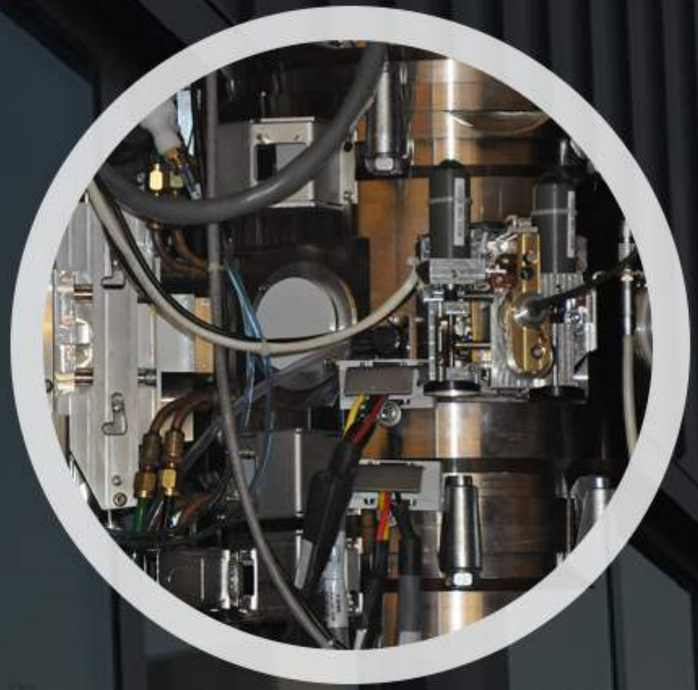


NCCAT

cryoEM short courses

SINGLE PARTICLE MARCH 2-6

MORNING LECTURES & ROUNDTABLES

AFTERNOON HANDS-ON PRACTICALS

NMR

CoMD/ NMR

X-ray

NYX @NSLS-II

Protein Production

COMPPÅ

NIH P41 - National Biomedical Technology Research Resources (BTRR)

National Synchrotron Light Source II

BROOKHAVEN NATIONAL LABORATORY

19-ID

NYX

NRAMM

photo by Swapnil Bhatkar

TRANSFORMATIVE HIGH RESOLUTION CRYO-ELECTRON MICROSCOPY PROGRAM

CryoEM

https://commonfund.nih.gov/CryoEM

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**.

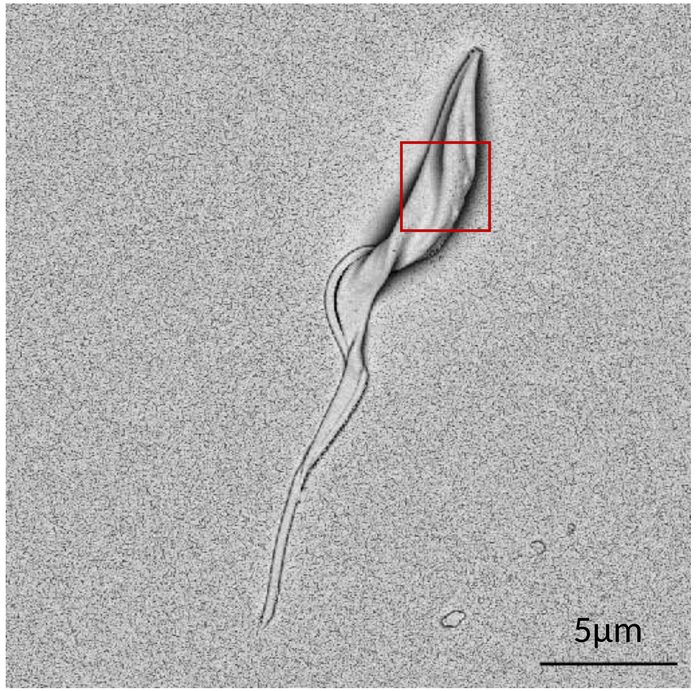
Lightning talks
Flash talks

60-90 seconds each

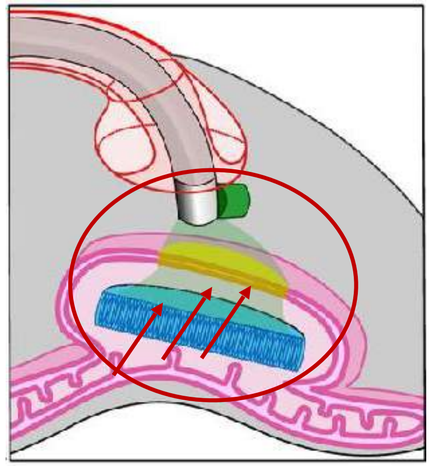
EMILIA ARTURO
LA JOLLA INSTITUTE FOR IMMUNOLOGY

AGNESE BARONINA
UNIVERSITY OF OXFORD

IRINA BREGY
UNIVERSITY OF BERN



Tripartite Attachment Complex (TAC)



PhD Bucket List:

- Single Particle Analysis of two TAC components
- Single Particle Analysis of on mitochondrial DNA replication Factor
- Cryo Electron Tomography of the TAC
- Mapping particle structures into the tomograms

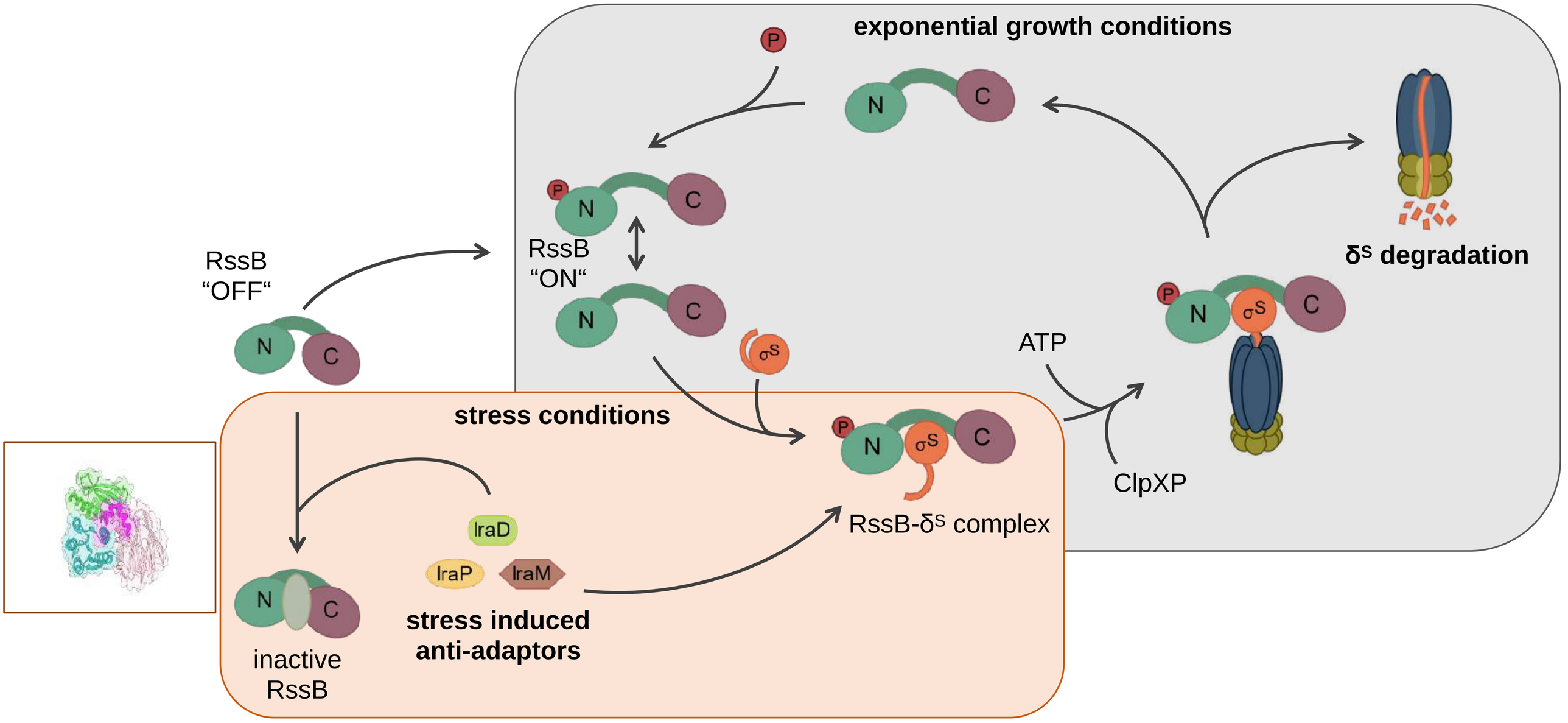
Irina Bregy
University of Bern
Institute of Anatomy
Institute of Cell Biology
Switzerland

