

ADVANCING CRYO-EM IN DRUG DISCOVERY



CLAUDIO CIFERRI (Head of Cryo-EM, Genentech)

EMBO Course September 2019

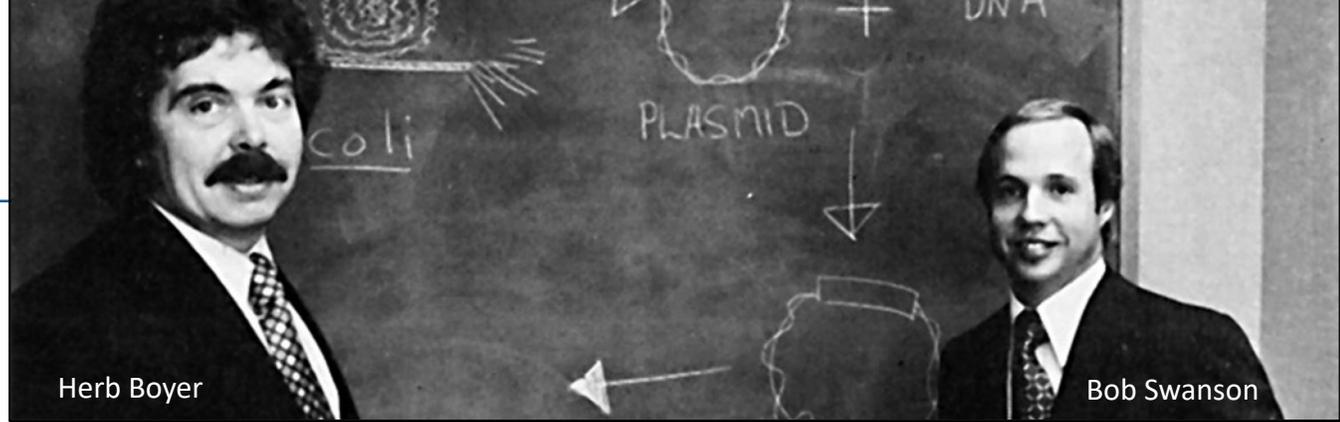
Genentech Inc.

"The Beginnings of Biotech" (Founded 1976)

- 1977: Produced first recombinant human protein
- 1978: Cloned human insulin
- 1979: Cloned human growth hormone
- 1982: First ever recombinant drug approval: insulin

- 1984: Started Structural Biology Group
- 1987: First published crystal structure

- 1990: Started formal four-year postdoc program



1997: First of 15 approvals for monoclonal Abs (latest: 2017)

2015: Decision to set up in-house cryo-EM (November)

2015: First published EM structure (December)

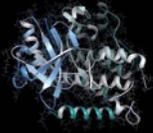
2017: Talos installation complete

2018: Krios installation complete (January)



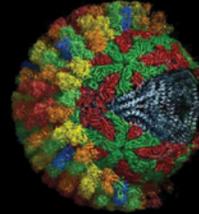
Cryo-EM as an integral part of Structural Biology

Cryo-EM is an evolving technology that requires talent, state-of-the-art equipment as well as a cutting-edge data processing infrastructure



X-Ray Crystallography

- Sample (mg amounts) must be crystallized.
- Generally suitable for <100kDa proteins.
- Atomic resolution but requires crystals. One conformational state per structure.
- Synchrotrons can collect dozens of X-ray datasets/week. Fast Data processing (minutes/days)



Cryo-EM

- Sample (µg amounts) is frozen in its native state.
- Generally suitable for larger Molecular weight complexes
- Near-atomic resolution, but fast sample preparation. Can describe heterogeneity/ flexibility.
- Data collection time is incompressible. We collect 1-2 datasets/week. Data processing is also slower (days/weeks).

Getting the first structure greatly reduces the effort for the following.

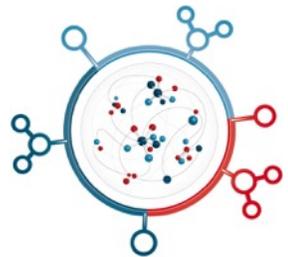
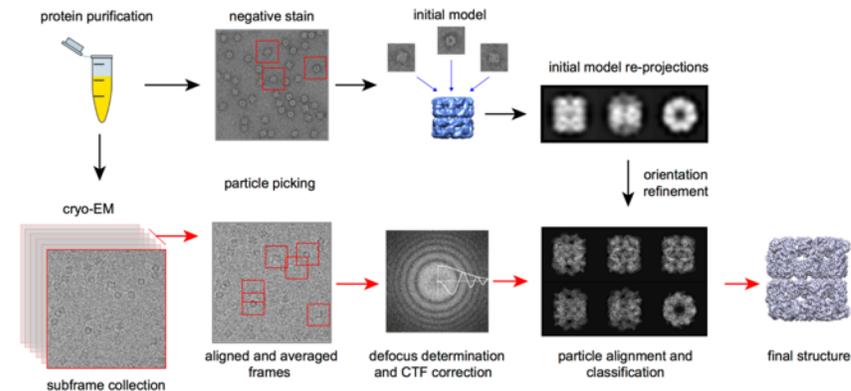
Talent Acquisition



State of the art Equipment

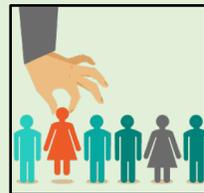


Data Collection/Processing Pipeline and Software Development

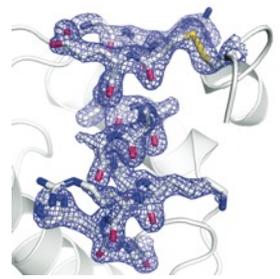


Acquiring the Right Talent to reach a Critical Mass

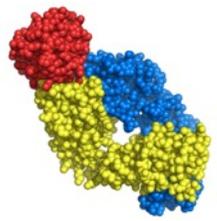
To Strengthen the Data Collection, Data Processing and Sample Preparation



Chris Arthur
Alberto Estevez
Open Position



Alexis Rohou
Lionel Rouge
Ben Barad
Iris Young



Marissa Matsumoto
Garrett Gross
Rina Fong



Marc Ksconsak

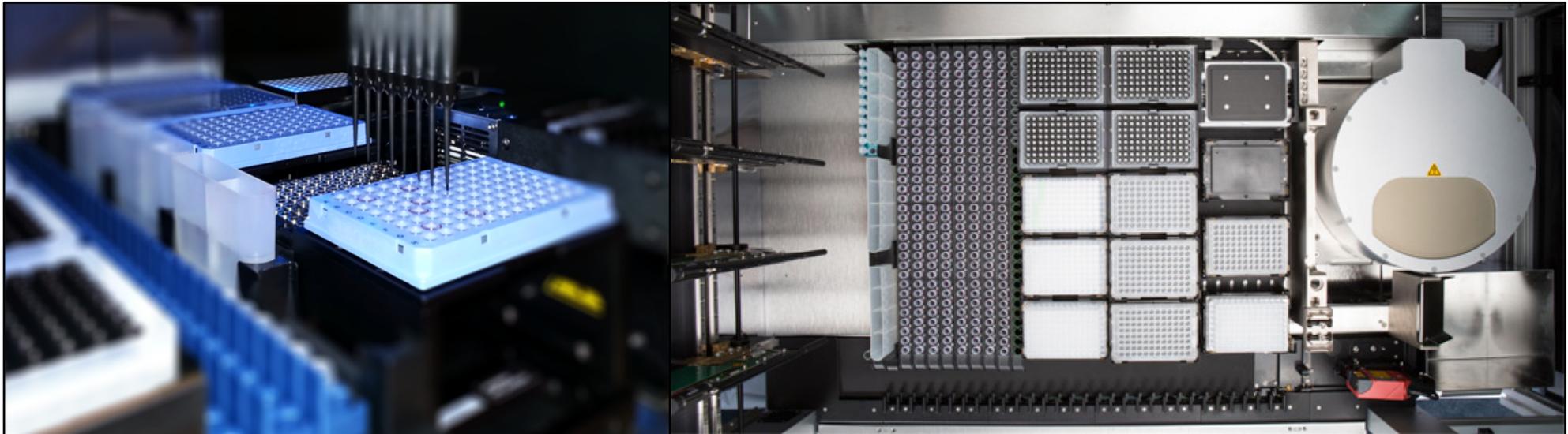


Nikit Kumar

Cryo-EM Challenged and Limitations:

Getting the right Sample is a must: accelerating the process of screening is Key!

- The sample requirements for cryo-EM generally several times less than X-ray crystallography (0.1-1 mg).
- 96 sample automated runs from *E.coli*, BEVS and Mammalian cells (>10,000 proteins/year).
- Highly Sensitive (0.1mg/L) & different purifications tags and cellular localization
- Detergent Screening (Membrane Proteins)

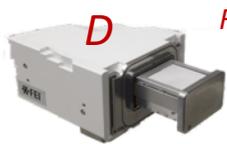


Genentech cryo-EM group and facility by 2020

Online July 2017



Talos 200C



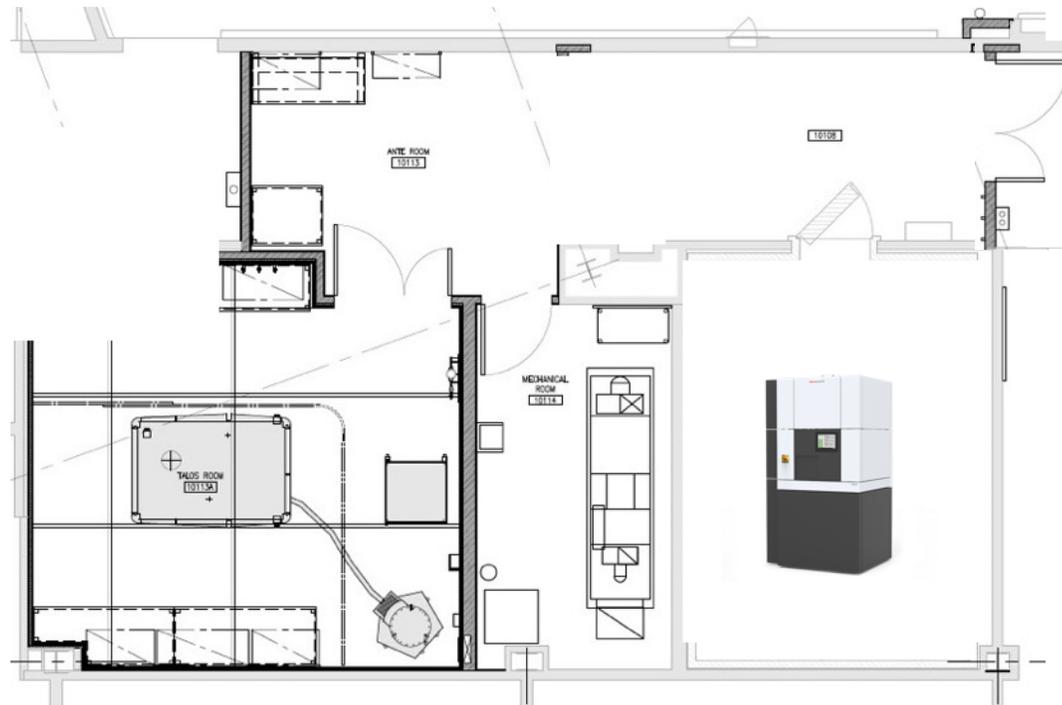
CETA-D CCD camera

February 2019

- Negative Staining
- Micro-ED

Screening Microscope: B10-113-115

Strategically positioned in FRC. Workhorse for negative and cryo-EM screening. (200KeV)



Expected 2019



K2 Direct Detector

- Cryo-EM screening
- (Autoloader + Direct Detector)

Genentech cryo-EM group and facility by 2020

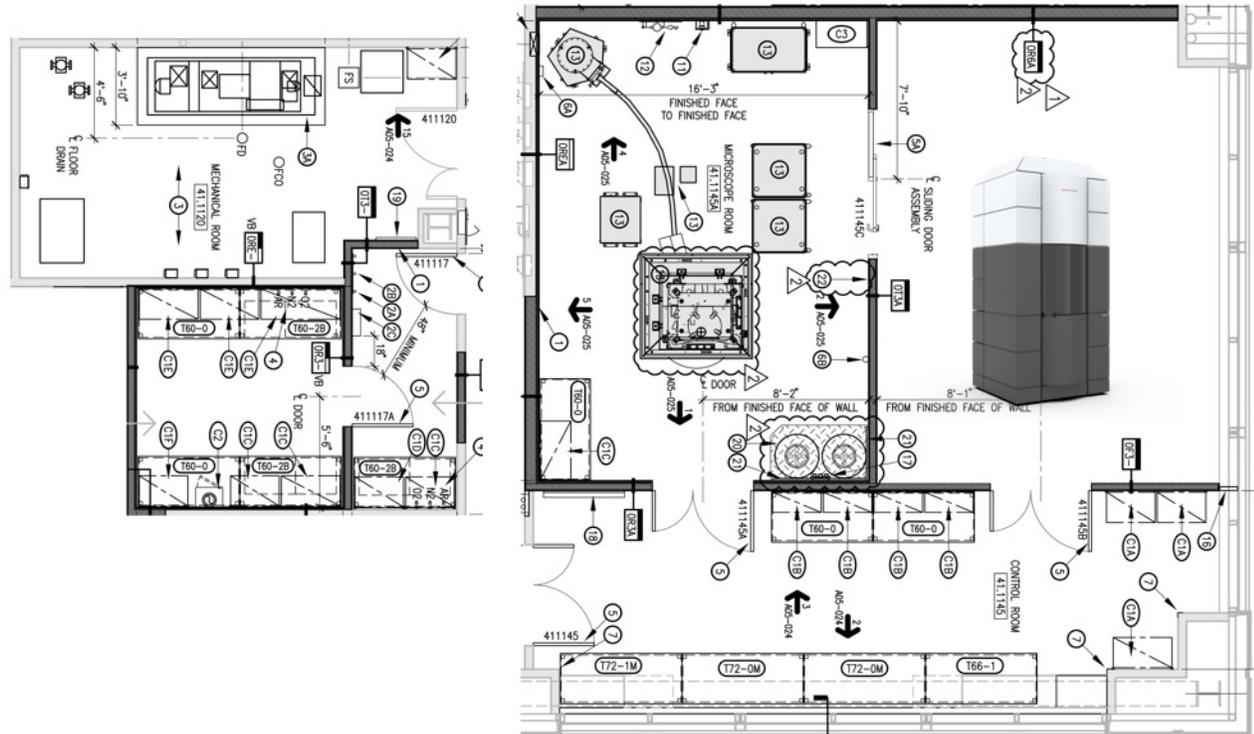
Online January 2018

By 2020



Titan Krios

High-Resolution cryo-EM with Direct Detector and Energy Filter,
Volta Phase Plate



