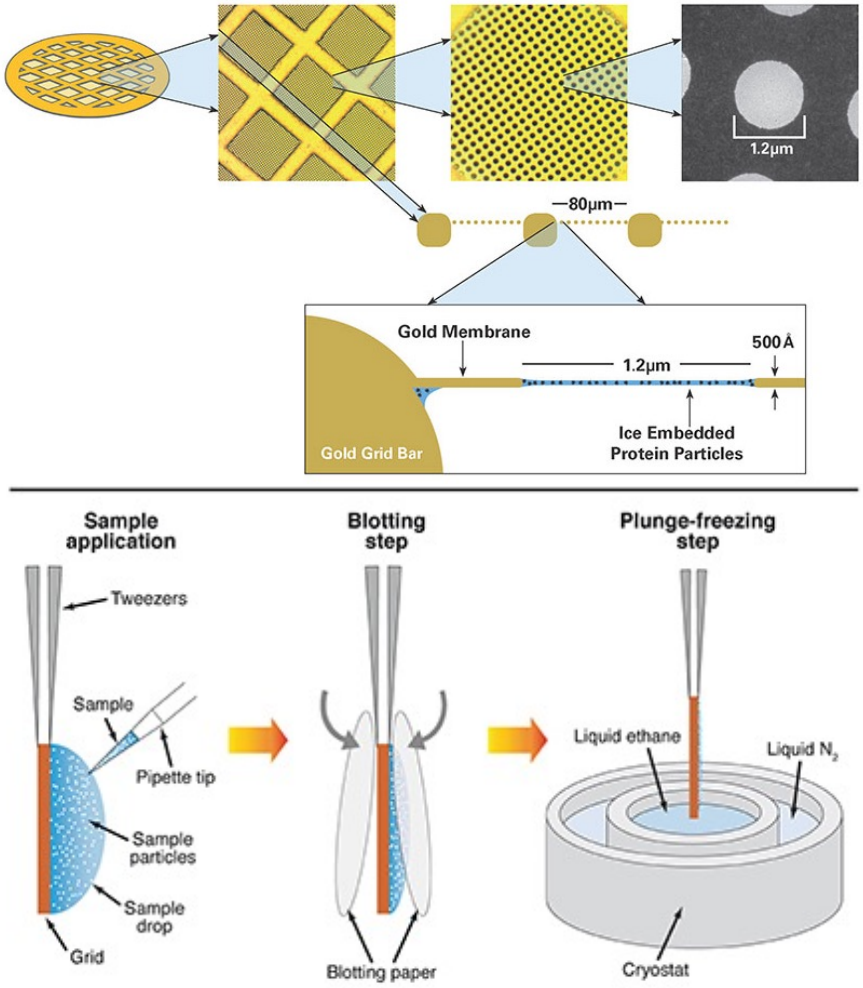




Homogenous sample, ready for cryo-EM grid preparation/screening

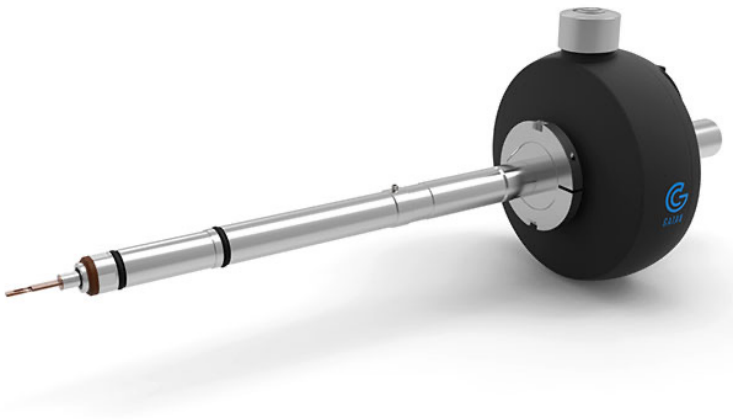
Cryo-EM grid preparation

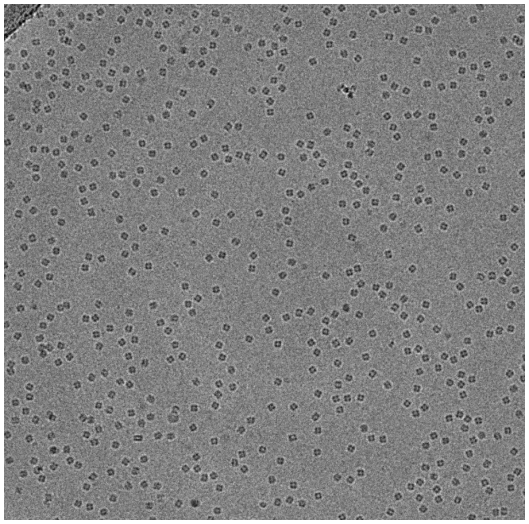
- **TFS Vitrobot Mark IV**
- Gold standard in cryo-EM grid preparation
- Samples passing negative stain analysis
- Local cryo-EM storage