@OchsenreiterL
@ BregyIrina

irina.bregy@ana.unibe.ch

CHRISTIANE BRUGGER
BROWN UNIVERSITY

How to cope with stress – lessons from *E. coli*



ELEONORA DI ZANNI
WEILL CORNELL MEDICAL COLLEGE

SARAH DOORE
MICHIGAN STATE UNIVERSITY

Bacteriophage-host interactions and virus structures

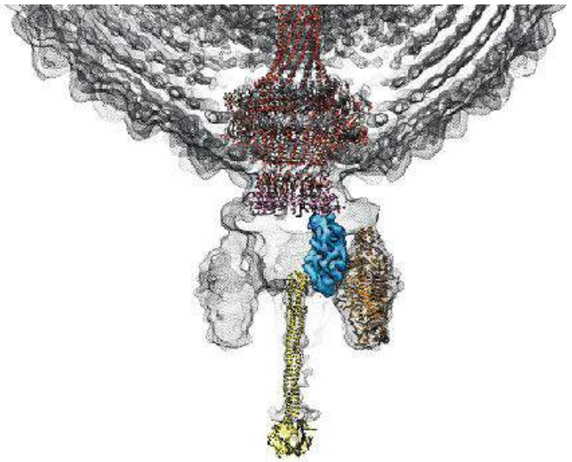
Sarah Doore

March 2, 2020

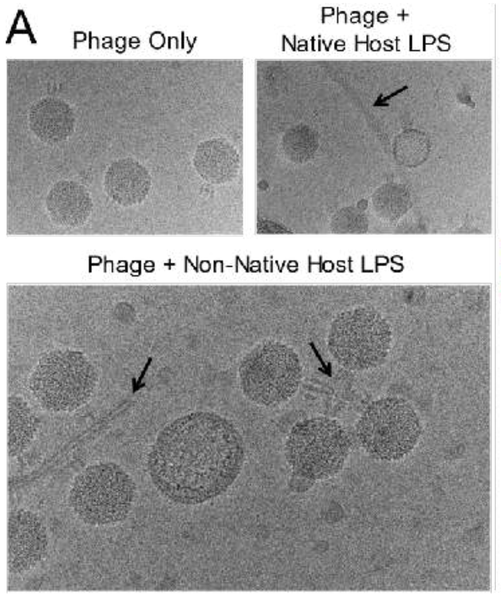
NCCAT SPA Short Course

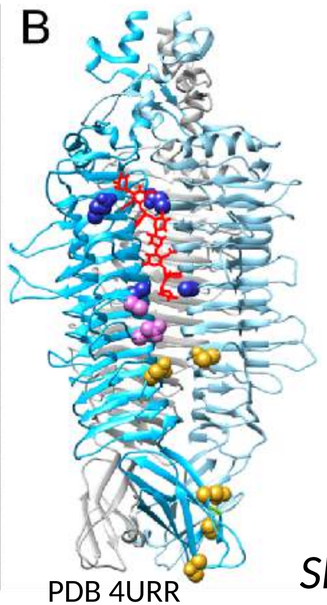
Phage-host interactions

Shigella virus Sf6



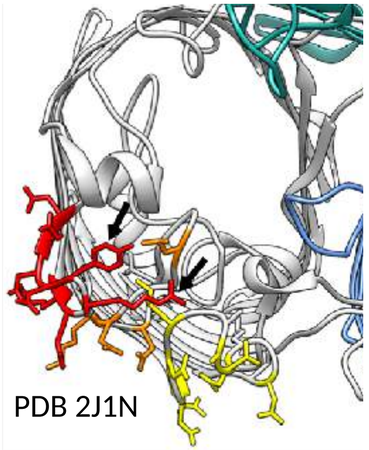
Parent (2012) *Virology* 427:177





PDB 4URR

Shigella virus Sf22



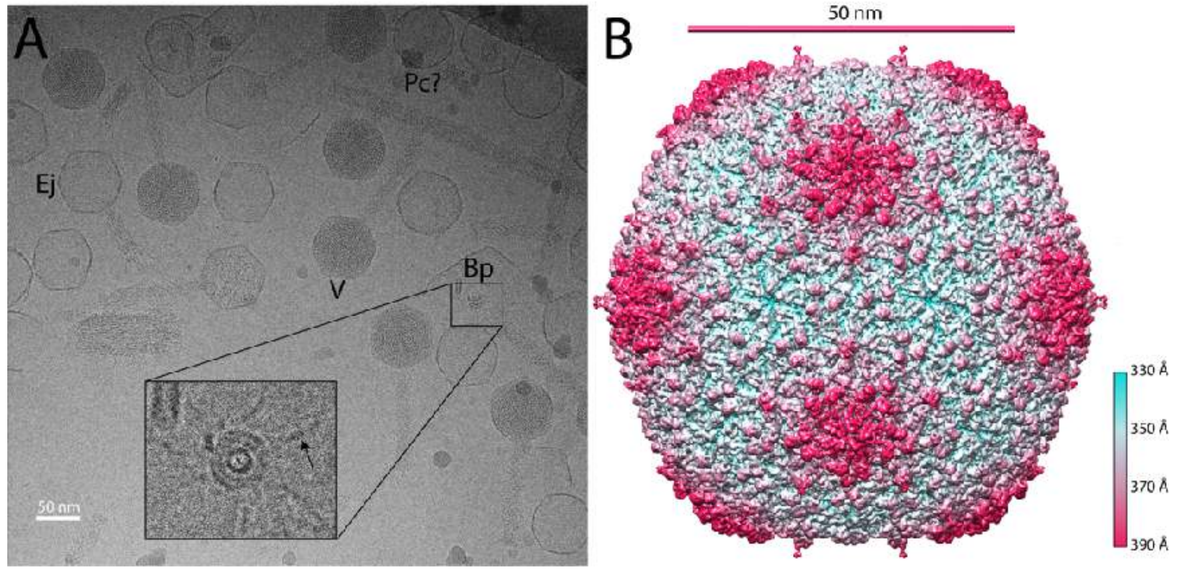
PDB 2J1N

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?

Virus structures

Shigella virus Sf14 – uncommon capsid geometry



High resolution capsid structure of the mature virion?

… of the procapsid?

… of ejected particles?

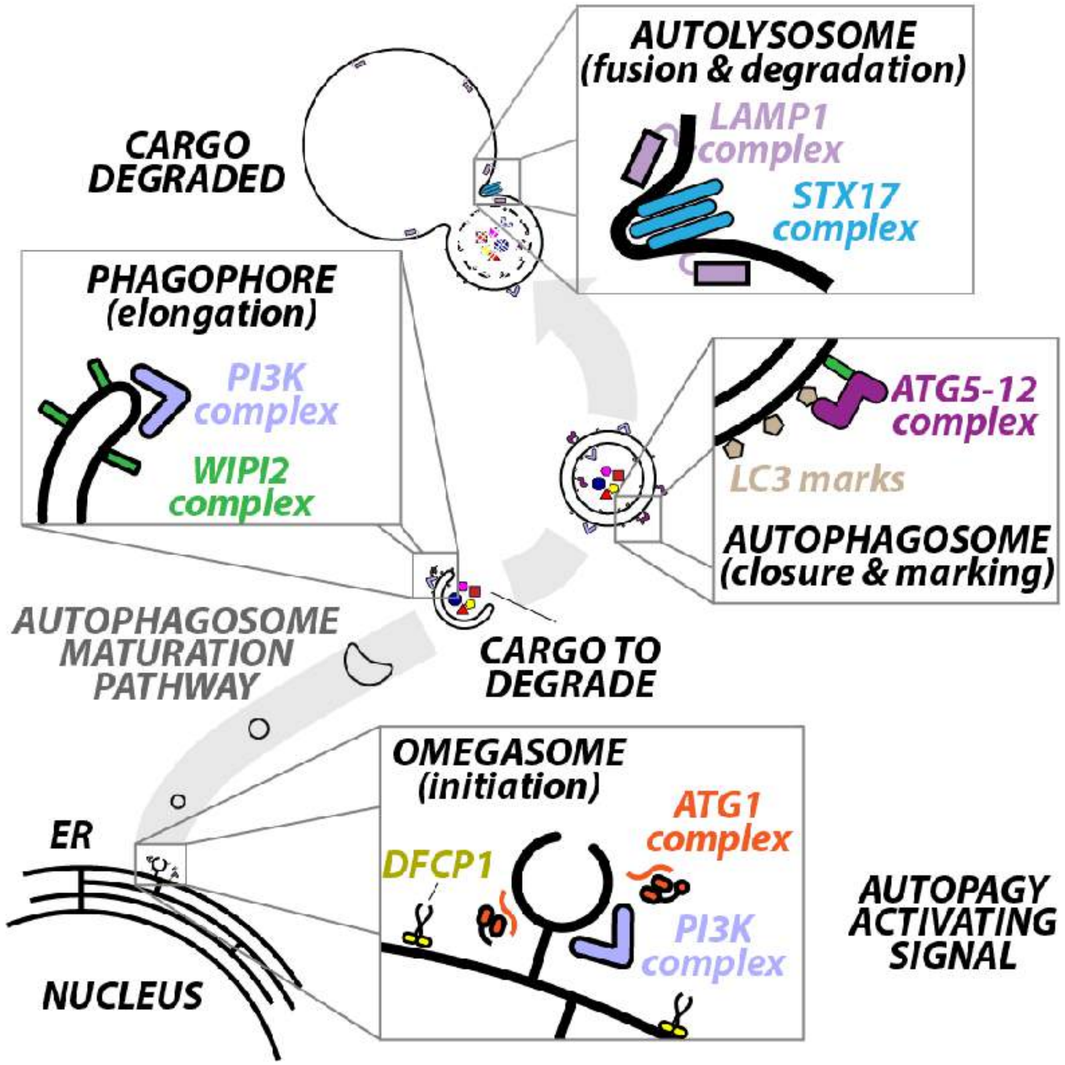
Resolve structures of the tail, especially the baseplate and tail fibers?

ANDREW GRASSETTI
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Structural analyses of molecular machines in autophagy

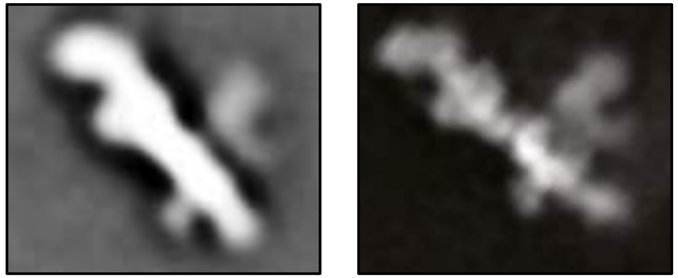
Andrew Grassetti
Postdoctoral Associate
Davis Lab
MIT