Titan Krios
Volta Phase Plate



K2 Bioquantum



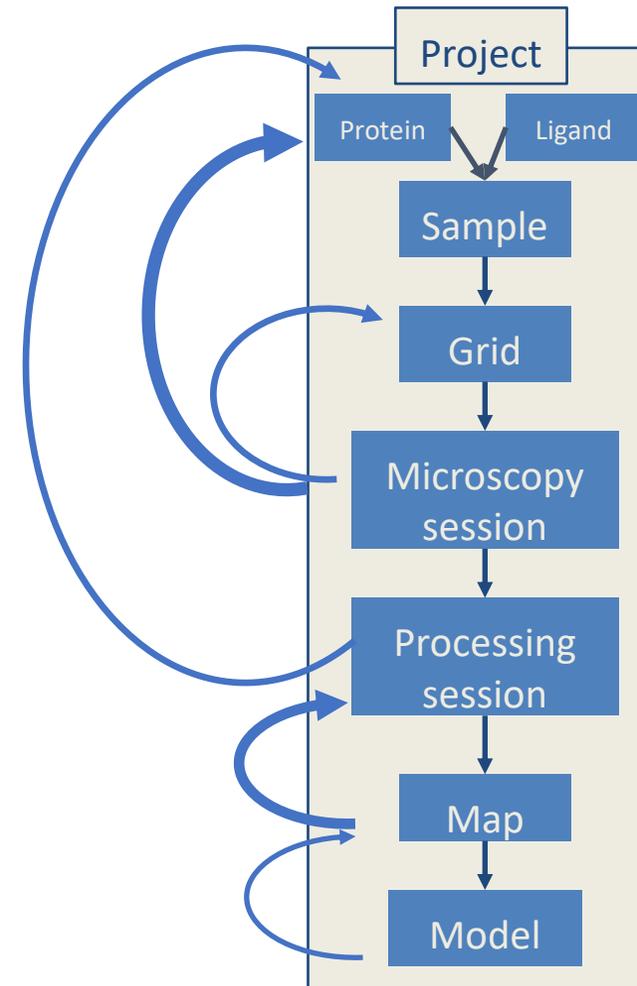
K3 Bioquantum

CryoEM Workflow

The workflow is highly iterative

Unlike many facilities, we have to keep track of everything from protein to model.

Easy to lose track of what's already been done/tried, especially when number of projects >> number of staff

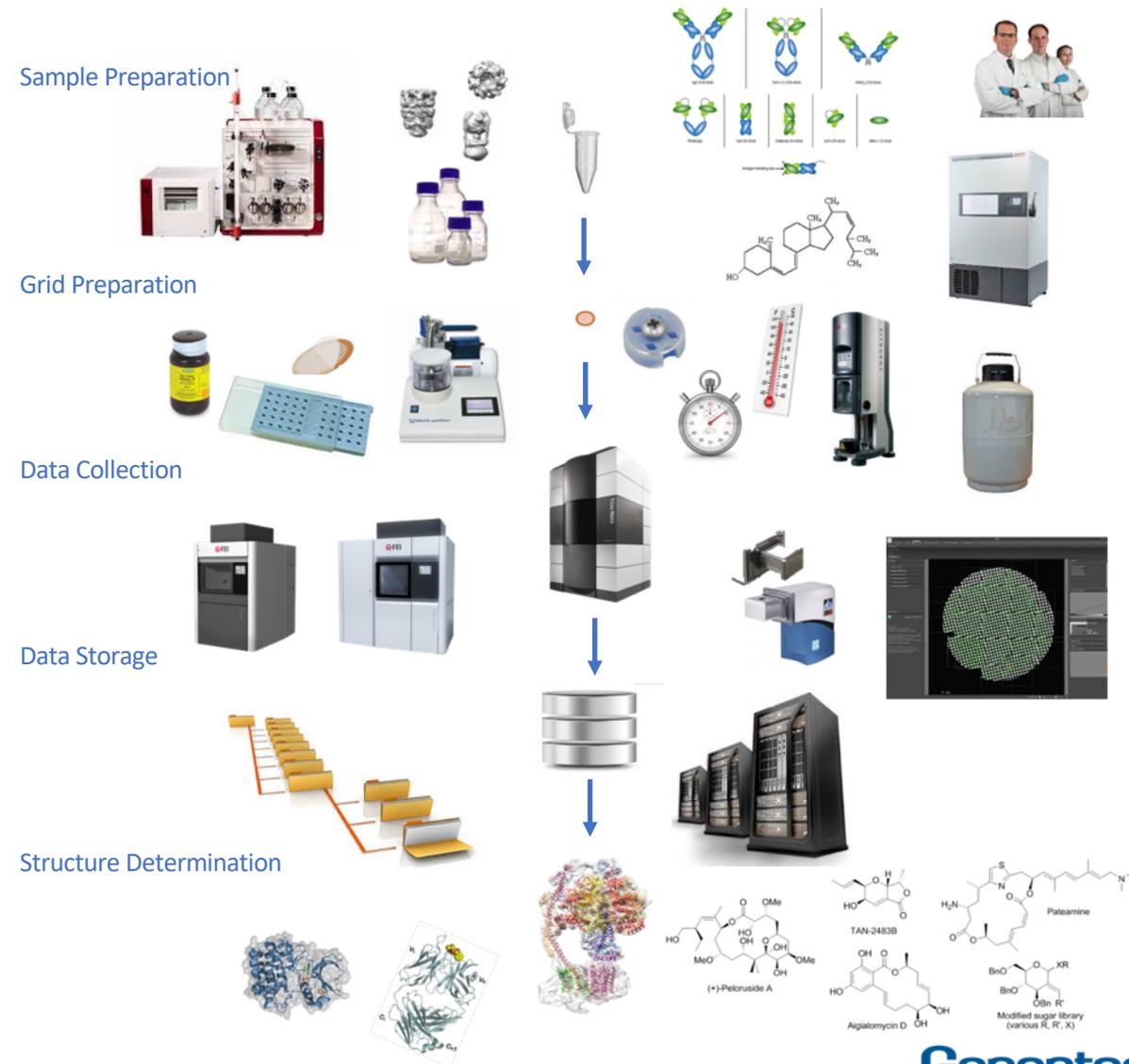


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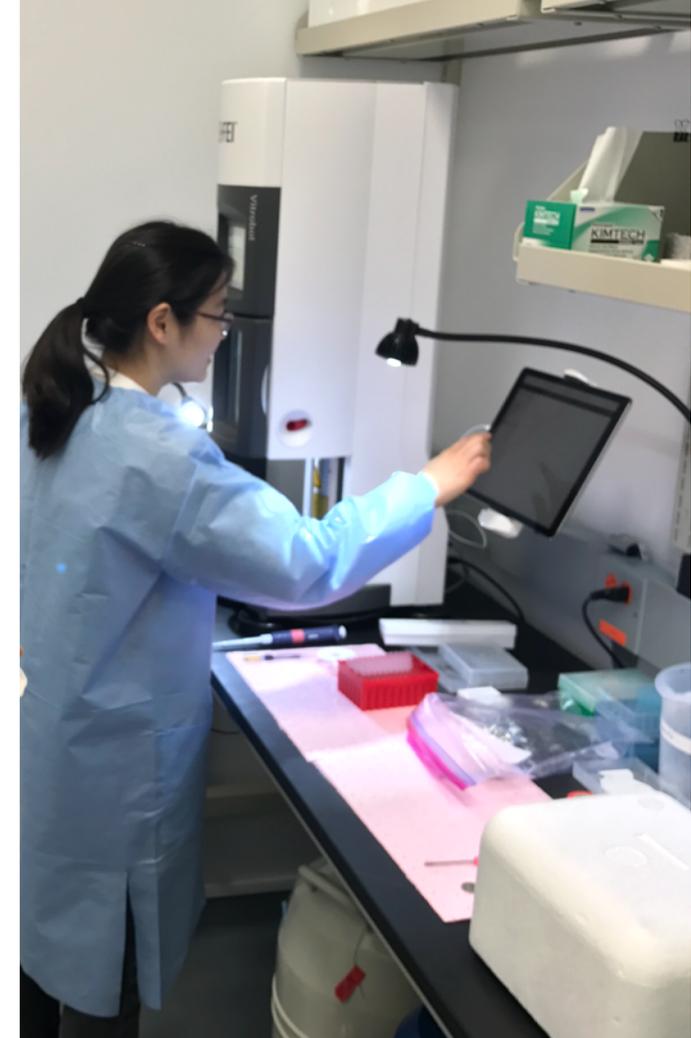


gP2S a cryoEM LIMS

The screenshot displays the CryoEM LIMS interface. At the top, the user is identified as Jacek Ziemiński. The main content area shows a list of microscopy sessions for the GroEL project. Each session entry includes the session name, the number of images, pixel size, defocus, and the date and time. The sessions are sorted by date.

Session Name	Number of images	Pixel size	Defocus	Date and Time
Microscopy XYZ	3,102	1.3 Å	0.9 - 2.7 µm	2017.02.13 16:45:23 #790991299
Using Grid 1	Microscope GNE Krios 1	Detector GNE Quantum K2	Microscopist Chris Arthur	
Microscopy XYZ	3,102	1.3 Å	0.9 - 2.7 µm	2017.02.13 16:45:23 #790991299
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Using Grid 1	Microscope GNE Krios 1	Detector GNE Quantum K2	Microscopist Chris Arthur	

gP2S in the Wild...



gP2S is available & free

Available now as a Docker

Open-source license

X-ray crystallography support coming 2019



docker

hub.docker.com/r/arohou/gp2s



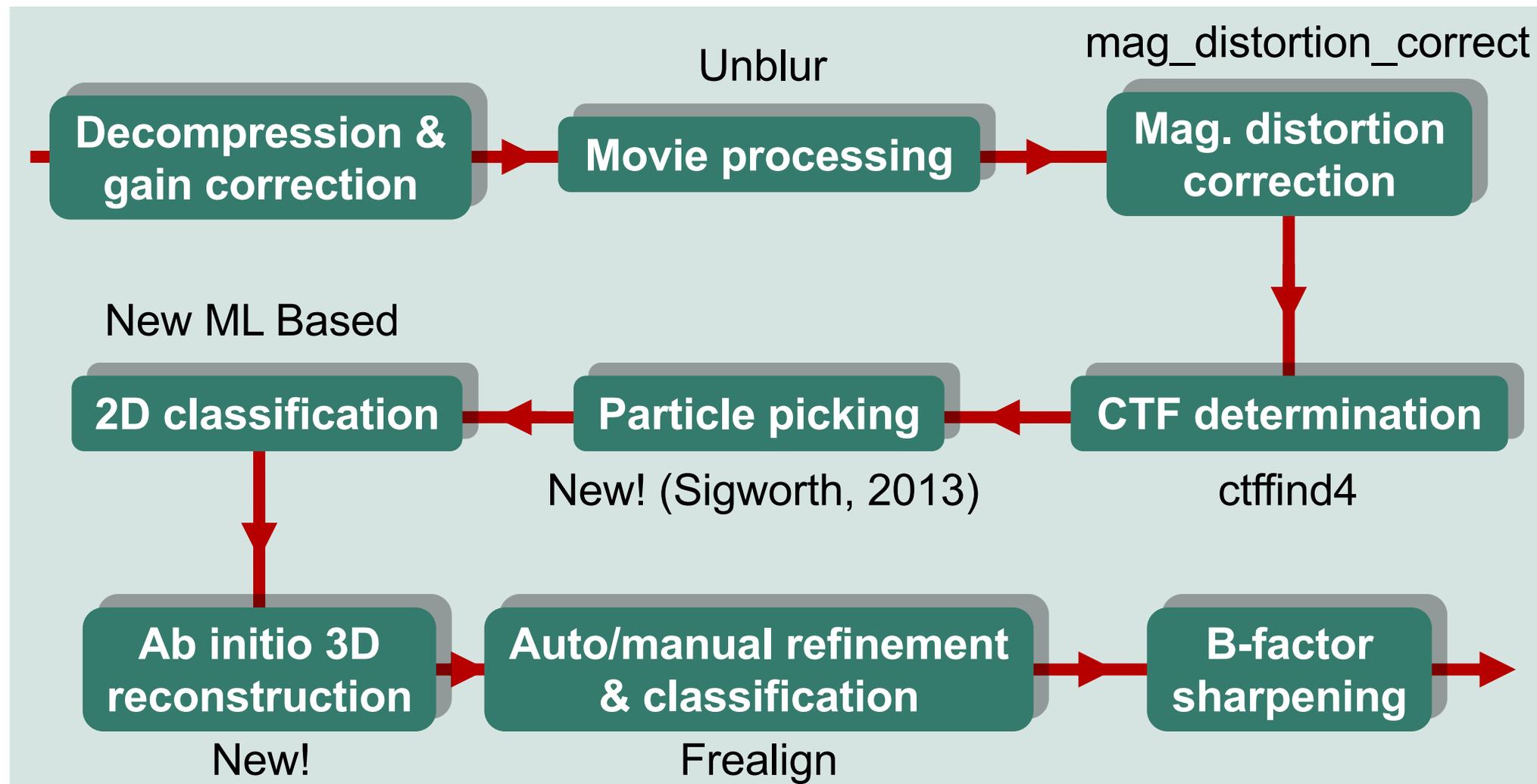
GitHub

github.com/arohou/gP2S

The next frontier: integrations

- EMDB & PDB deposition
- Data transfer & backup
- Equipment
 - Thermo microscopes
 - Thermo vitrobot
 - Gatan K2
- Microscope control software
 - SerialEM
 - EPU
 - Latitude
- Image processing software





cisTEM is fast!

