cryoEM short courses

SINGLE PARTICLE MARCH 2-6
MORNING LECTURES & ROUNDTABLES
AFTERNOON HANDS-ON PRACTICALS
The program aims to broaden access to high-resolution cryoelectron microscopy (cryoEM) for biomedical researchers, by creating national service centers, and cultivating a skilled workforce, through the development and implementation of cryoEM training material.
Lightning talks
Flash talks
60-90 seconds each
EMILIA ARTURO
LA JOLLA INSTITUTE FOR IMMUNOLOGY
IRINA BREGY
UNIVERSITY OF BERN
PhD Bucket List:
- Single Particle Analysis of two TAC components
- Single Particle Analysis of mitochondrial DNA replication Factor
- Cryo Electron Tomography of the TAC
- Mapping particle structures into the tomograms

Tripartite Attachment Complex (TAC)

Irina Bregy
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CHRISTIANE BRUGGER
BROWN UNIVERSITY
How to cope with stress – lessons from *E. coli*

RssB "OFF"

RssB "ON"

RssB-δS complex

δS degradation

exponential growth conditions

ATP

ClpXP

stress conditions

inactive RssB

stress induced anti-adaptors
ELEONORA DI ZANNI
WEILL CORNELL MEDICAL COLLEGE
Bacteriophage-host interactions and virus structures

Sarah Doore
March 2, 2020
NCCAT SPA Short Course
Phage-host interactions

*Shigella* virus Sf6


How do bacteriophages interact with their specific hosts?

*Shigella* spp. are Gram-negative bacteria – many phages recognize lipopolysaccharide + an outer membrane protein. How and where?

PDB 4URR

Shigella virus Sf22

PDB 2J1N
Virus structures

*Shigella* virus Sf14 – uncommon capsid geometry

High resolution capsid structure of the mature virion?
... of the procapsid?
... of ejected particles?

Resolve structures of the tail, especially the baseplate and tail fibers?
Structural analyses of molecular machines in autophagy

Andrew Grassetti
Postdoctoral Associate
Davis Lab
MIT
Autophagy overview and preliminary data
Single particle cryo-EM on influenza polymerase
Replicating polymerase to structure- workflow

biochemical optimization
- RNA template design
- use of specific NTPs, NTP analogues
- reaction time and conditions

cryo-EM sample preparation & data collection

Am. Sulphate precipitation
→ Ni2+ Affinity
→ Strep tag affinity
→ Heparin

Tecnai-12
→ Talos Glacios

Krios
ESRF or EMBL HD

data processing & structure determination
Structural studies of hypomodified ribosomes

Sohail Khoshnevis

Department of Biology, Emory University and
Department of Biochemistry, Emory University School of Medicine, Atlanta, GA
Ribosomal RNAs are post-transcriptionally modified at functionally important sites

Hypomodification affects the function of ribosomes. But why?
Dr. Kyle Kroeck
EM density of AcrB in a native cell-membrane nanoparticle.

Structure of lipid bilayer and protein–lipid interactions
DAVID LIN
IOWA STATE UNIVERSITY
Models of Bruton’s tyrosine kinase (Btk) autoinhibition and activation
MARIA MARTINEZ MOLLEDO
INSTITUT PASTEUR
Maria Martinez Molledo
Postdoctoral researcher
IECB (Bordeaux, France)
Research interest:

Structural and functional characterization of human membrane proteins, nutrient uptake systems and potential drug targets.
Motion correction (MotionCor2) of 7,365 movies

CTF estimation (Gctf) of 7,365 movies

Manual exposure curation (7,317 good movies)

Manual reference picking (~1,800 particles)

Progressive particle extraction and template optimization (~1,350,000 particles)

Rounds of 2D classification and 2D selection (<300,000 particles)

Rounds of Ab initio 3D references (32,031 particles)

NU-Refinement of the good classes (32,031 particles)

3D auto-refine

CTF particle refinement

RELION 3.0  cryoSPARC v2.12.4

Class 0
22,244 particles
29.30%

Class 1
21,649 particles
28.51%

Class 2
32,031 particles
42.19%
Structural studies of co-translational targeting machinery

Smriti Sangwan
Post-doc in Peter Walter’s lab at UCSF
Graduate studies in X-ray crystallography at UCLA in David Eisenberg’s lab
Co-translational protein targeting at the ER

1. IRE1 binds SRP-bound ribosomes preemptively
2. IRE1 binds ribosomes engaged with the translocon
3. Proteins fail to fold properly
4. IRE1 binds unfolded proteins and oligomerizes
5. mRNA decay/ XBP1 splicing

Reduced ER load, upregulation of chaperones

How does IRE1 interact with the co-translational targeting machinery?
What does active IRE1 look like?
How do these interactions change under stress?
Cryo-EM studies of IRE1 native pull downs from HEK293 cells

HEK293 cells expressing flag-tagged IRE1

↓

Isolate microsome fraction

↓

Purify intact complexes from cells by immunoprecipitation solubilise in detergent

**Supernatant**  |  **Pellet**
---|---
**IRE1** | ![IRE1 Image]
**Sec61** | ![Sec61 Image]
**Ribosome** | ![Ribosome Image]
**SRP** | ![SRP Image]
KYE STACHOWSKI
OHIO STATE UNIVERSITY
KARTHIKEYAN SUBRAMANIAN
WEST VIRGINIA UNIVERSITY
THE GOLDEN AGE

HP1029

CRBP1

CAIP

Lpp20

[Images of various proteins and icons representing food and drinks]
THE DARK AGE
ZHEN XU
UNIVERSITY OF IOWA
NCCAT
Single Particle Short Course

The University of Iowa
Carver College of Medicine
Protein & Crystallography Facility
Zhen Xu, Ph.D.

March 2\textsuperscript{nd}, 2020

A HAWKEYE’S VIEW OF CAMPUS
Cryo-EM study of Protein-DNA complex