Autophagy overview and preliminary data



preliminary data

PMID: 31591221

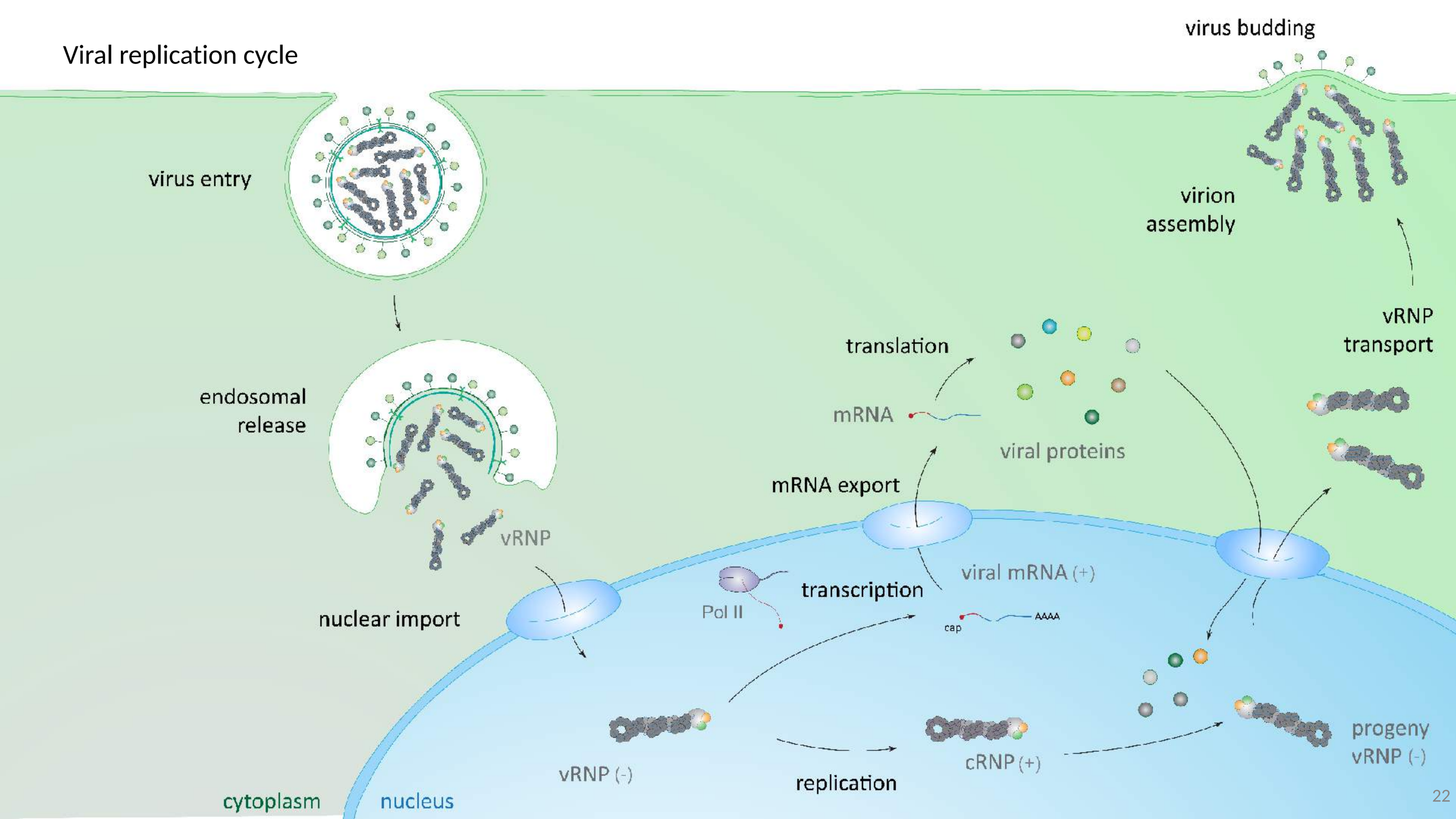


SISSY KALAYIL
EUROPEAN MOLECULAR BIOLOGY LABORATORY

Sissy Kalayil, PhD
Postdoc (10/2018 - Present), Stephen Cusack's Lab
European Molecular Biology Laboratory, Grenoble

Single particle cryo-EM on influenza polymerase

Viral replication cycle



Replicating polymerase to structure- workflow

biochemical optimization

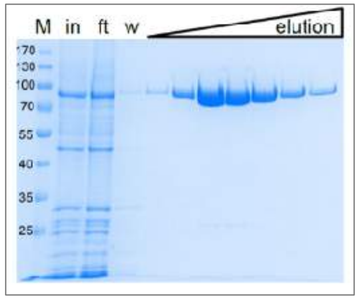
- RNA template design
- use of specific NTPs, NTP analogues
- reaction time and conditions

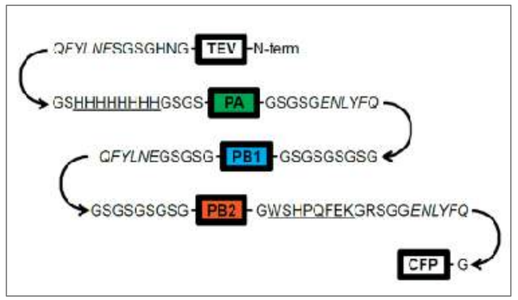
Am. Sulphate precipitation

Ni2+ Affinity

Strep tag affinity

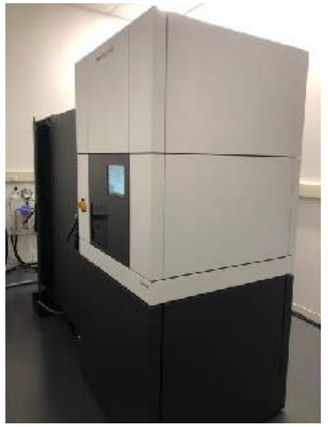
Heparin





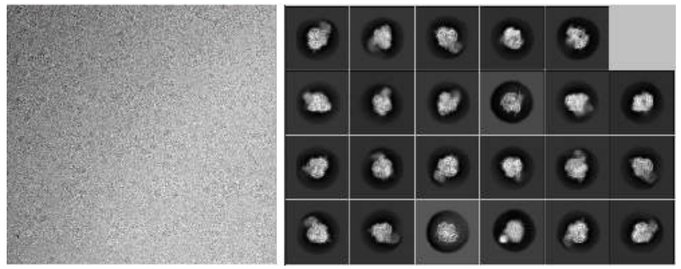
cryo-EM sample preparation & data collection

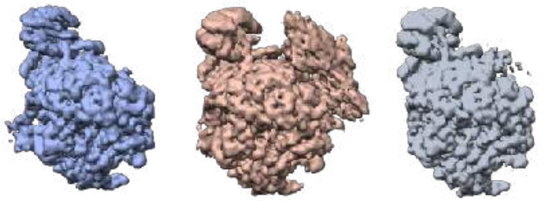
Tecnai-12



Talos Glacios

data processing & structure determination





Krios

ESRF or EMBL HD

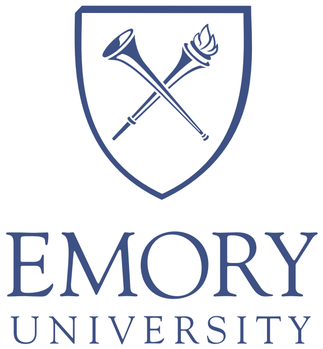
SOHAIL KHOSHNEVIS
EMORY UNIVERSITY

Structural studies of hypomodified ribosomes

Sohail Khoshnevis

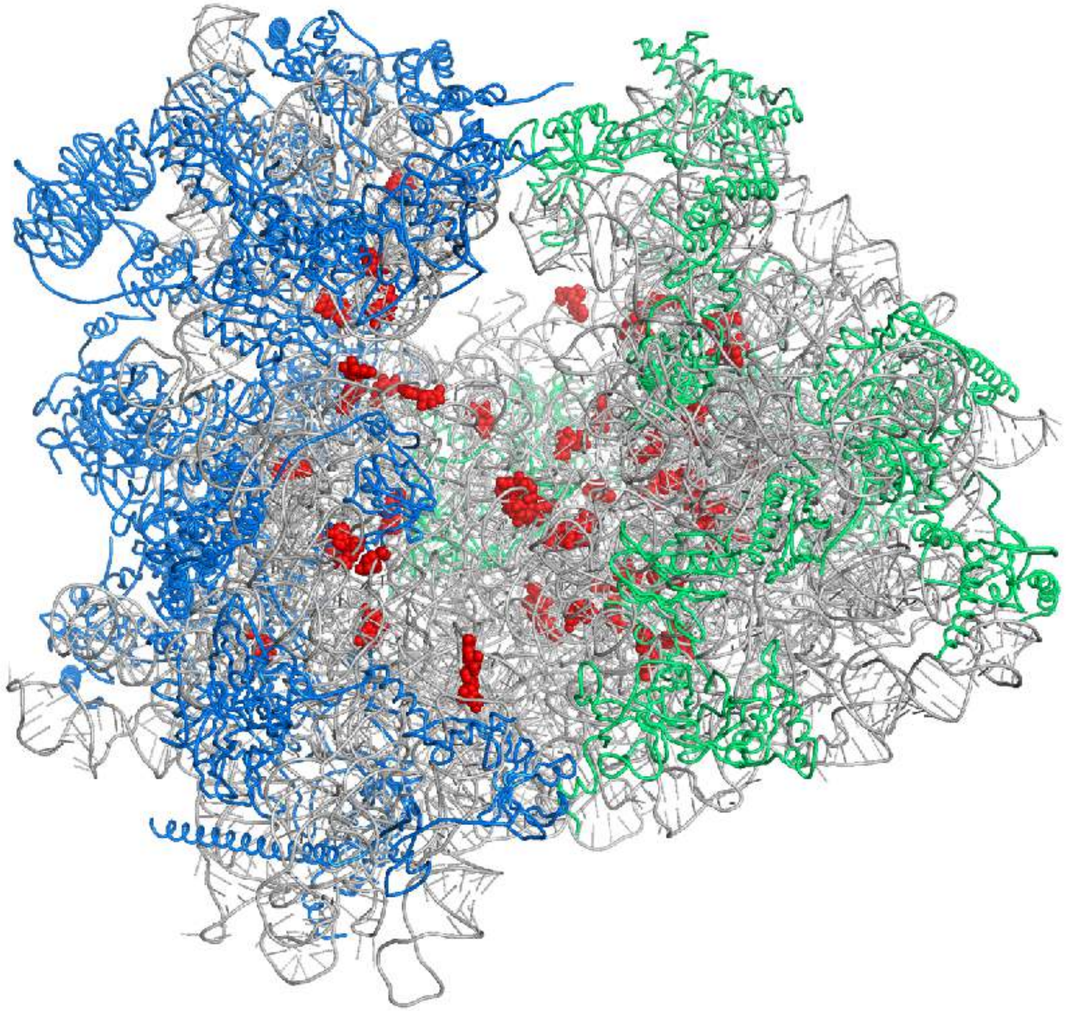
Department of Biology, Emory University and

Department of Biochemistry, Emory University School of Medicine, Atlanta, GA



Ribosomal RNAs are post-transcriptionally modified at functionally important sites