2.2 Å resolution cryo-EM structure of β -galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,^{1*} Alan Merk,^{1*} Soojay Banerjee,¹ Doreen Matthies,¹ Xiongwu Wu,² Jacqueline L. S. Milne,¹ Sriram Subramaniam^{1†}

Processing on a single workstation

2 x Xeon (44 cpu cores)

512 GB Memory

16TB SSD

cisTEM is fast!

Processing on a single workstation

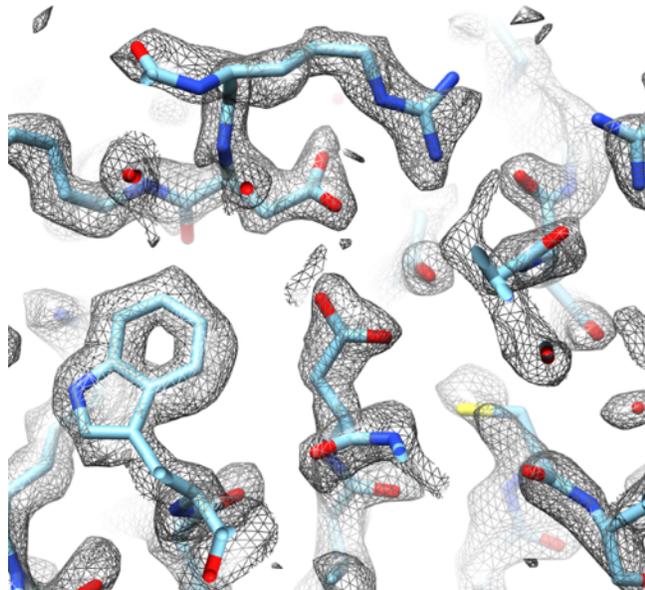
2 x Xeon (44 cpu cores)

512 GB Memory

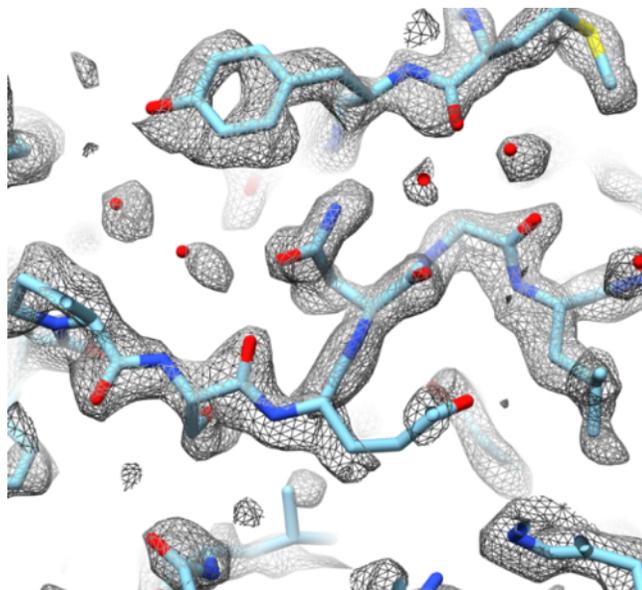
16TB SSD

Processing Step	Details	Time (hours)
Movie Processing	1539 movies, 38 frames, super-resolution	1.1
CTF Determination	on images (not frames)	0.1
Particle Picking	131,298 particles	0.1
2D Classification	50 classes, 28 selected with 119,523 particles	0.8
Ab-initio 3D	40 iterations	0.8
Auto refinement	8 iterations, final resolution 2.2 Å	1.4
Manual refinement	1 iteration (incl. defocus), final resolution 2.2 Å	0.4
Total		4.7

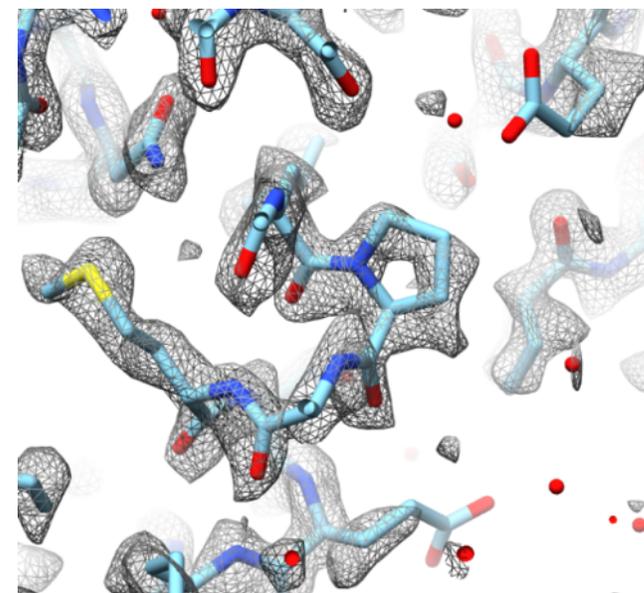
*Bartesaghi et al. 2015,
reprocessed*



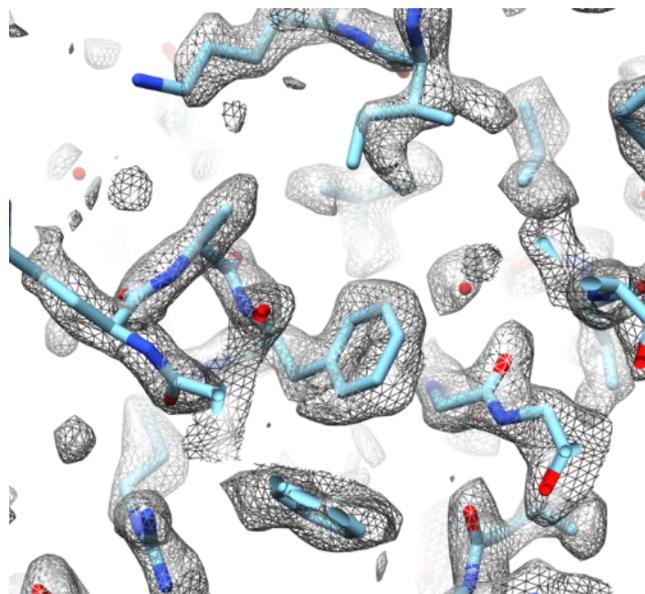
Trp, Asp...



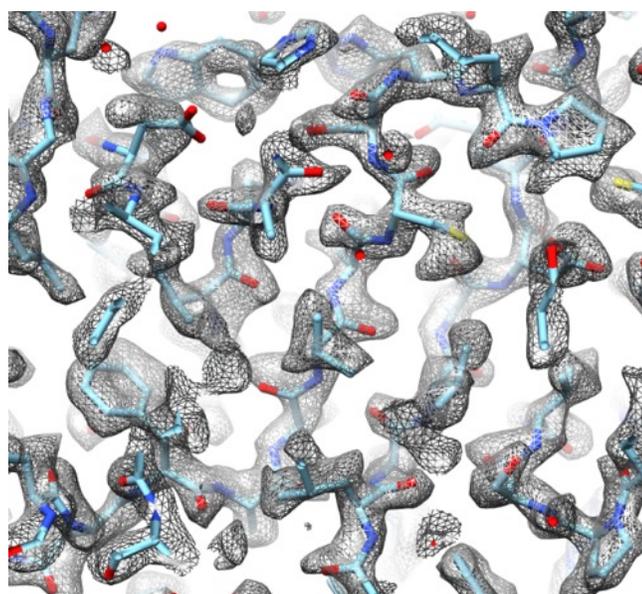
Tyr, Asn, carbonyl, H₂O



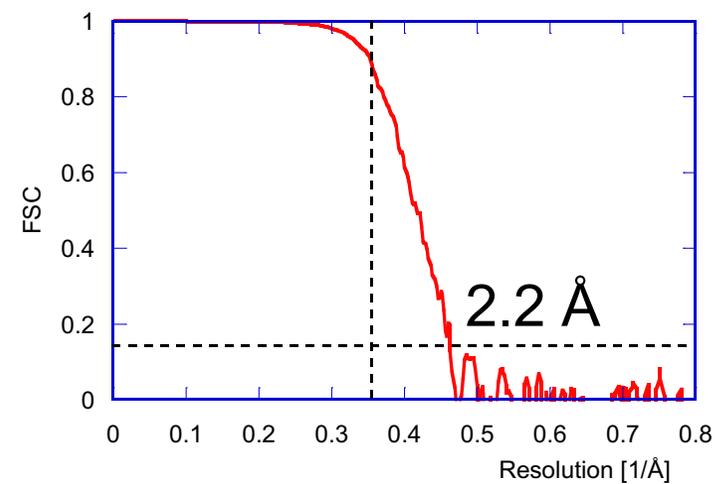
Pro, Met...



Phe, Ile, α -helix



Trp, carbonyl, β -sheet



(no masking)

Bartesaghi et al. 2015,
reprocessed

gP2S is available & free

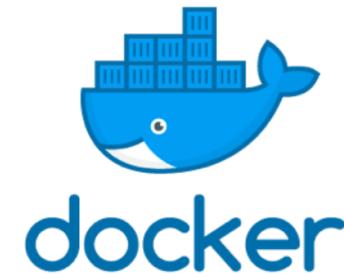
Free (beer & freedom) software:

- cisTEM for fast, easy 3D reconstruction

Available now from cistem.org

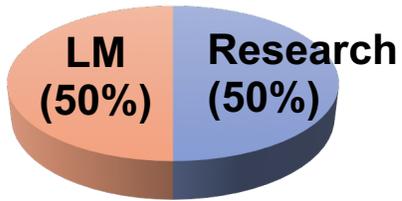
- gP2S for lab/facility information management

Available now from [github](https://github.com), [docker hub](https://hub.docker.com/)



Cryo-EM enters our SBDD Pipeline in early 2018

2015-2017



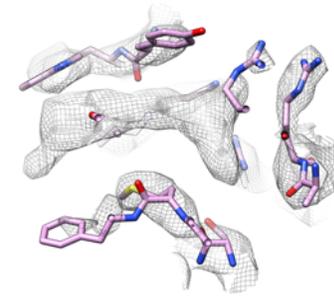
Talos:

- screening
- optimization
- epitope mapping

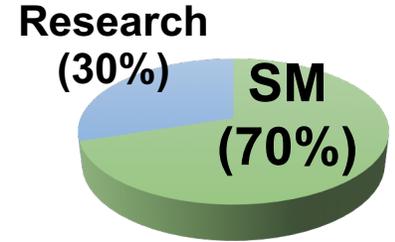


Krios:

- high-resolution
- priority to SM pipeline projects



2018



SM binding site localization

Structure-based drug design

Binding pocket mapping (side chains)

Resolution

20 Å

15 Å

4.5 Å

3.5 Å

2.5 Å



Cryo-EM structures are already relevant for SBDD

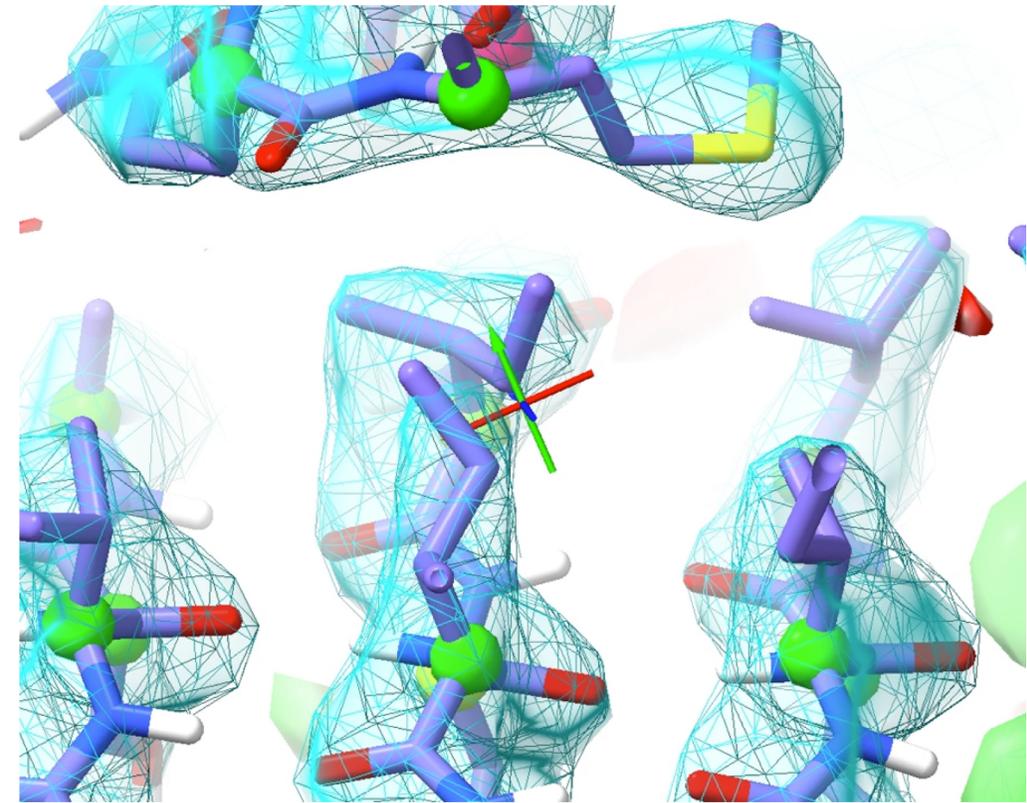
19

Can we support SM projects as fast as X-ray crystallography?