cryo-EM screening

- **JEOL JEM2100plus**
- 200kV, LaB6 source, cryo-polepiece, side entry holder
- TVIPS CMOS TemCam-XF416ES camera, 48 frames/s
- serial EM low dose software for SPA analysis and Tomography





Advance screening and high resolution data collection in CEITEC, Brno

- Access through CIISB
- Peer reviewed proposal
- Regular remote access

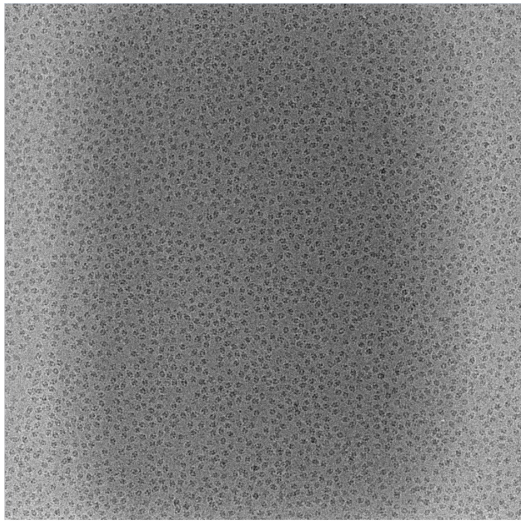
IOCB
JEOL JEM2100plus
200 kV LaB6
CMOS TVIPS
Basic screening

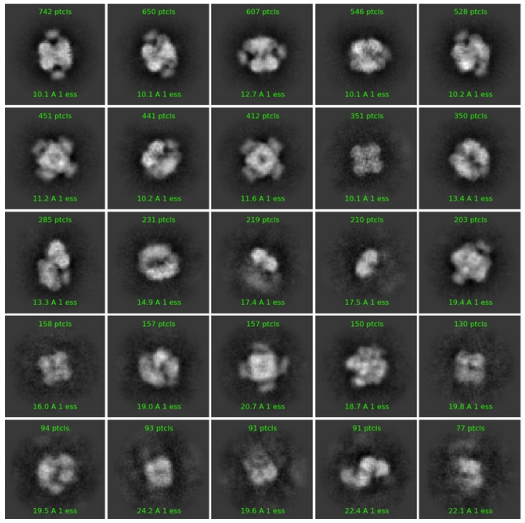
CEITEC
TALOS ARCTICA
200 kV FEG
K2 direct electron detector
12 positions autoloader
Automatic data acquisition
~4A resolution range in 1 day

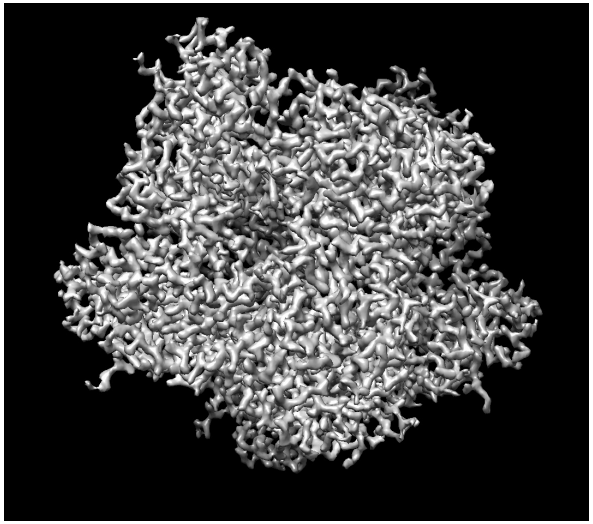
Europe facilities (CEITEC)
TITAN KRIOS
300kV FEG
direct electron detector
12 positions autoloader
Automatic data acquisition
high resolution data