Small subunit

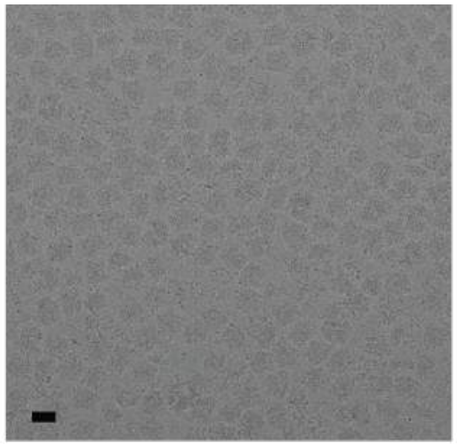


Large subunit

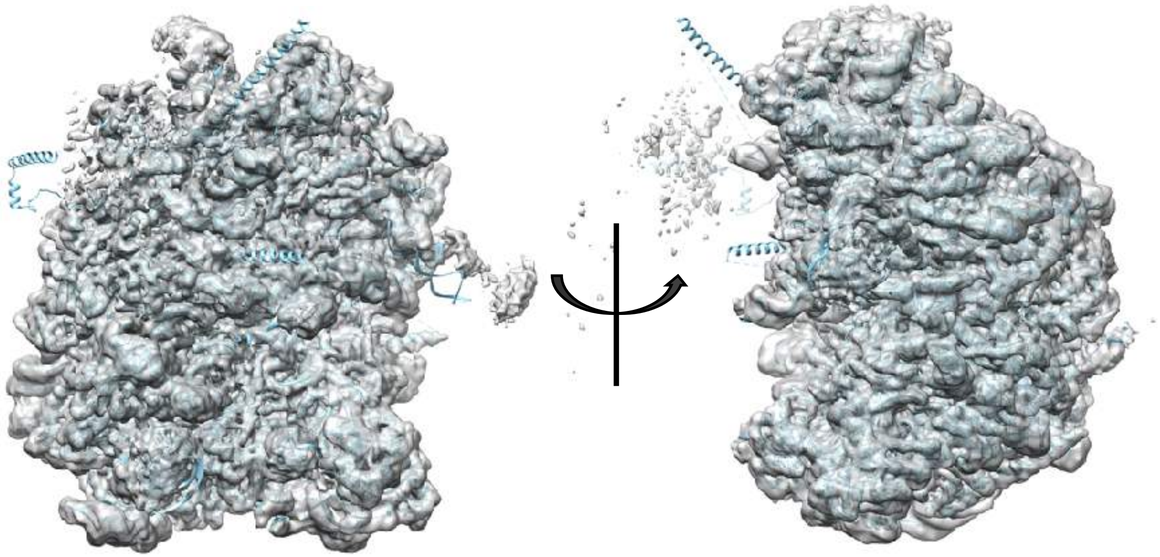
Ben-Shem A. *et al.* (2011) *Science*, PDB:4V88

Hypomodification affects the function of ribosomes. But why?