- By X-ray crystallography we can collect several structure/hours and solve several structures/day or week
- By cryoEM we can only collect only 1-2 datasets per week
 - We need several hundreds of thousands particles to obtain a final reconstruction
 - About 10-20K movies per dataset
 - We can currently collect 150 movies/hour (K2 camera) and project 300-400 with the K3 camera
 - Need a large amount of data storage (5-20TB/week)
 - Data processing requires days/weeks
- **We need to choose systems where we can make the difference and select fewer critical compounds for SMDD**

Can we support SM projects as fast as X-ray crystallography?

- We use WARP for on the fly data processing and CisTEM (sometime help from Relion) to solve all our structures
- Classical crystallography software (Coot, Phenix, DIALS)
- We need to work very closely with Medchem and Compchem to prioritize compounds and structures (fewer more relevant/informative structures).
- Molecular Dynamics with MOE and Molecular Dynamic Flexible Fitting with Isolde.



A real case scenario...

Chronic Pain

Neuroscience Project Goal: Make a revolutionary new Pain Drug.



- Affects 1 in 5 individuals
- Chronic pain can be debilitating
 - examples*
 - Neuropathic pain
 - Inflammatory pain
- Results in a \$600 billion cost to society annually

Neuroscience Project Goal:

- Make a *revolutionary* new pain drug

GOF: Altered Nav1.7 gating (Excessive pain)



Inherited Erythromelalgia (IEM)



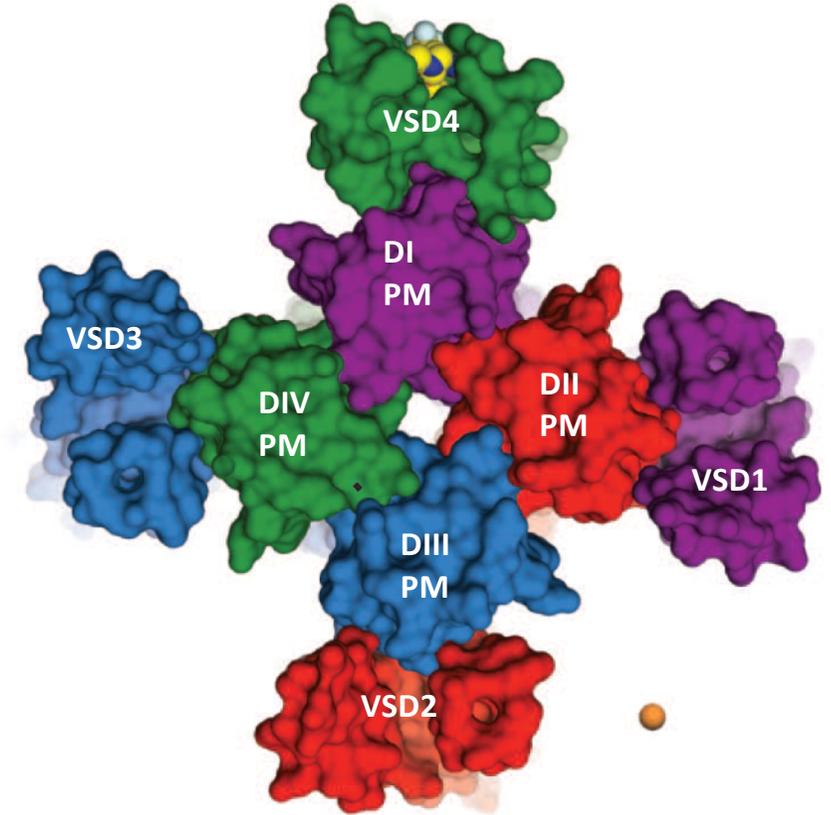
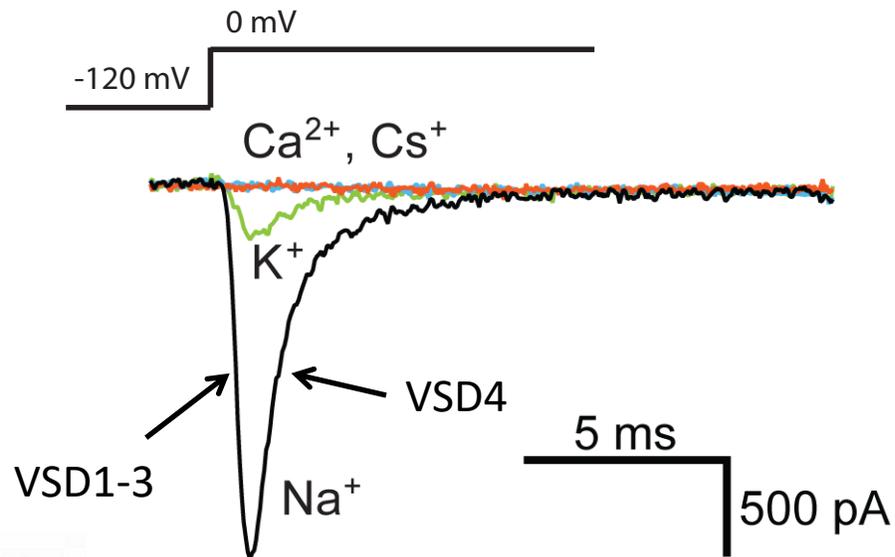
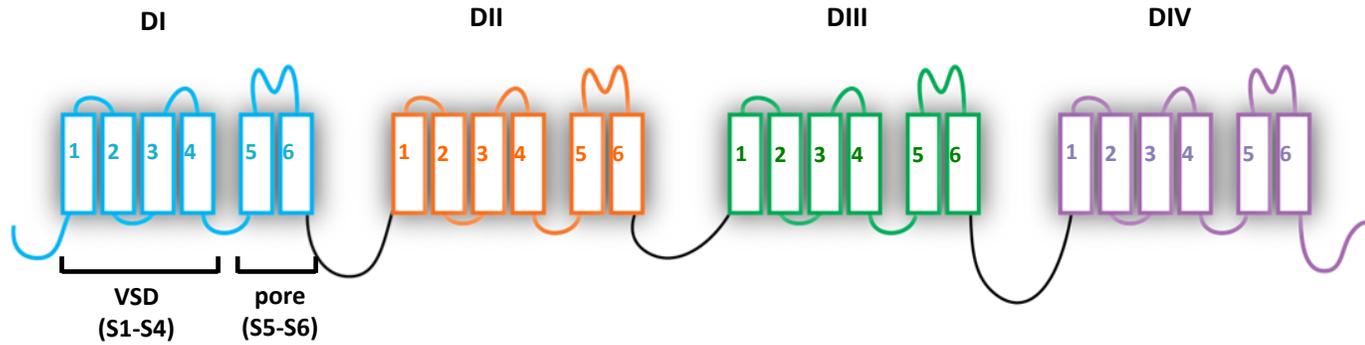
Paroxysmal Extreme Pain Disorder (PEPD)

LOF: no Nav1.7 expression (no pain)



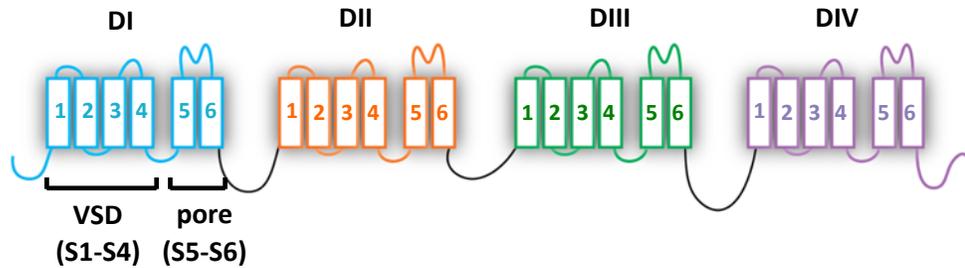
Congenital Insensitivity to Pain (CIP)

Nav channels drive action potentials

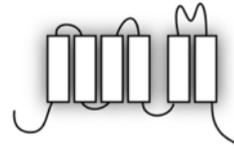


A strategy to enable human Nav1.7 structures

Nav1.7

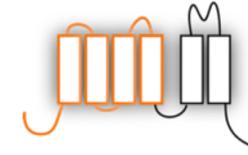


NavAb (homotetramer)

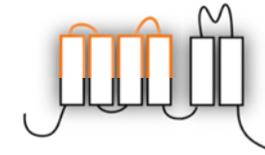


Nav1.7-NavAb chimera

VSD2-NavAb

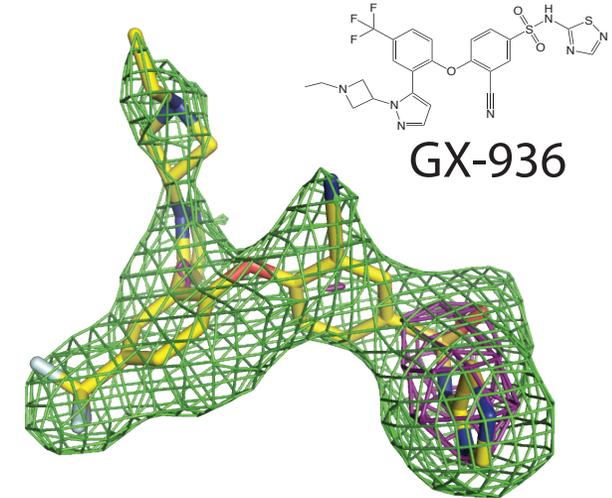
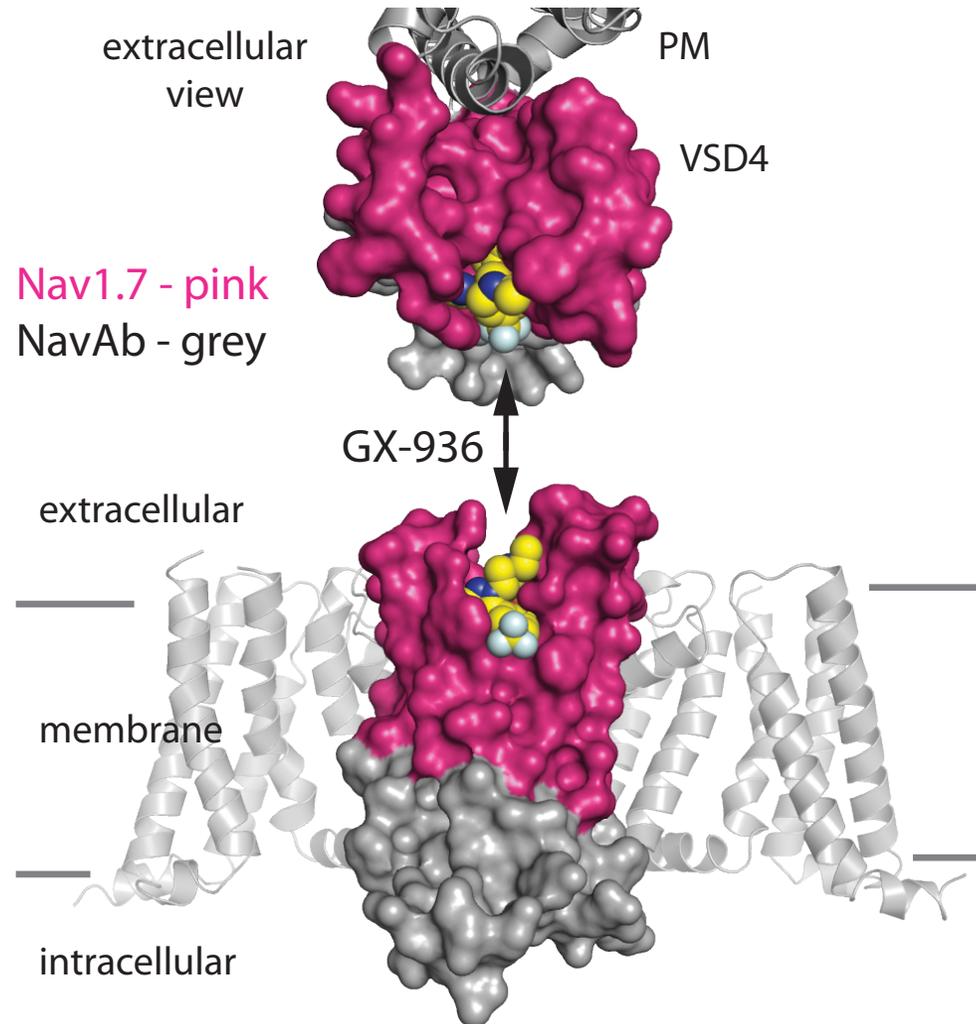


VSD2ex-NavAb



hNav1.7	
Size	24 TMs/2000 residues
Expression	~10,000 copies/cell
Purification yield	0.001 mg/L
Auxiliary subunits	mandatory
PTMs	extensive
Structure precedent	none