Cryo-EM data analysis

- 2Ds, 3Ds, 3D refinement

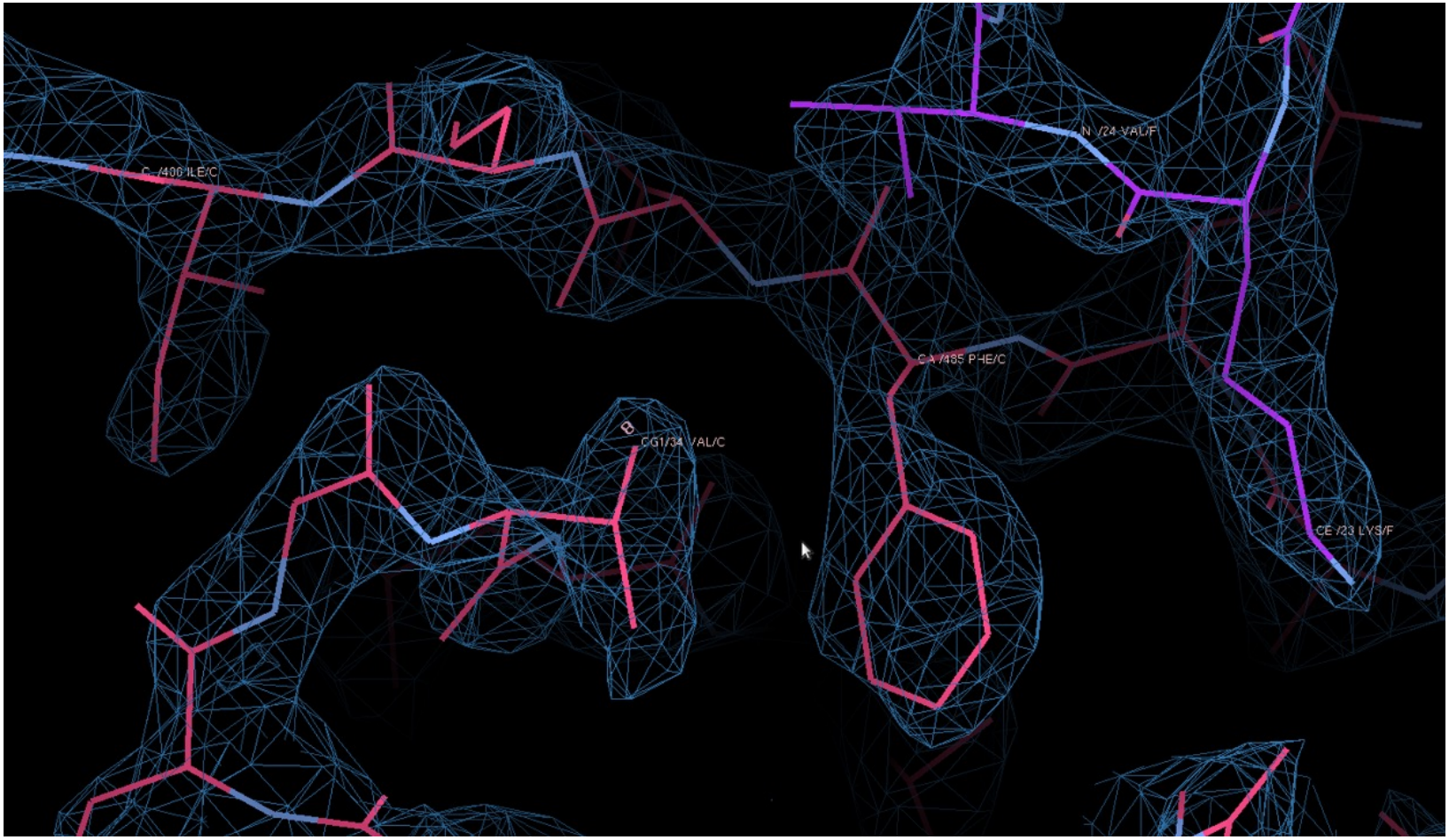






Cryo-EM data analysis

- 2Ds, 3Ds, 3D refinement
- ~2.5Å resolution cryoEM map (Iva Pichova group)
- ~6 months



Cryo-EM computing at IOCB

- Teeming up with IOCB computing core; Hector Martinez-Seara
- Cryo-EM WIKI on hpcg.uochb.cas.cz
- **SBGrid license available** sbgrid.org