A

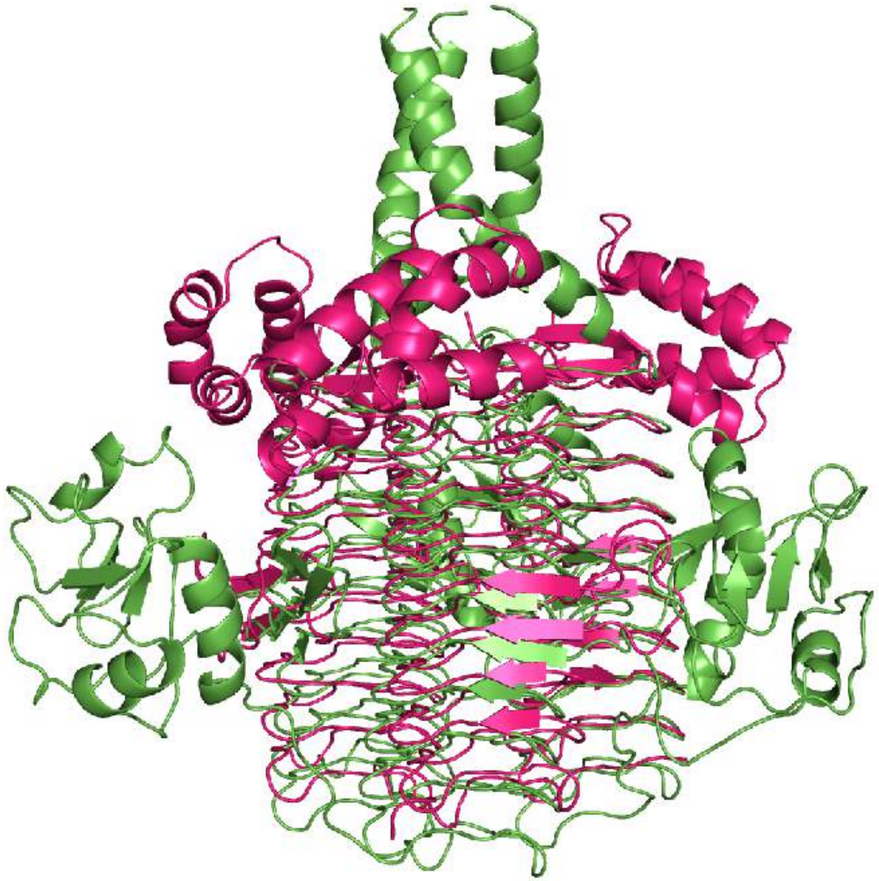


B



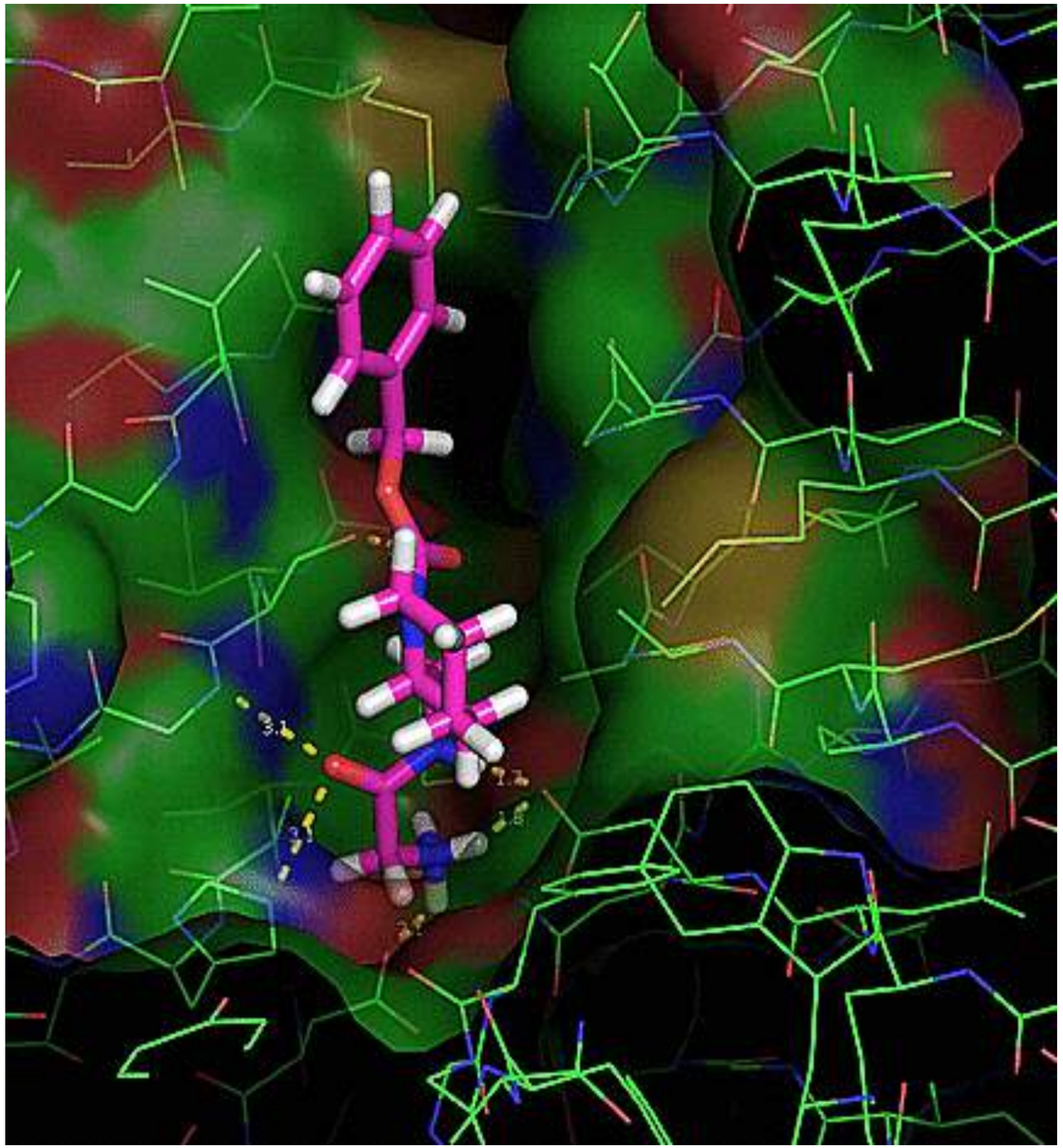
KYLE KROECK
VIRIGINIA COMMONWEALTH UNIVERSITY

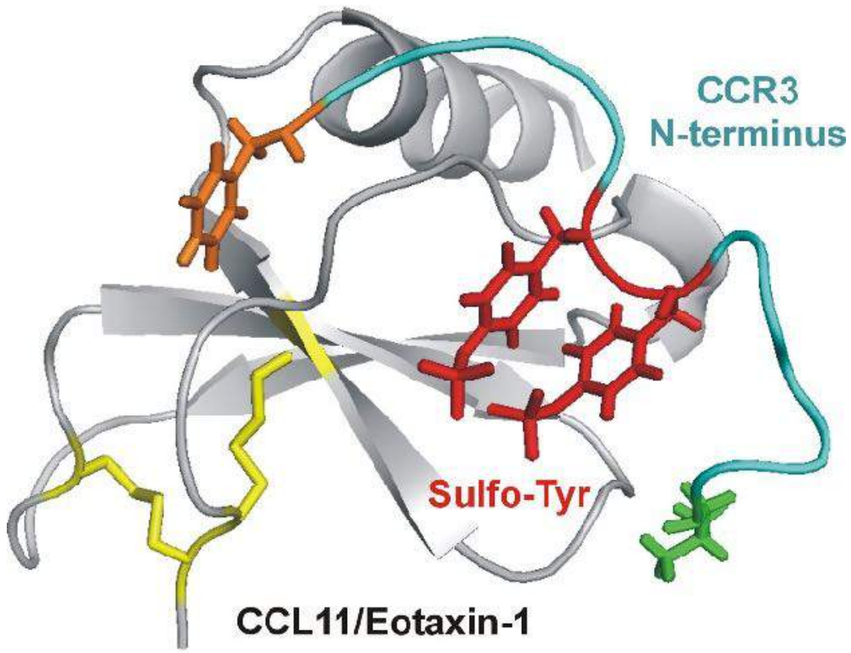
Dr. Kyle Kroeck

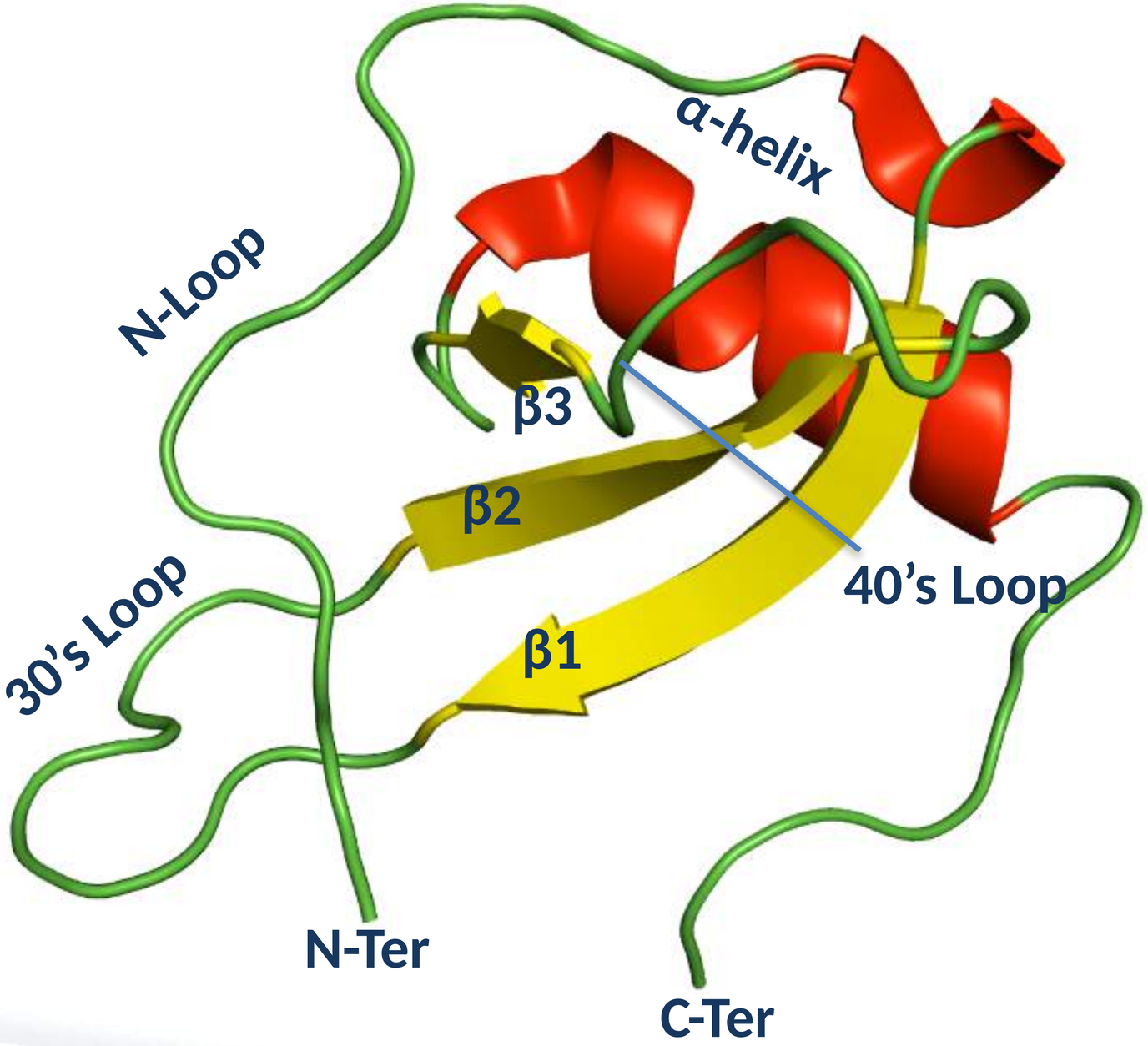


LpxA

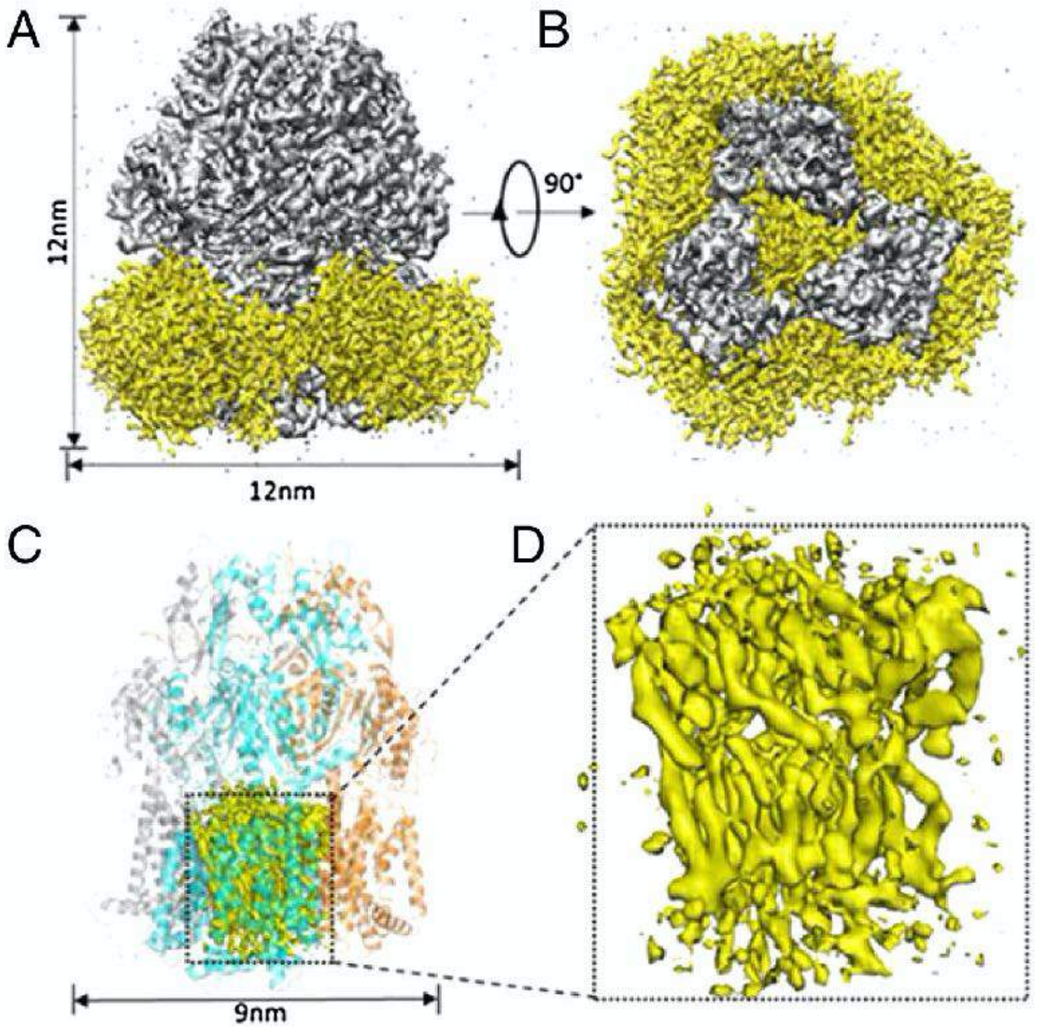
LpxD



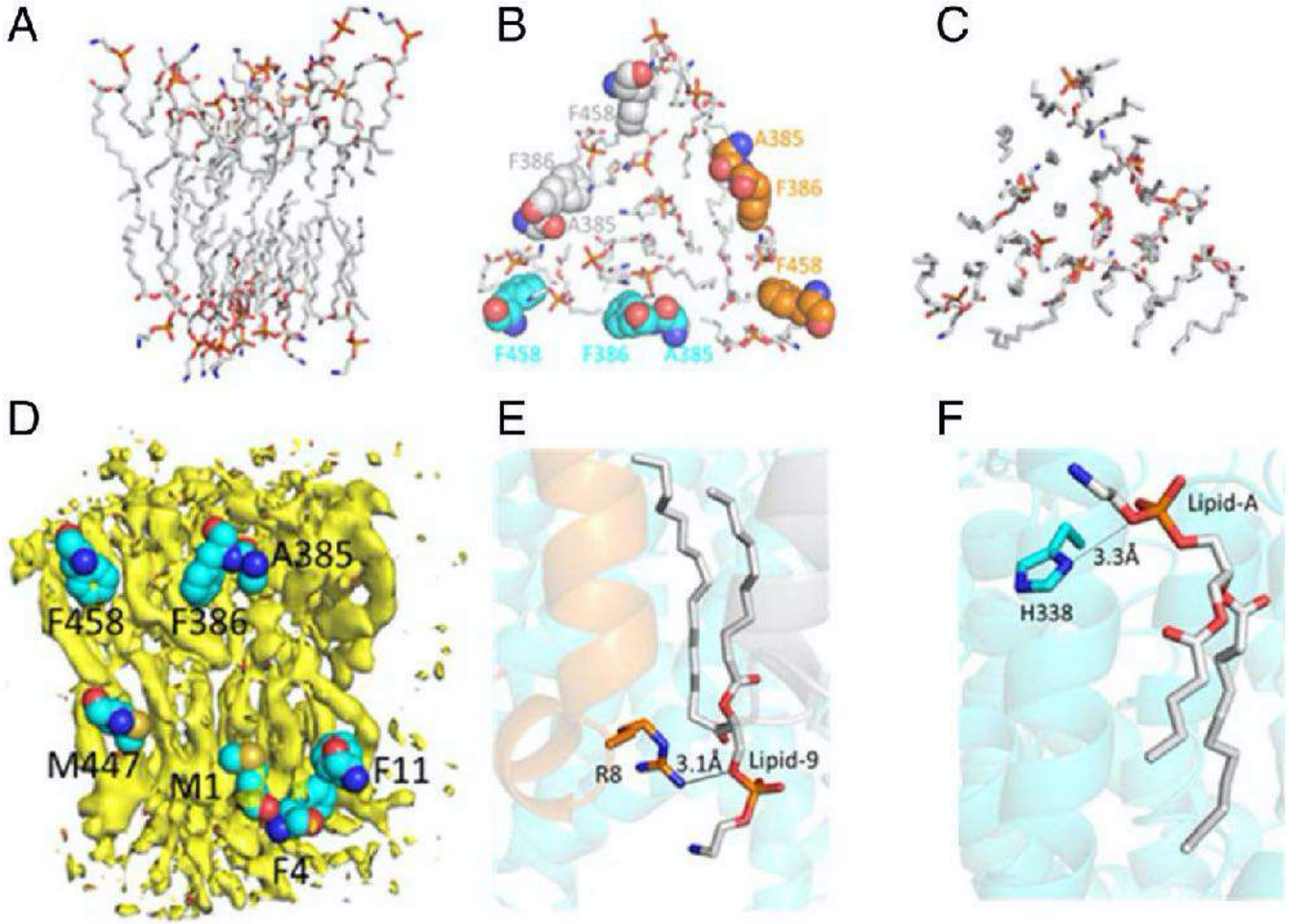

CCR3
N-terminus
Sulfo-Tyr
CCL11/Eotaxin-1


α-helix
N-Loop
β3
β2
30's Loop
40's Loop
β1
N-Ter
C-Ter

EM density of AcrB in a native cell-membrane nanoparticle.