4 years of effort, >600 constructs, 1 structure



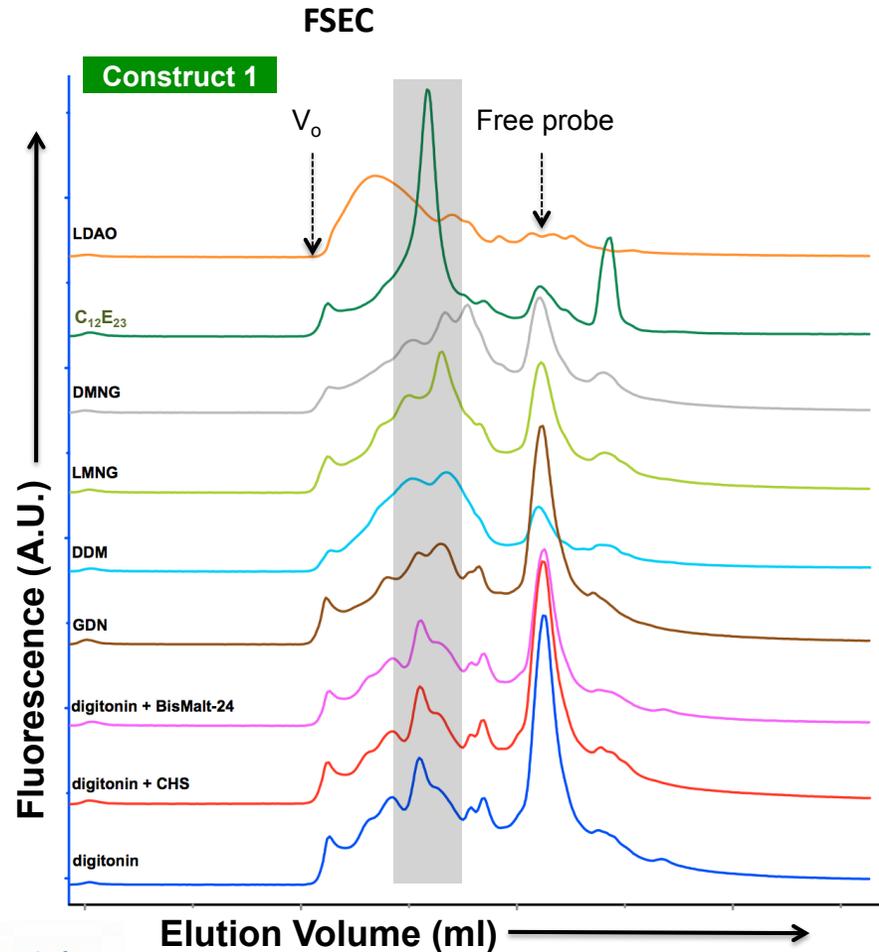
Since 2014 no additional X-ray structures

Payandeh et al., 2011; Ahuja et al. 2015

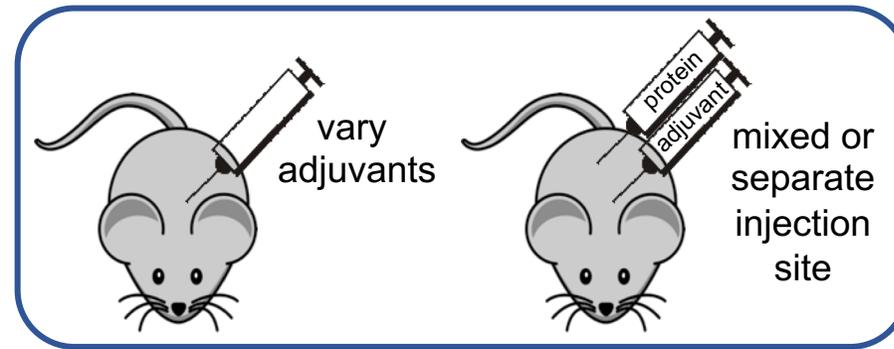
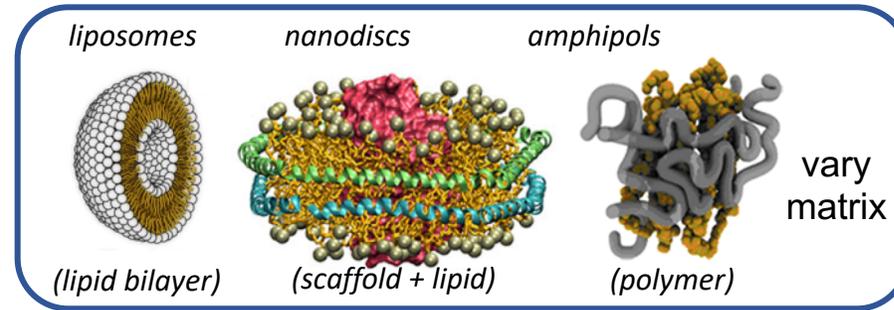
Genentech
A Member of the Roche Group

NavAb Fabs as structural chaperones for cryoEM

Nav1.7: 120 kDa tetramer



Multiple reconstitutions and immunization strategies



Cryo-EM of Nav1.7-NavAb chimera for SM program

120 kDa tetramer + 2 Fabs

Total ~ 220 kDa

Detergent solubilized

Krios, energy filter, K2

13,263 movies

282,532 particles (after 2D classification)

No 3D classification

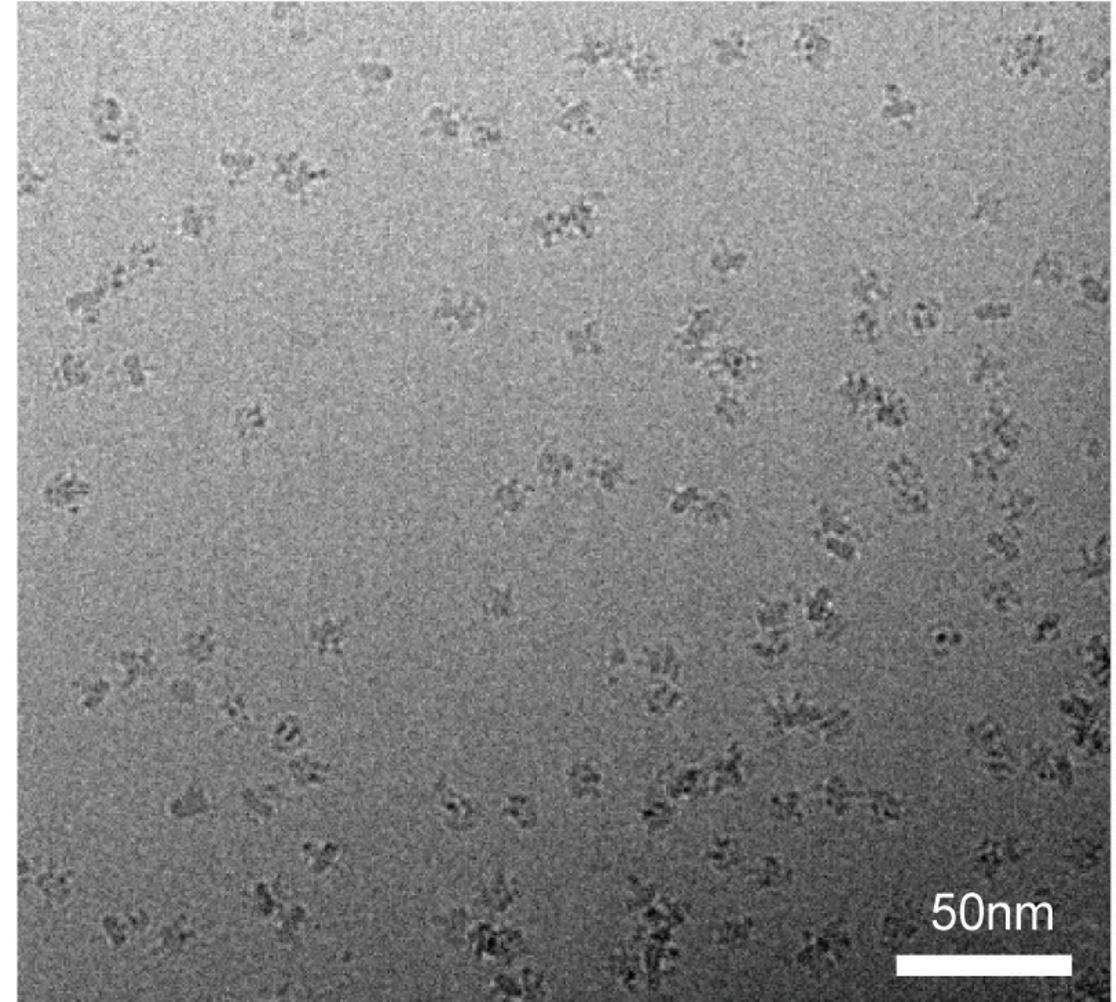
All processing in **cisTEM**

Max. resolution in refinement: 4.2 Å

Frames 2-30 (1.2 – 36 e/Å²)