To run RELION GUI

```
source /opt/uochb/soft/spack/20210921-git/share/spack/setup-env.sh
spack load relion@3.1.3
spack load fftw@3.3.9+mpi
spack load cuda@11.0.2
spack load openmpi@4.1.1~legacylaunchers

relion
```

https://hpc.nih.gov/apps/RELION/relion31_tutorial.pdf

IMPORT_MOVIES

MOTION CORRECTION MC

CTFFIND

PARTICLE PICKING TOPAZ

PARTICLE_EXTRACTION

2D_CLASSIFICATION

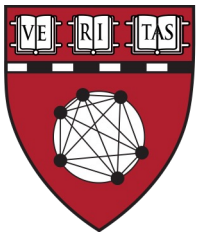
PARTICLE_SELECTION

3D_CLASSIFICATION

3D_REFINEMENT

TIPS from other HPC centers:

- NIH



SBGrid
CONSORTIUM

SERVICE vs TRAINING

‘Every user should get the maximum out of their data collection session’

SERVICE SCHEME

- Sample preparation
- Screening
- Data collection
- Data processing
- Collaboration based

cons:

- no knowledge and skill transfer
- depends on facility capacities

TRAINIG SCHEME

- Training of basic users
- Training of independent super-users
- Knowledge and skill transfer
- Super-users can train basic users
- Motivated users
- Growing EM-community
- More time for facility development

cons:

- initial lag time

Cryo-EM team

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EM team

Romana Hadravová
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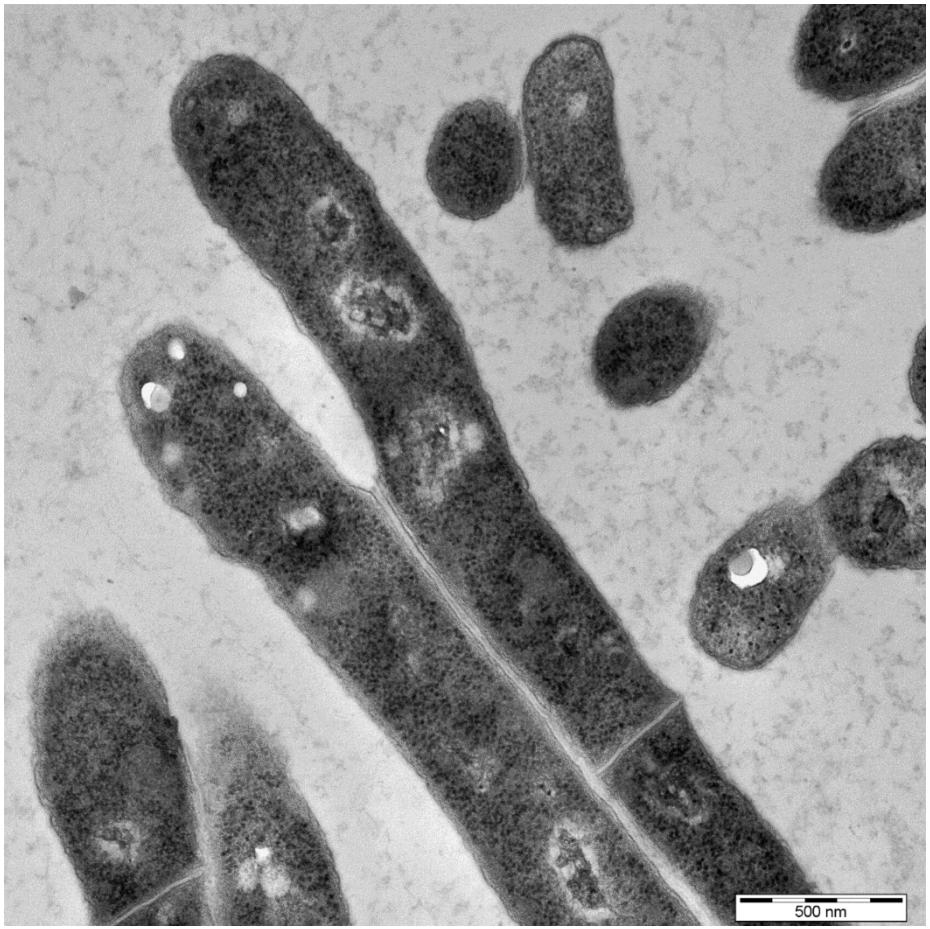
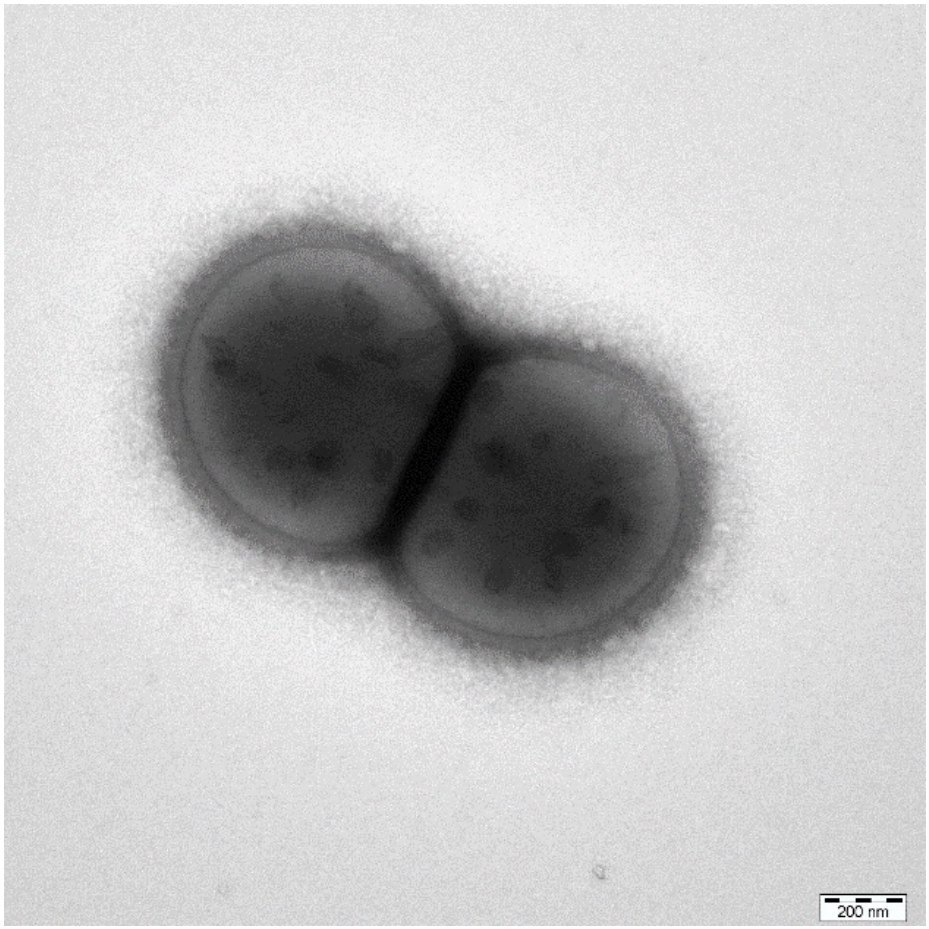
Ultrathin sections