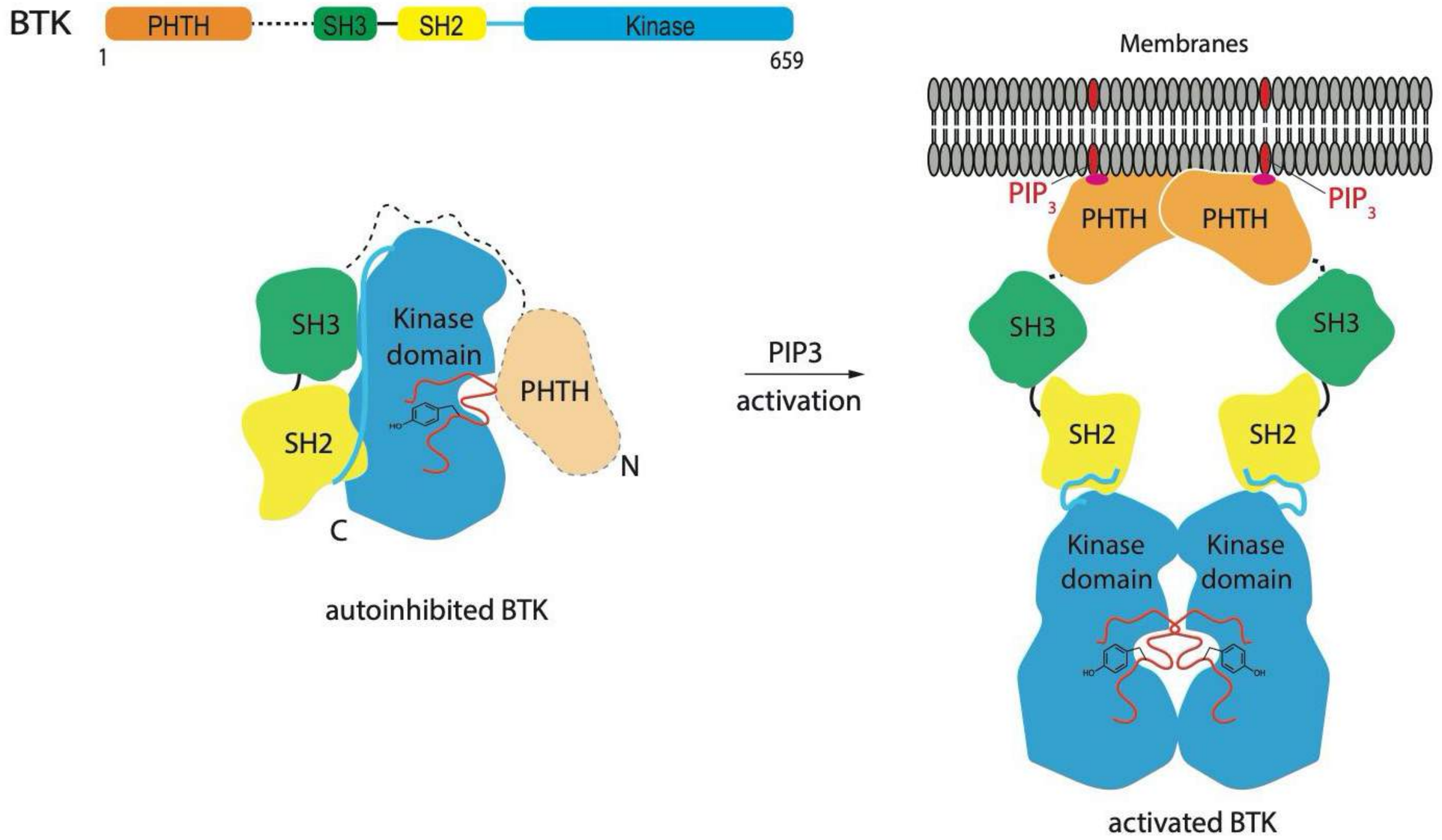


Structure of lipid bilayer and protein–lipid interactions



DAVID LIN
IOWA STATE UNIVERSITY

Models of Bruton's tyrosine kinase (Btk) autoinhibition and activation



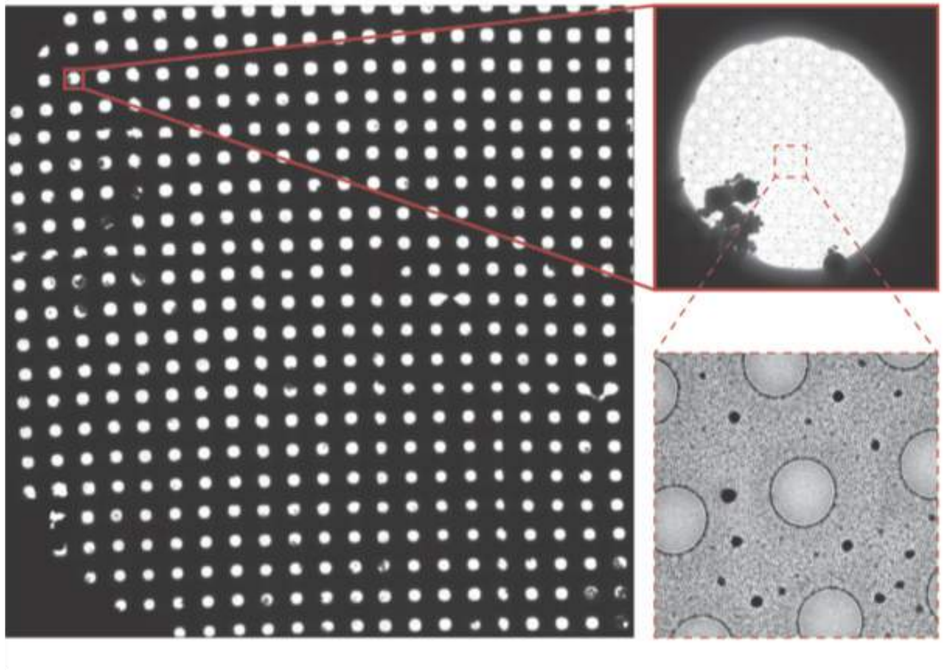
MARIA MARTINEZ MOLLEDO
INSTITUT PASTEUR

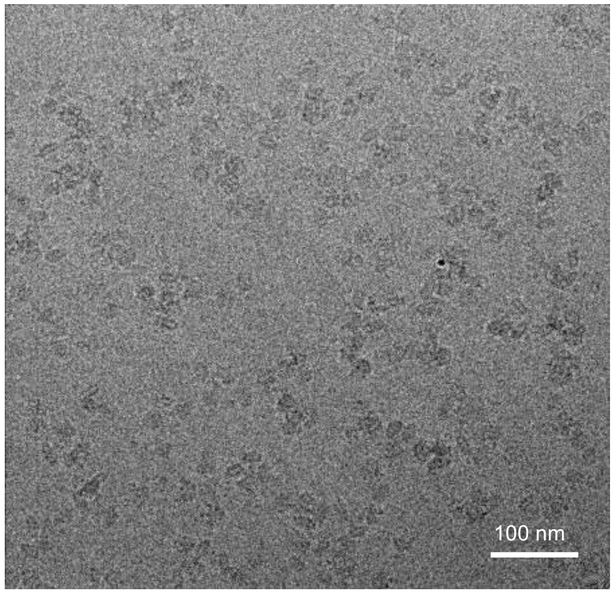
Maria Martinez Molledo
Postdoctoral reasearcher
IECB (Bordeaux, France)

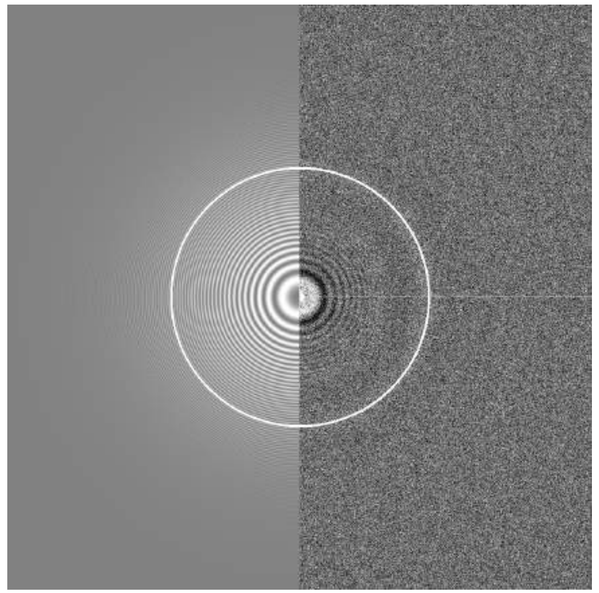
Institut Européen de Chimie et Biologie
I E C B