FSC = 0.143 @ ~2.9 Å

FSC = 0.5 @ ~3.2 Å



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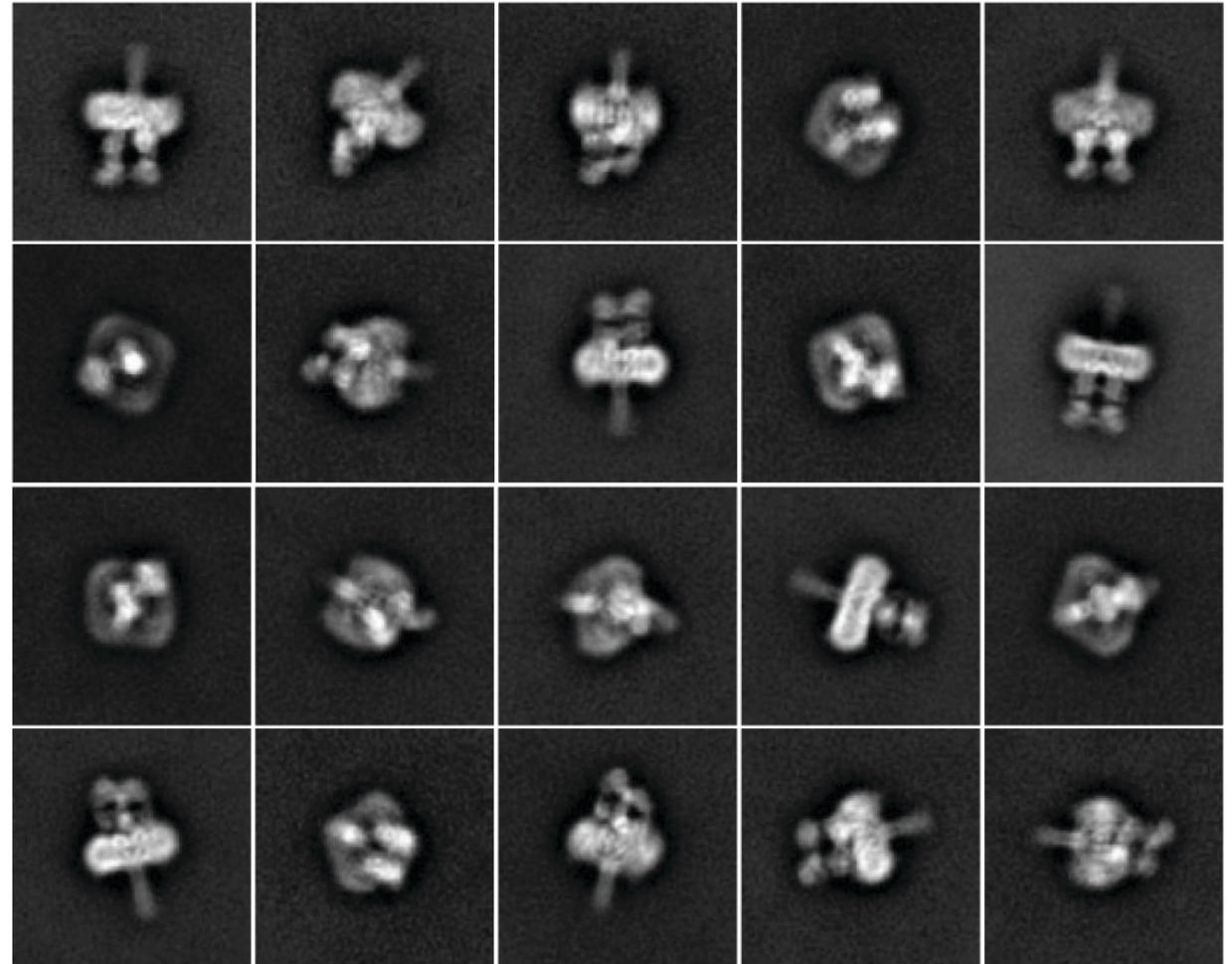
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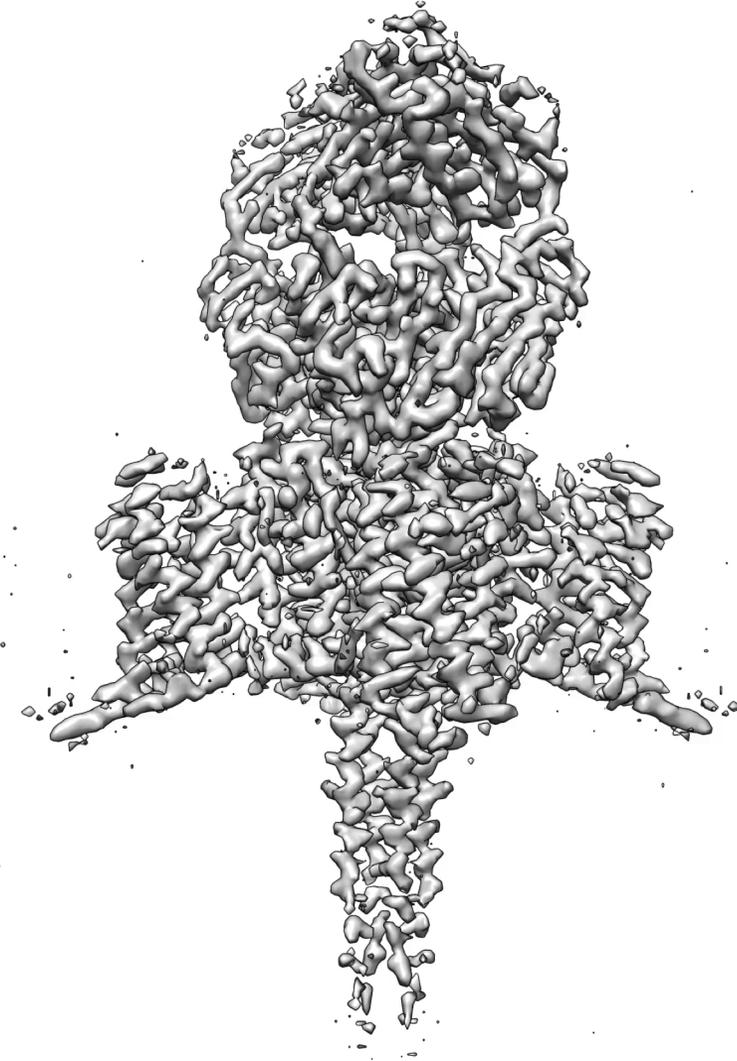
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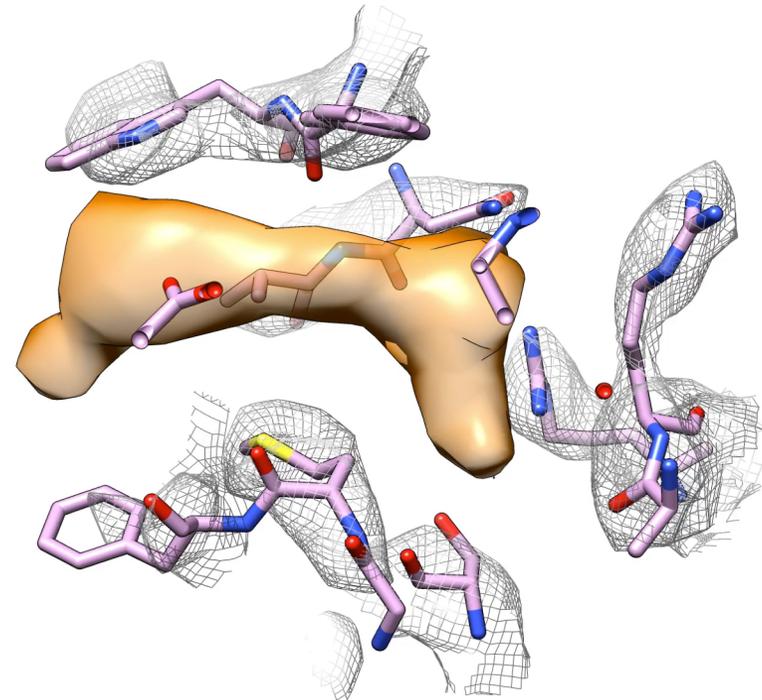
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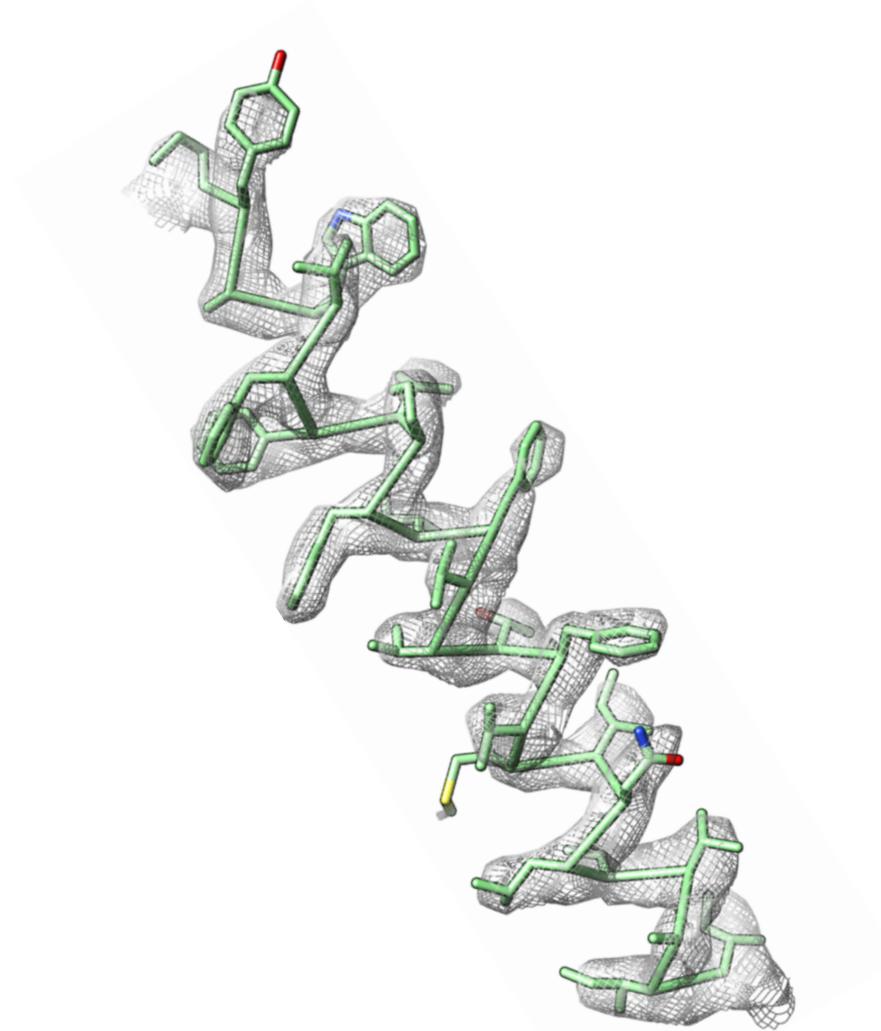
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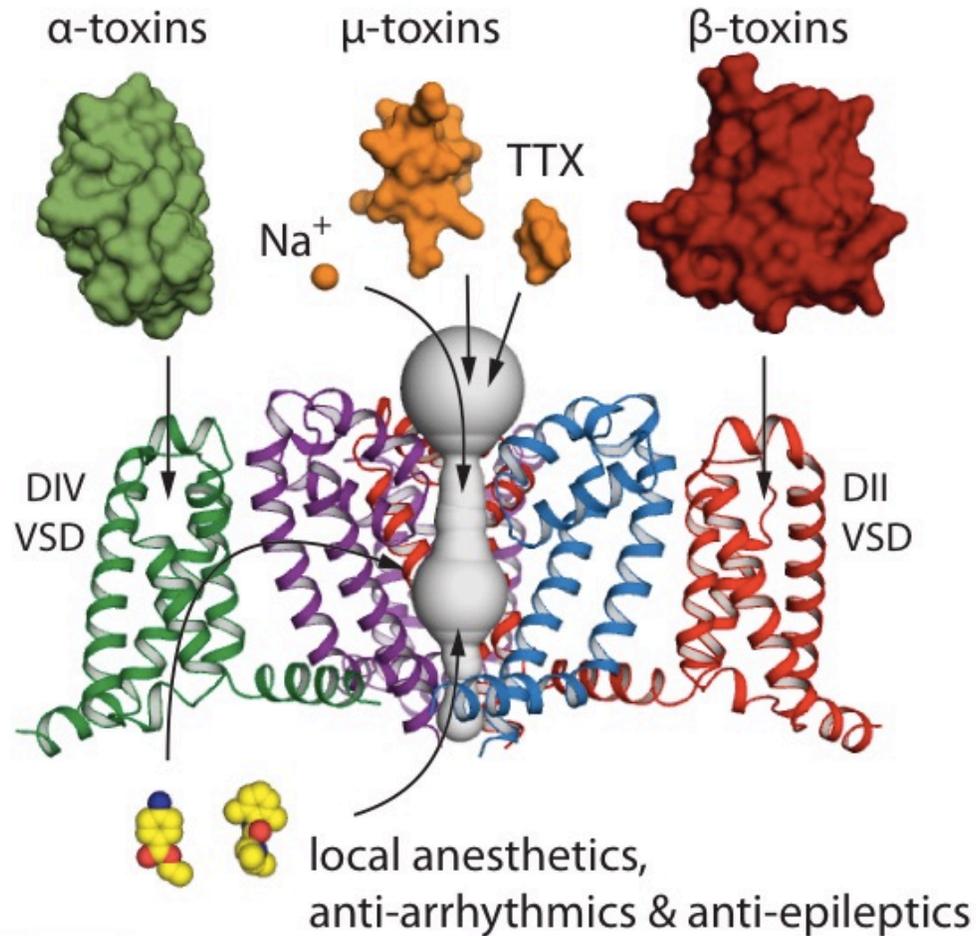
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Venom peptides illuminate sodium channel pharmacology



Christopher Koth

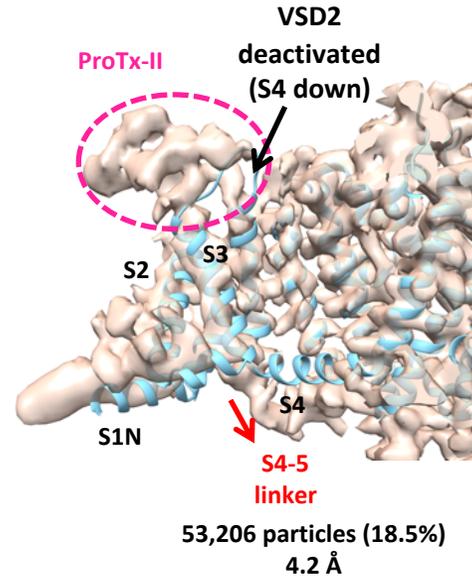
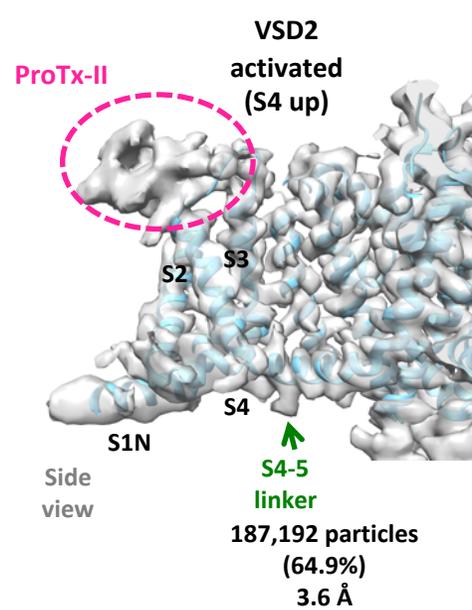


Jian Payandeh

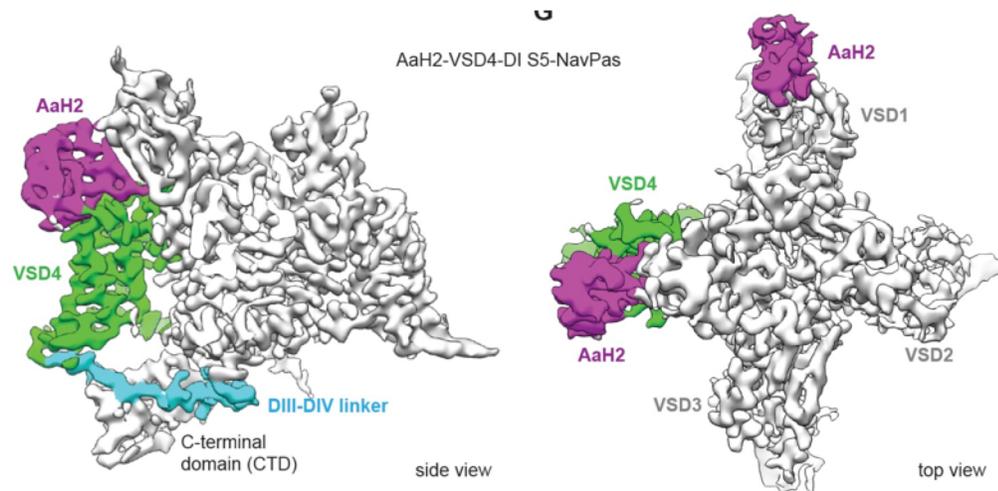
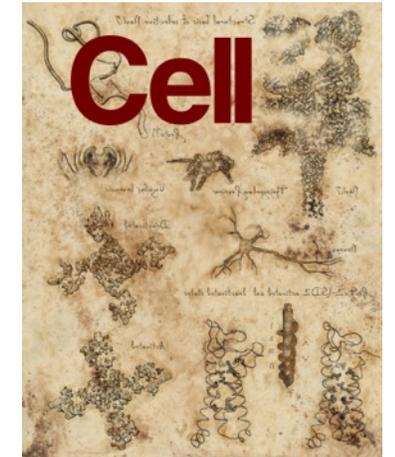


Ahuja *et al.* 2015

Nav 1.7 – Toxins structural studies



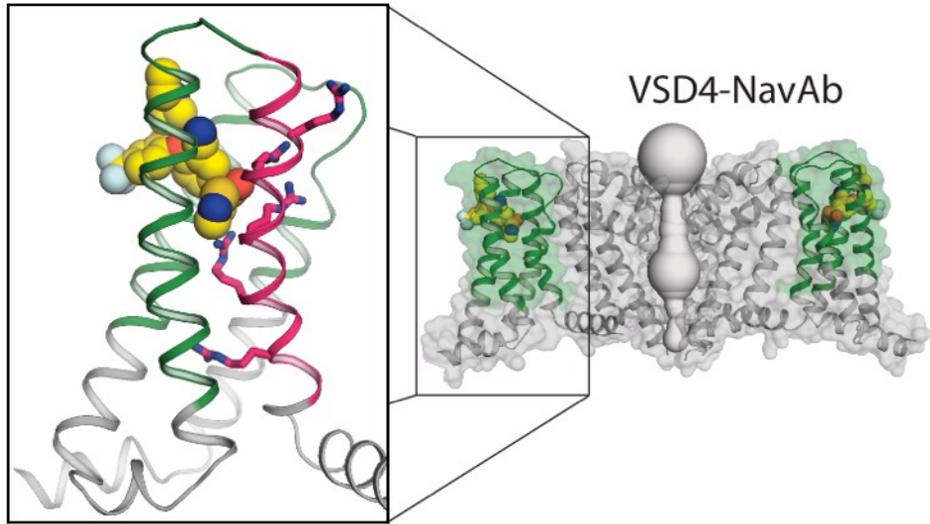
Hui Xu
(Postdoc)



Thomas Clairfeuille
(Postdoc)