- Cells
- Tissues
- Immunogold labeling

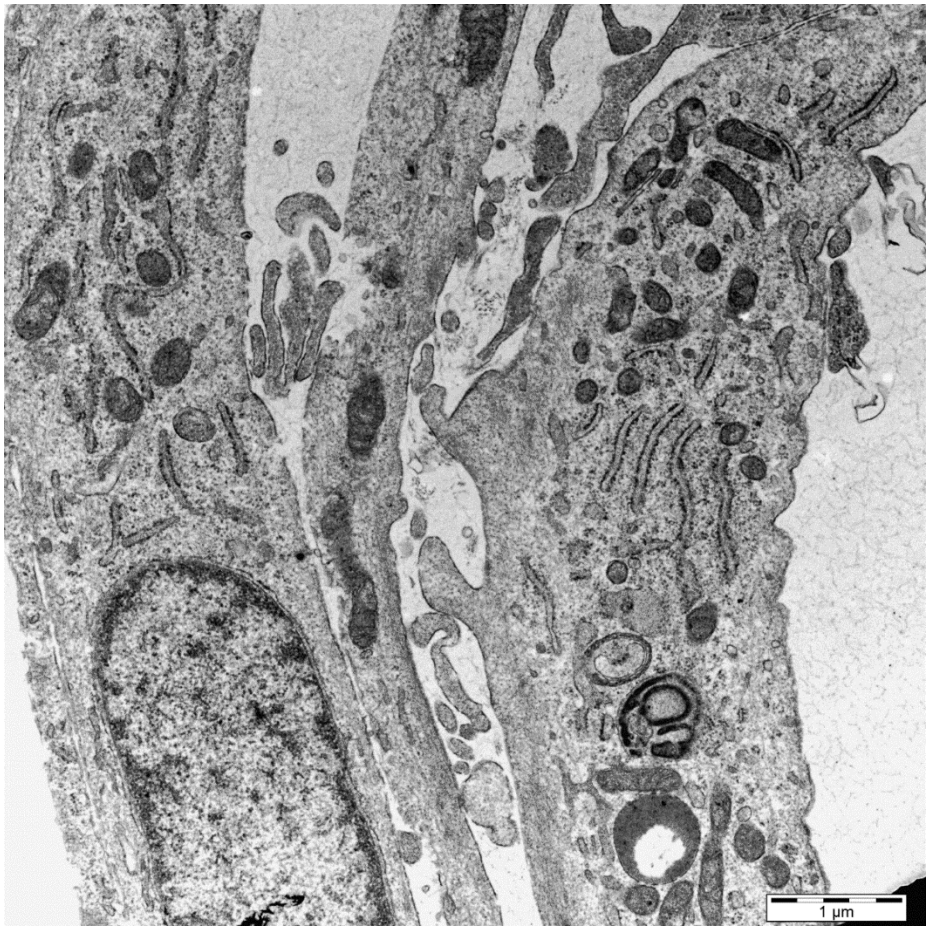
Materials



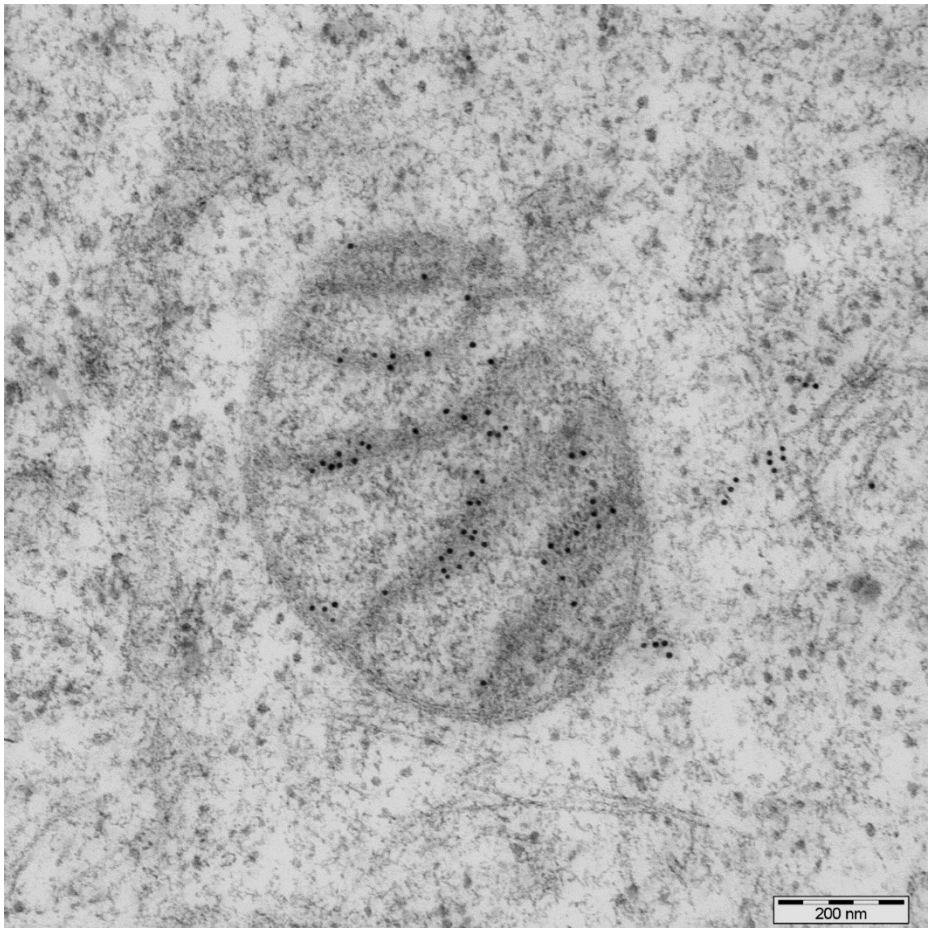
Mycobacterium