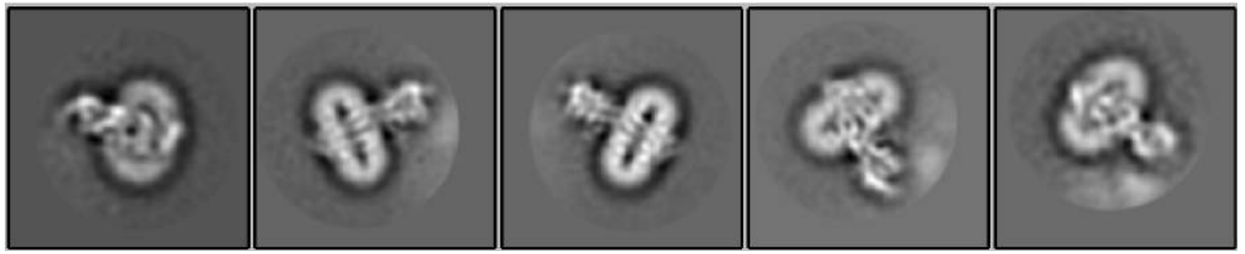
Research interest:

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)

↓

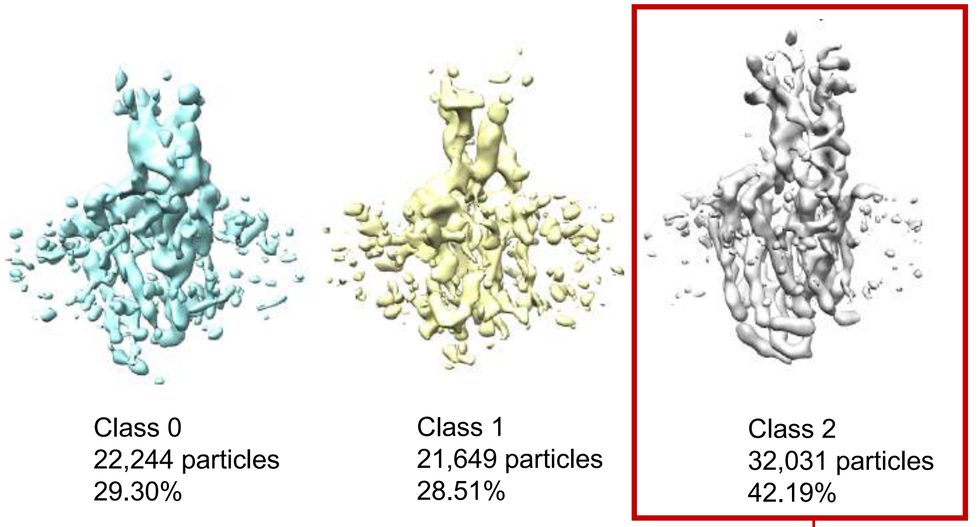
3D auto-refine

↓

CTF particle refinement

RELION 3.0

cryoSPARC v2.12.4

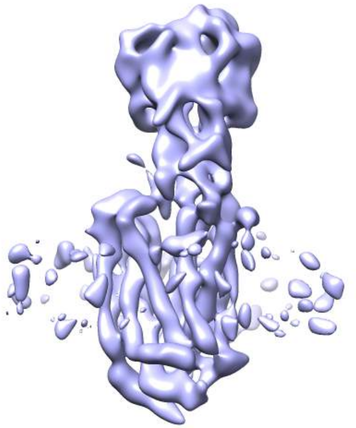


Class 0
22,244 particles
29.30%

Class 1
21,649 particles
28.51%

Class 2
32,031 particles
42.19%

NU-Refinement

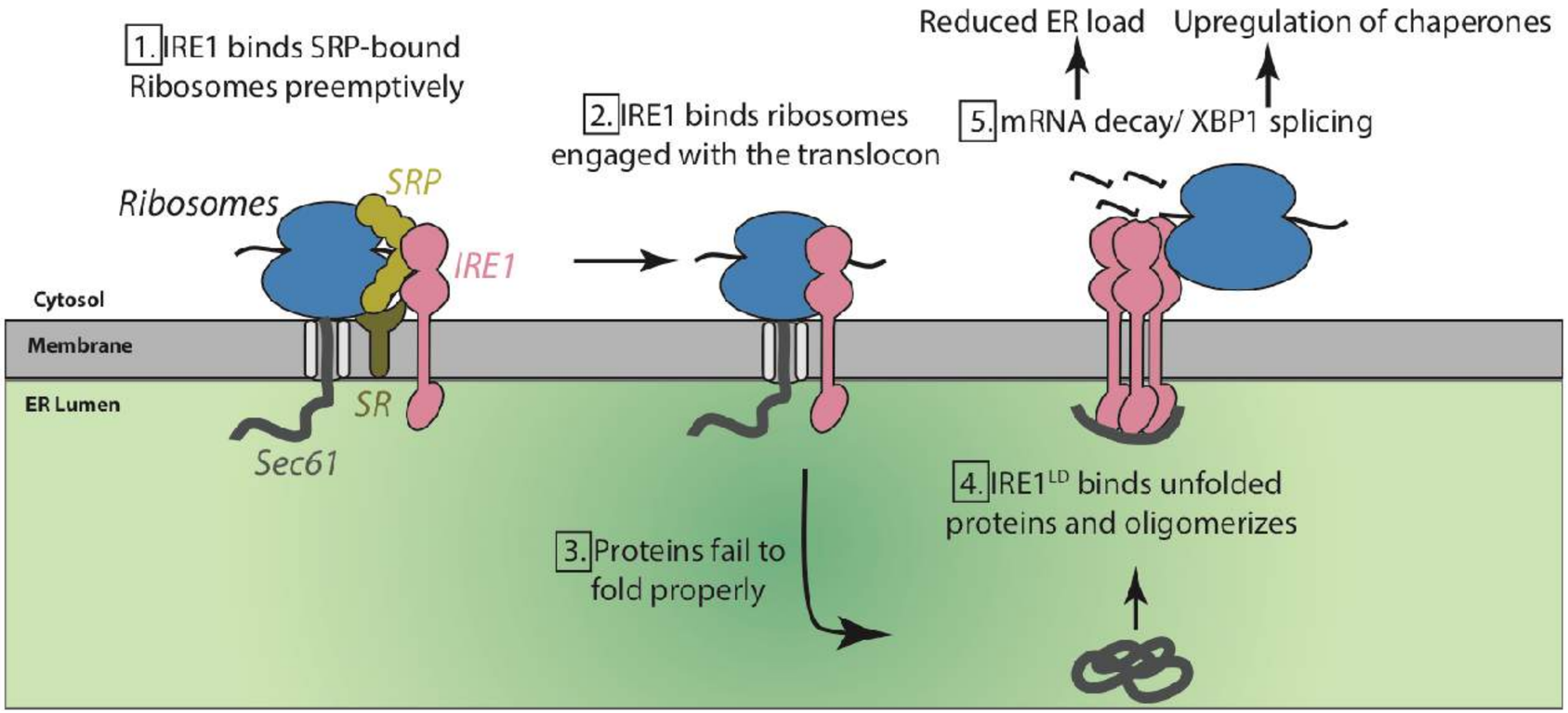


SMRITI SANGWAN
UCSF

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



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

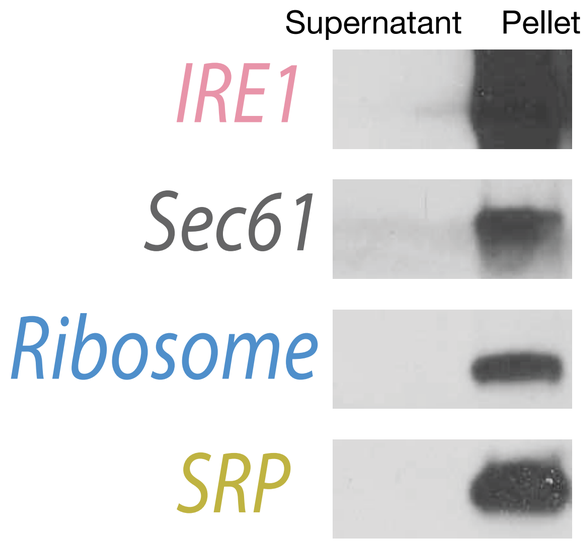
HEK293 cells expressing flag-tagged IRE1

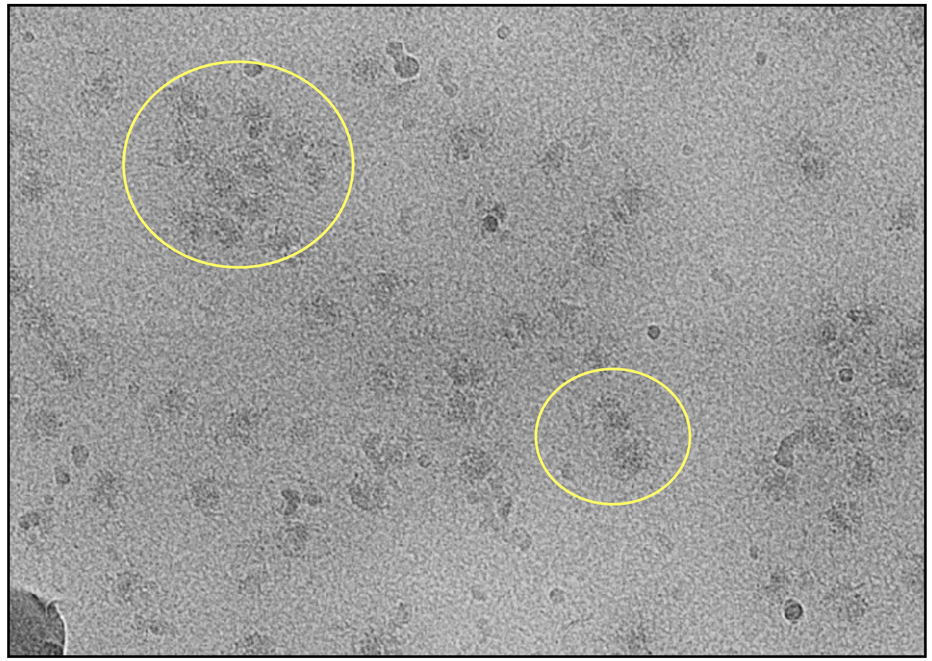
↓

Isolate microsome fraction

↓

Purify intact complexes from cells
by immunoprecipitation solubilise in detergent