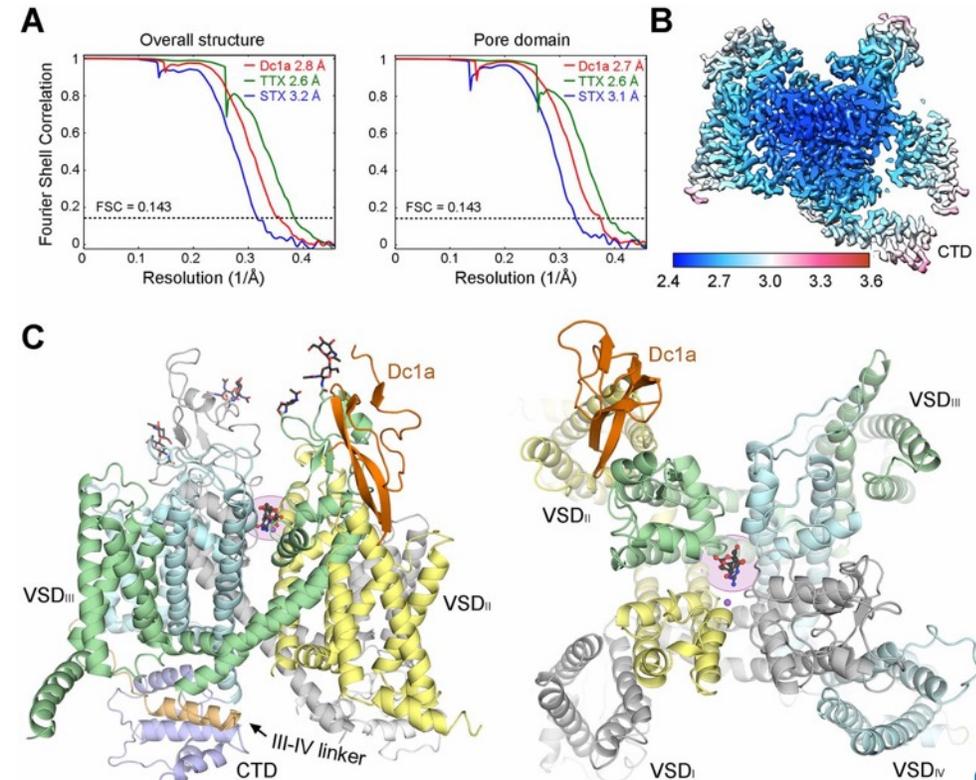


Strategies to achieve higher resolutions



Structural basis for the modulation of voltage-gated sodium channels by animal toxins

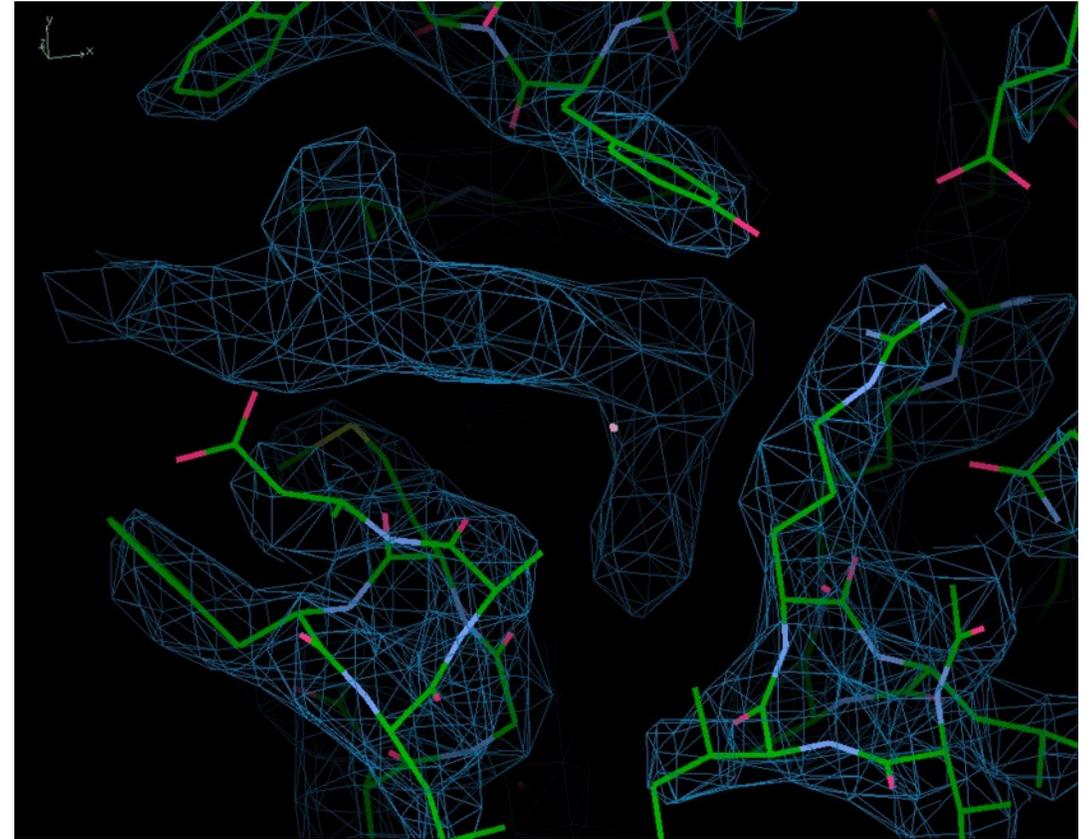
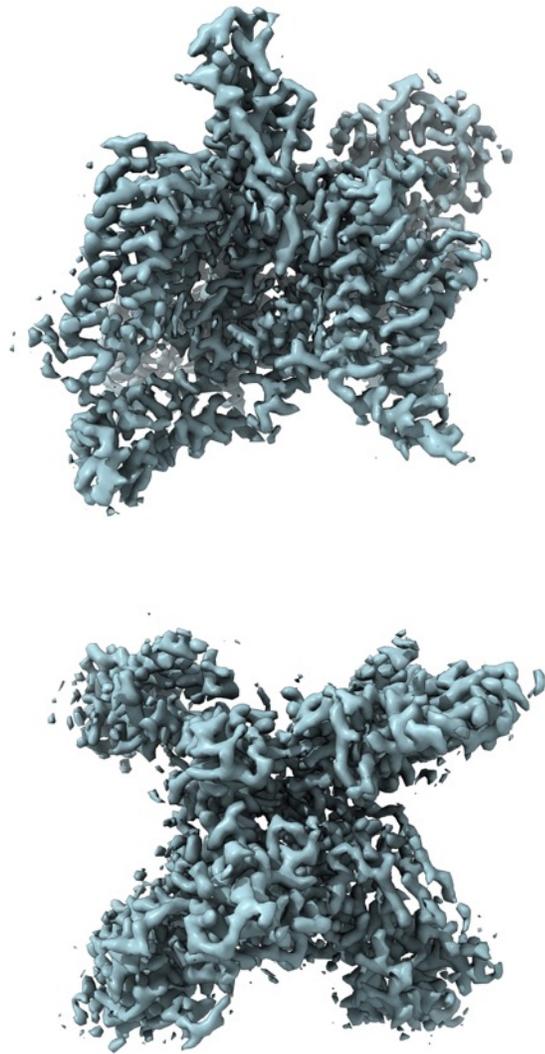
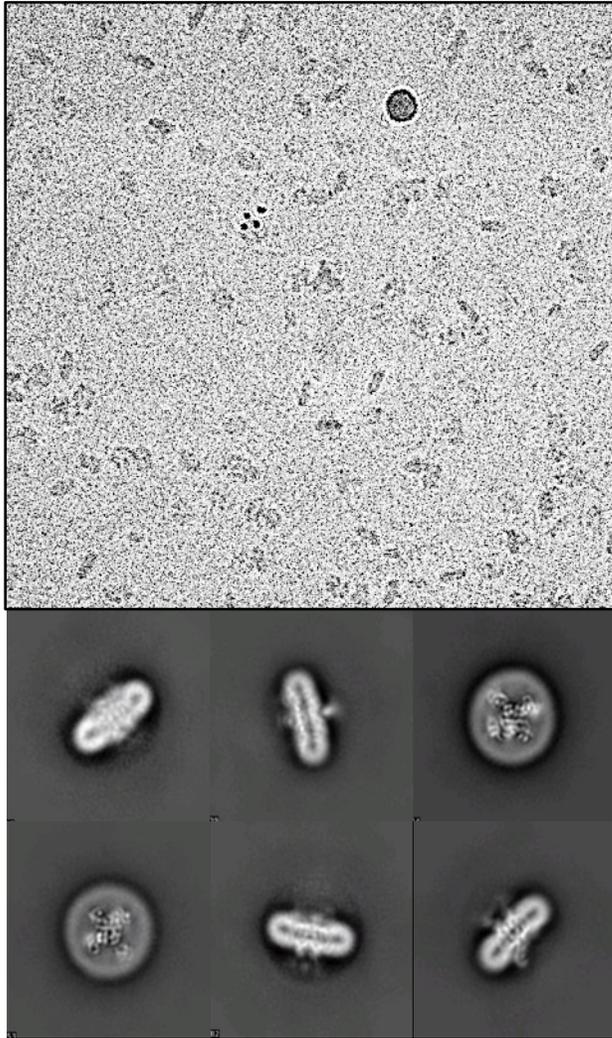
Huaizong Shen^{1,2,3*}, Zhangqiang Li^{1,2,3*}, Yan Jiang^{4*}, Xiaojing Pan^{1,2,3*}, Jianping Wu^{1,2,3†}, Ben Cristofori-Armstrong⁴, Jennifer J. Smith⁴, Yanni K. Y. Chin⁴, Jianlin Lei⁵, Qiang Zhou^{1,2,3‡}, Glenn F. King^{4‡}, Nieng Yan^{1,2,3†‡}



- Toxins + GNE compounds
- Matrix screening
- Include early SM series

~3Å

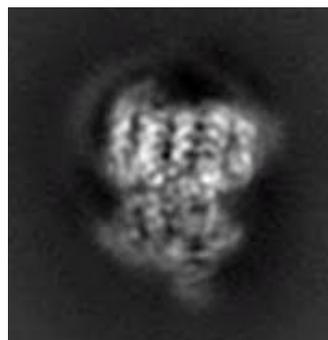
Cryo-EM of NavPas-SM at $\sim 3\text{\AA}$



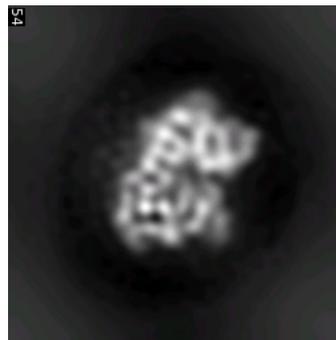
Could Cryo-EM Structurally Enable ALL SM targets?

Exploring cutting-edge technology to enable all SM projects

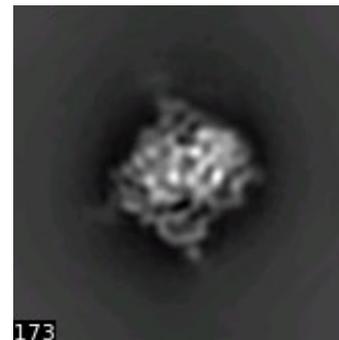
- 2019 • Cryo-EM group goal: Enable small targets (<100kDa)



(95kDa)



(70kDa)

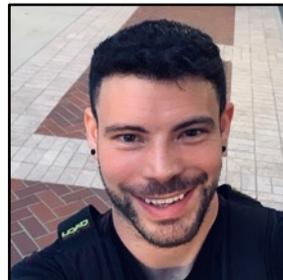


(80kDa)

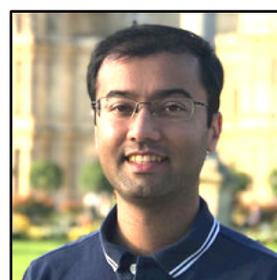
- Fab chaperone and Multimerization



Marissa Mastumoto



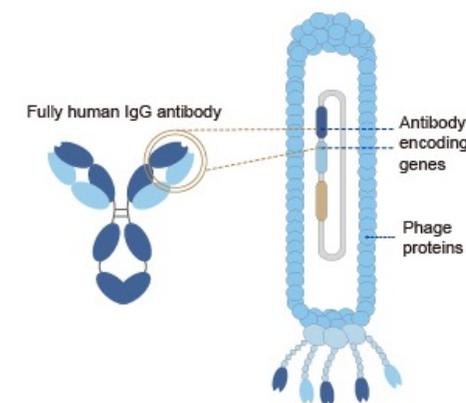
Garrett Gross



Nikit Kumar



Rina Fong



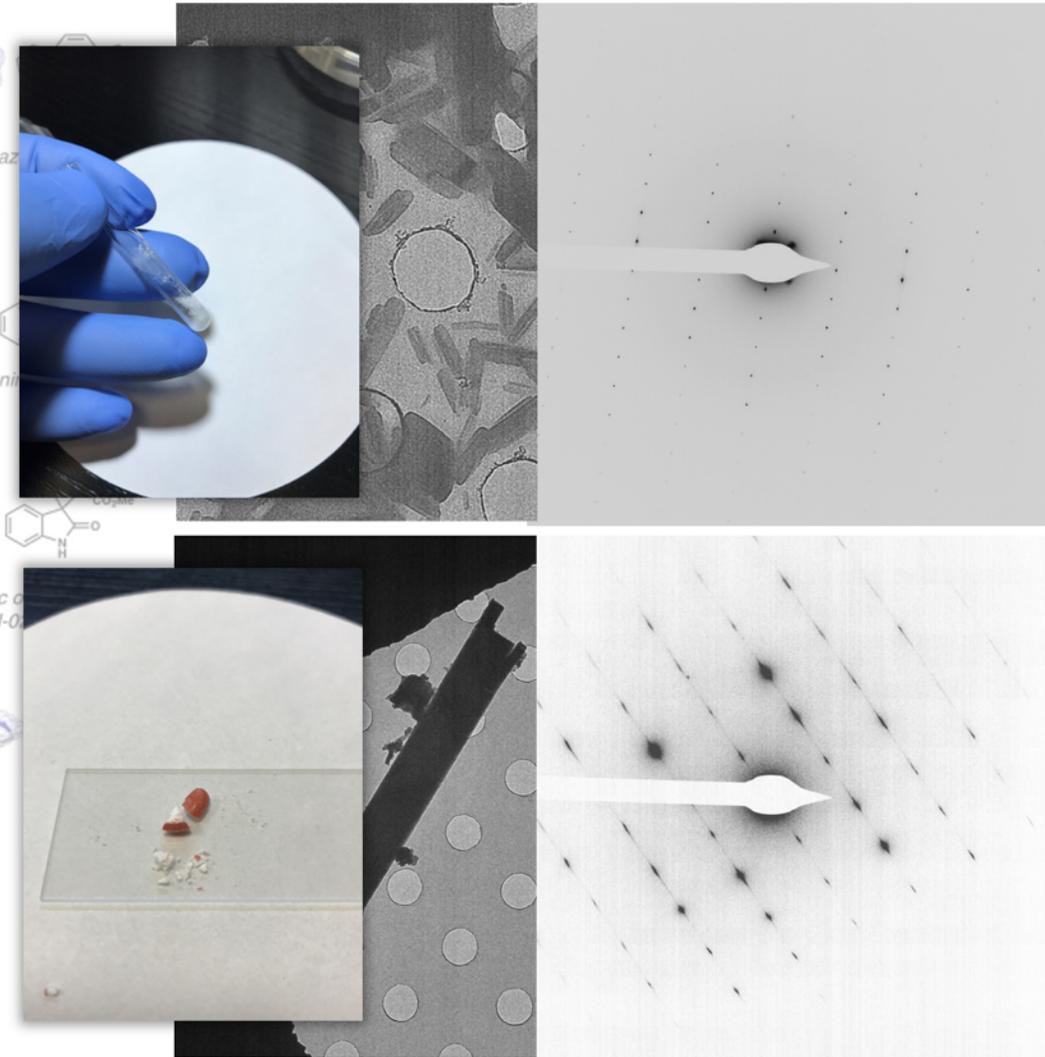
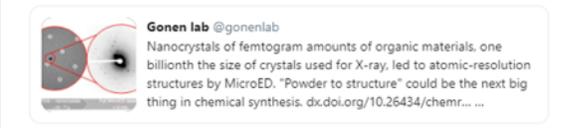
Next challenge: MicroED