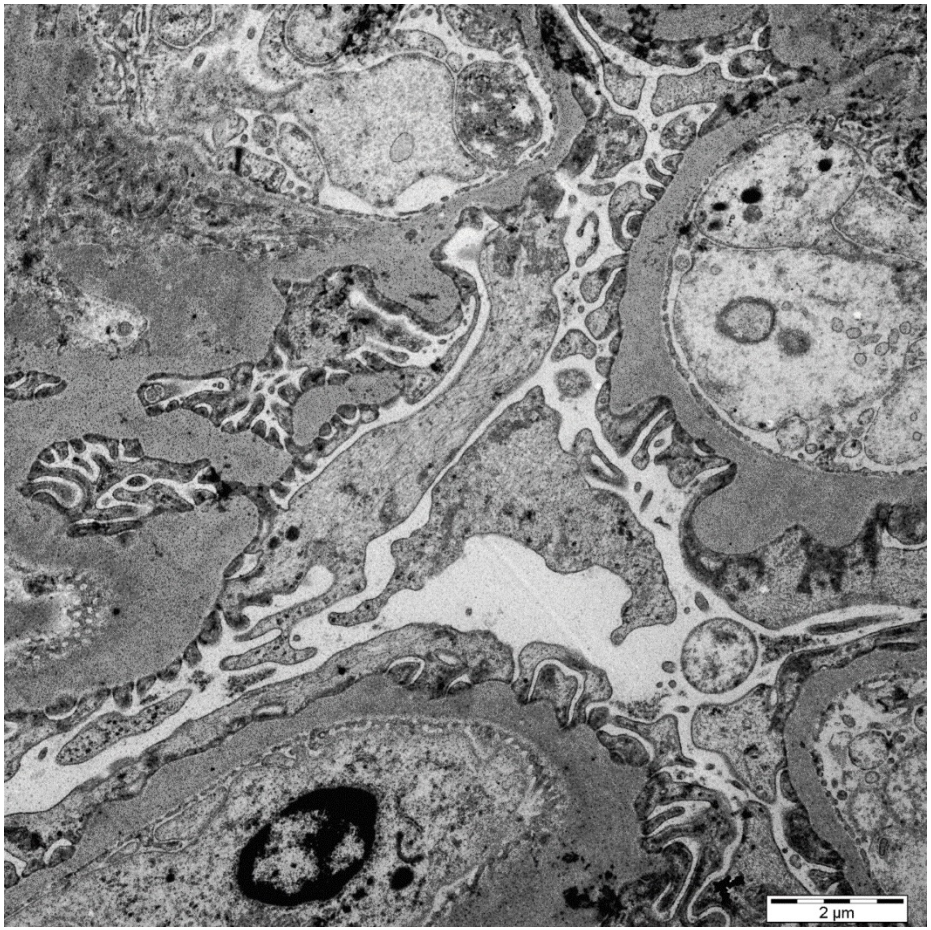
Staphylococcus Aureus



U251 cells

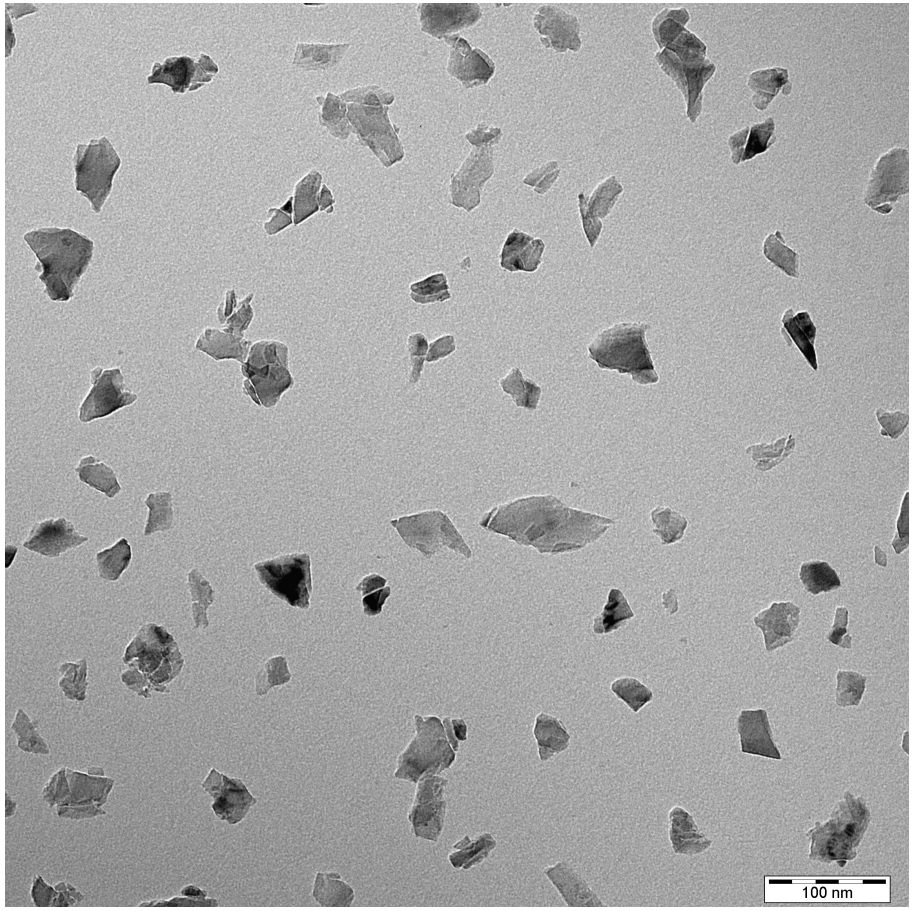


Gold immuno labelling

