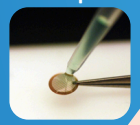
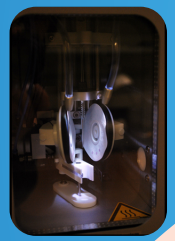
Molecular 3D-Structure Determination using Cryo-EM

BIOEM MOLECULAR AND CELLULAR BIOLOGY

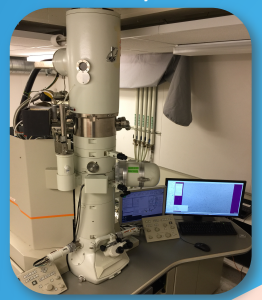
Purified sample



Vitrification via FEI Mark 3 vitrobot



Cryo-EM using the JEOL 2100 / DE20



CryoEM using the ThermoFisher Glacios/Gatan K3

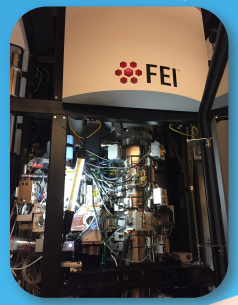
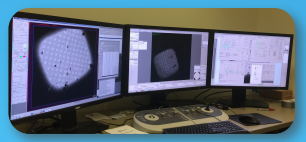
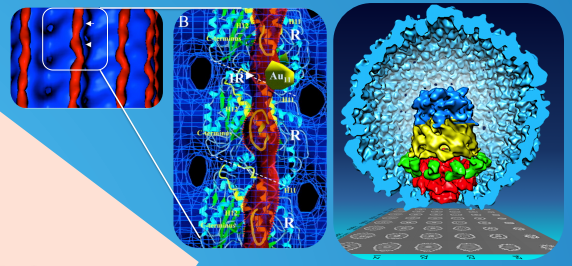


Image processing using GPU-enabled clusters





Sample

SAMPLE
- MW>150 kDa
- Homogeneous by SDS/PAGE
- Gel filtration purified
- Salt, pH, Buffer optimization
- 0.3 - 5 mg/ml

Screening

NEGATIVE STAIN
- Concentration check
- Homogeneity check
- Obtaining initial model
- JEOL 1230 100kV, 2K CCD

CRYO-SCREENING
- 10x negative stain concentration
- Freezing strategy to minimize Denaturation/aggregation
  - Minimal Buffer
  - Additives/Detergents
  - Grid types

Data Collection

GRID SCREENING
- Cryo-capable 200kV JEOL-2100F with DE-20 direct detector

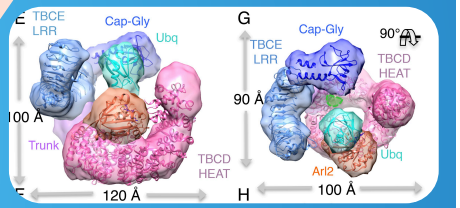
GRID SCREENING/HI-RES
- Thermo Scientific 200 kV Glacios with GATAN K3 detector and autoloader
- 12 screening samples/day
- 1000-3000 images/day

ULTRA HI-RES
- FEI Titan Krios at UCSF with Gatan K3 direct detector and phase plate
- 4 Days/month (BACEM)
- 1000-6000 images/day

Image data Processing

ON-THE-FLY DATA PROCESSING
- In-house workstation and storage computers
  - two dual-GPU, 12-cpu
  - two Quad-GPU 24-cpu
  - 130 TB storage array.
- On-the-fly Motion correction/ CTF estimation
- On-the-fly 2D class averages
- GPU based software available
  - SBGrid, Relion3, EMAN2

3-D Structure Analysis



STRUCTURAL ANALYSIS
- 2-D/3-D classification
- Mid –high resolution 3-D reconstruction
- *De novo* atomic model building
  - <1 nm : Secondary structure
  - 3-5 Å : Near-atomic/Backbone
  - <3 Å : Side chains/ligand

Contact and Consultation

Enoch Baldwin (Faculty Director)
epbaldwin@ucdavis.edu
Tel: 530-752-1108

Fei Guo, Ph.D. (Technical Director)
feiguo@ucdavis.edu
Tel: 530-752-1676