KYE STACHOWSKI
OHIO STATE UNIVERSITY

KARTHIKEYAN SUBRAMANIAN
WEST VIRGINIA UNIVERSITY

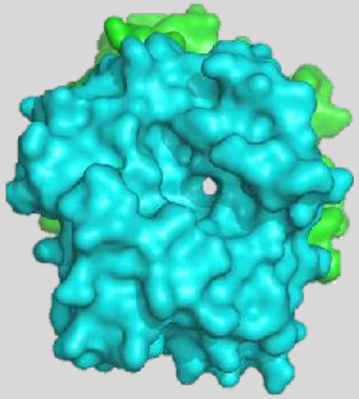
FRANCESCA VALLESE
COLUMBIA UNIVERSITY

FRANCESCA VALLESE

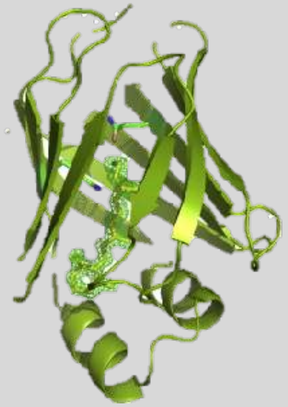
FROM ITALY WITH LOVE

THE GOLDEN AGE

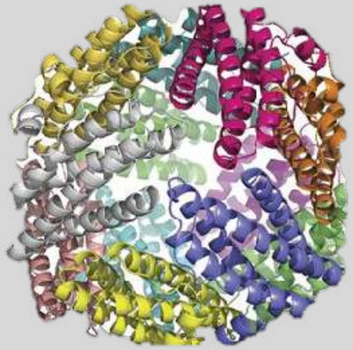
HP1029



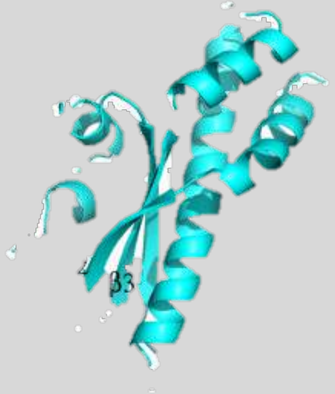
CRBP1

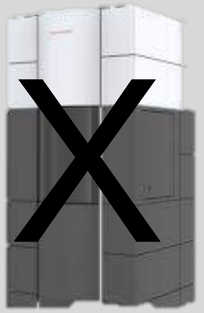


CAIP

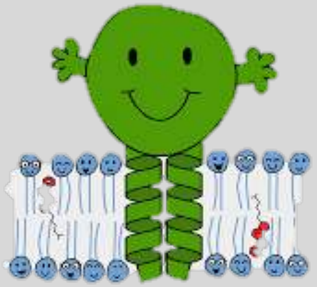


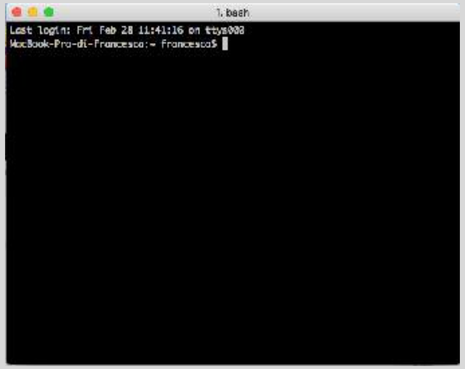
Lpp20

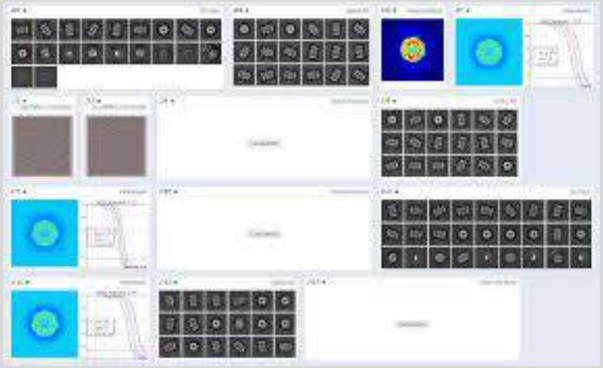


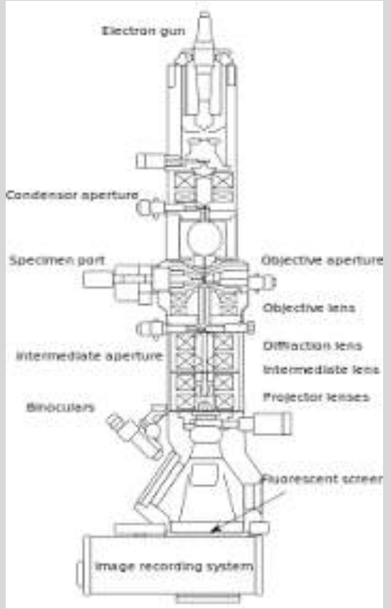


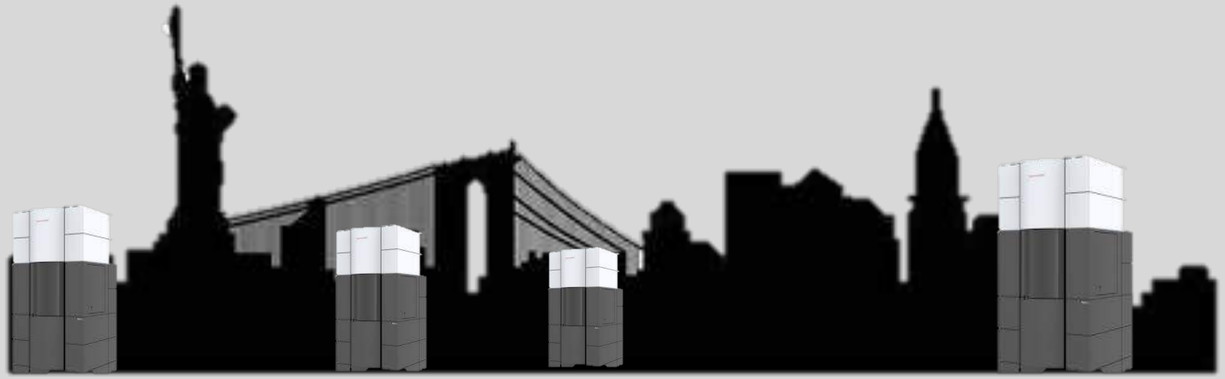
THE DARK AGE




1. bash
Last login: Fri Feb 28 11:41:16 on ttys000
MacBook-Pro-di-Francesco:~ francesco$

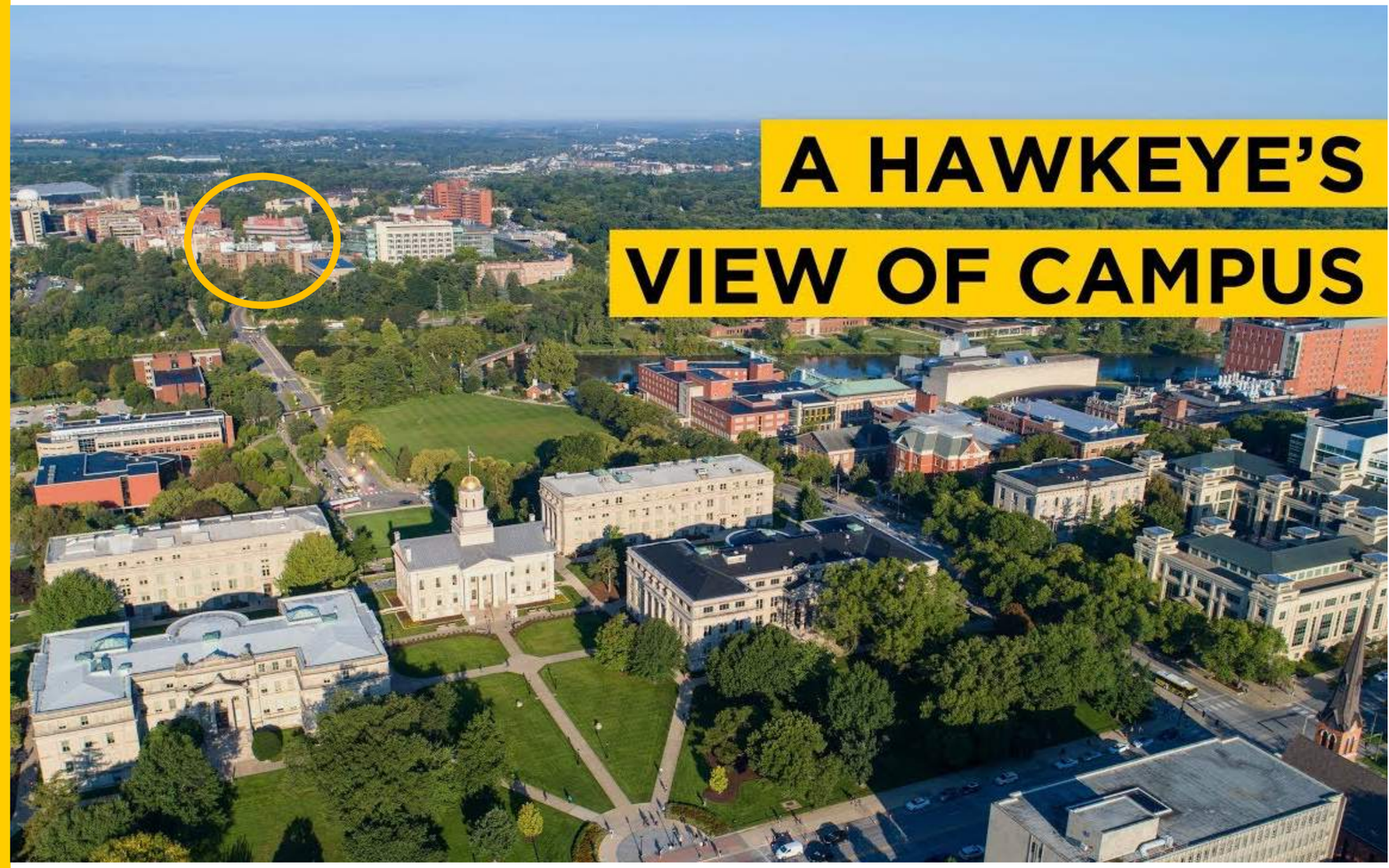



Electron gun
Condensor aperture
Specimen port
Objective aperture
Objective lens
Diffraction lens
Intermediate aperture
Intermediate lens
Projector lenses
Binoculars
Fluorescent screen
Image recording system



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 2nd, 2020

Cryo-EM study of Protein-DNA complex