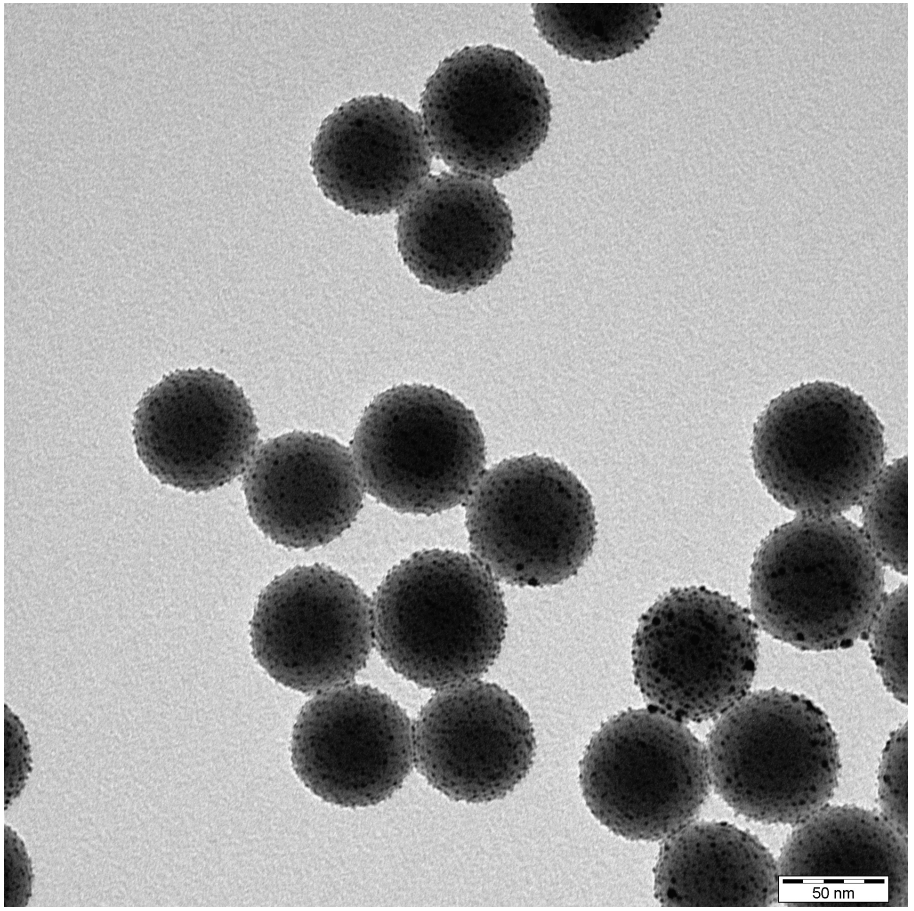


Kidneys

Materials



Diamond particles



Silicone particles
with gold nanoparticles

THANK YOU FOR YOUR ATTENTION !