To determine structures of SM, macrocycles and proteins



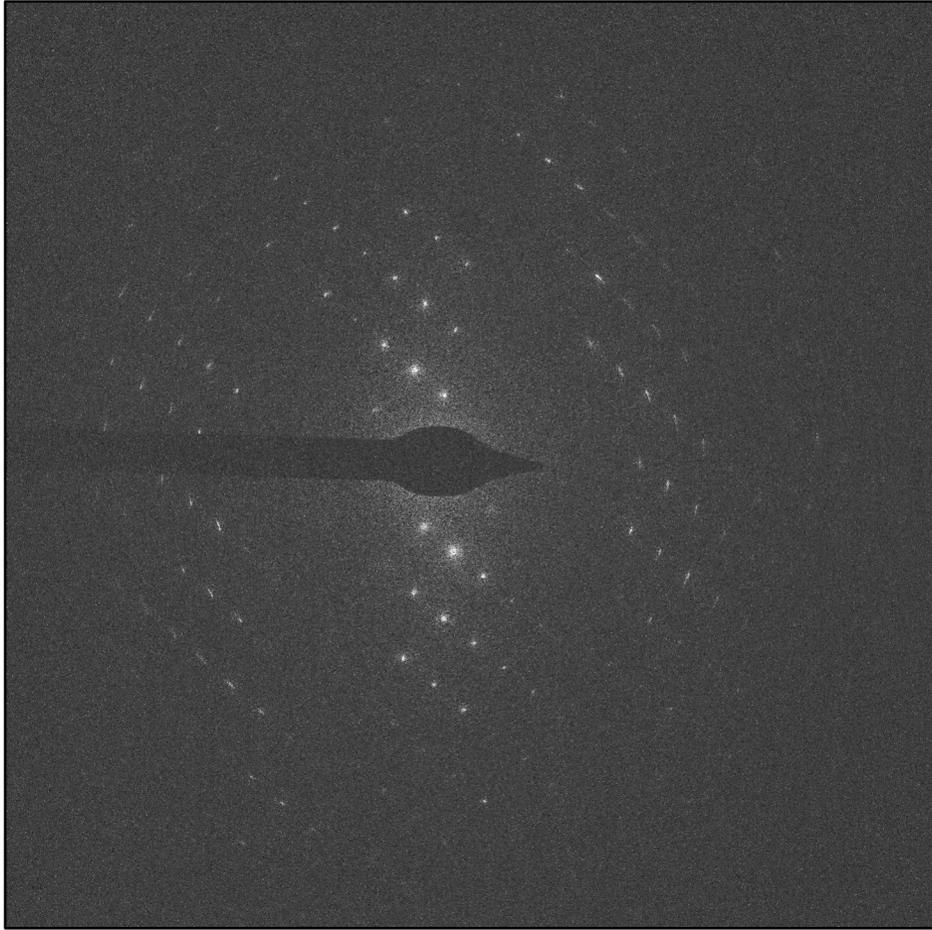
Supporting Information

ABSTRACT: In the many scientific endeavors by organic chemistry, unambiguous identification of a small molecule's structure is of paramount importance. Often, NMR and other powerful spectroscopic techniques developed to address this challenge. While these techniques rely on inference of connectivity, X-ray crystallography and neutron diffraction studies. In practice, X-ray crystallography is rarely applied in routine due to intrinsic limitations of both techniques. Here we report the use of MicroED to provide routine and unambiguous determination of a small molecule's structure with minimal sample preparation, we could in atomic resolution (<1 Å) crystal structures.

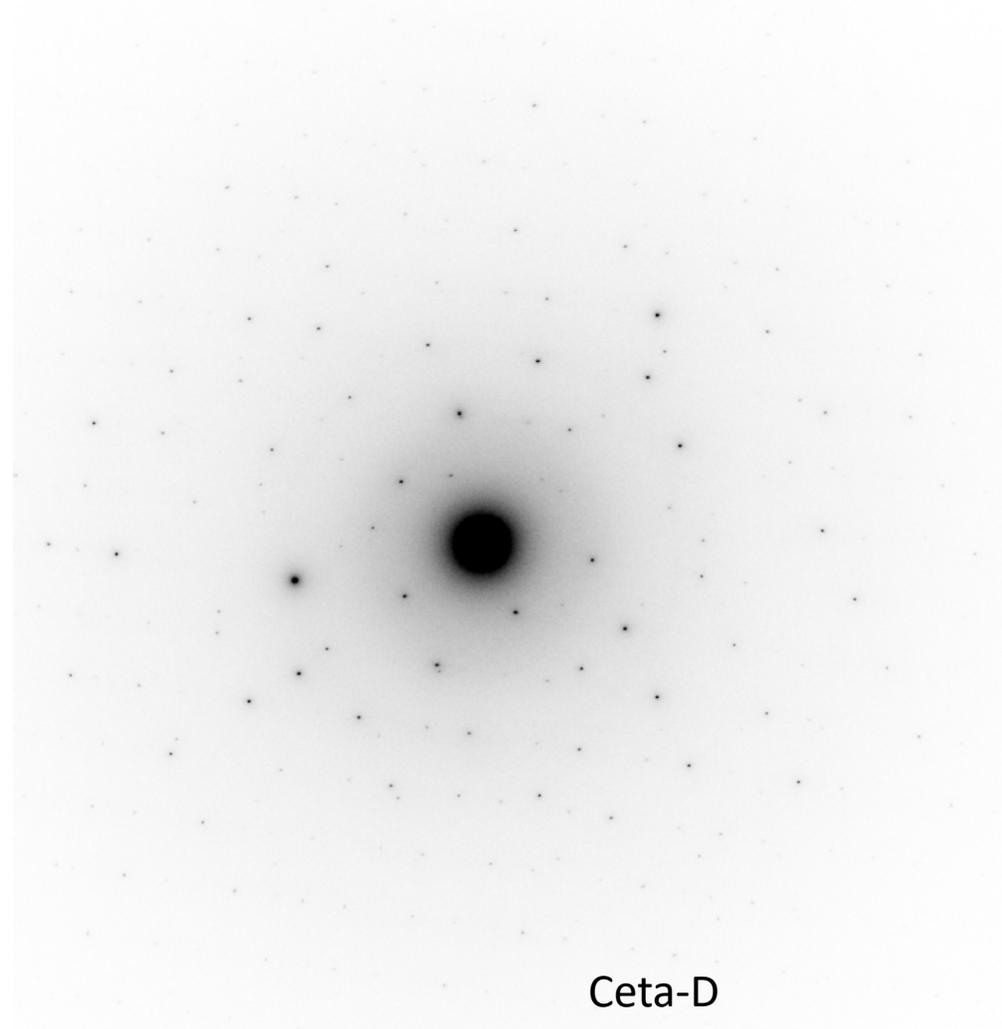


Next challenge: MicroED

To determine structures of SM, macrocycles and proteins



Ceta

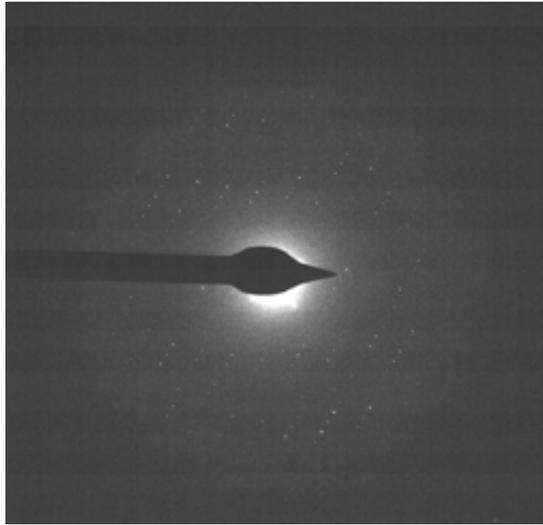


Ceta-D

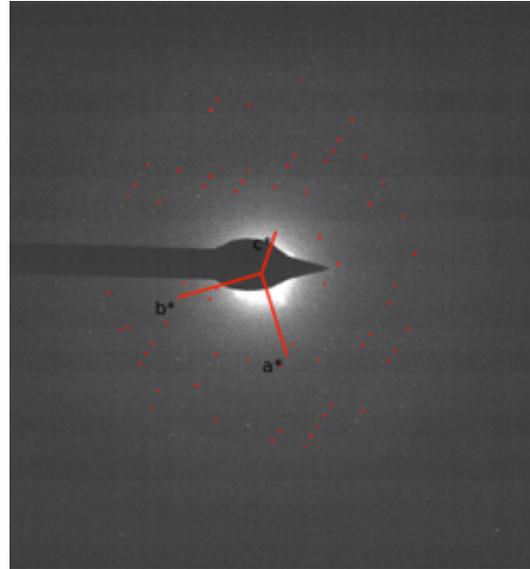
Next challenge: MicroED

To determine structures of SM, macrocycles and proteins

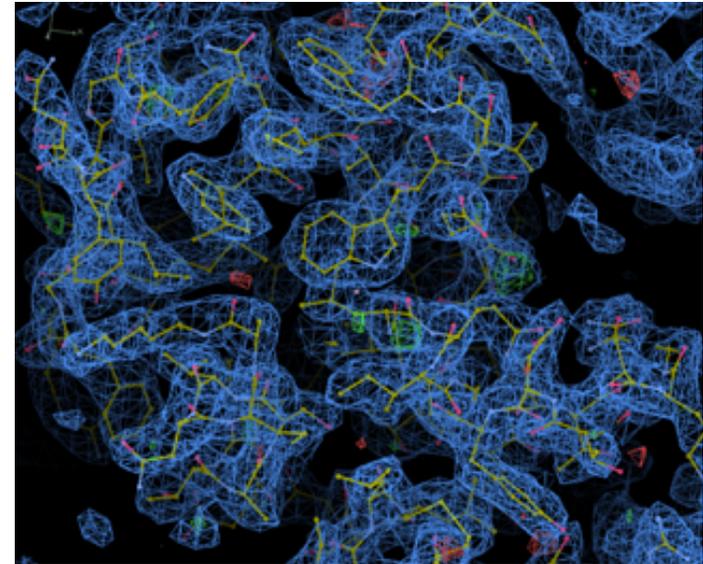
First example of MicroED structure determination at Genentech (Lysozyme at 2.5Å)



Raw image



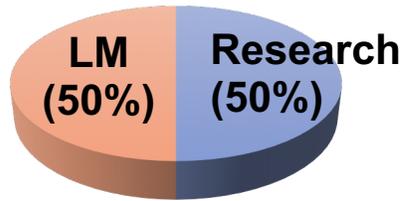
indexed



3-5 years Plan for cryo-EM at Genentech

Drammatically increase the number of targets, Double Equipment and Increase Critical Mass

2015-2017



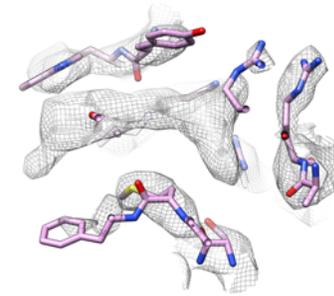
Talos:

- screening
- optimization
- epitope mapping

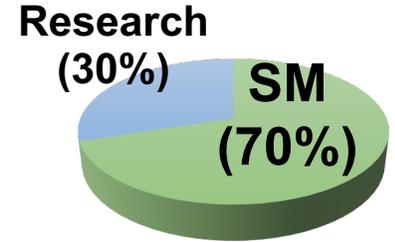


Krios:

- high-resolution
- priority to SM pipeline projects



2018



As of January 2018, SB has two EM instruments

By 2020

SM binding site localization

Structure-based drug design

Binding pocket mapping (side chains)



In Cell CryoEM for SMDD

Acknowledgements:



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Colin Garvey

Benjamin Budde

Annie Dosey

Bill Young

Raymond Ha

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BMR

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Sarah Hymowitz

Chris Koth

Jian Payandeh

James Kiefer

Wayne Fairbrother

Vishva Dixit

Krista Bowman

Melissa Starovasnik

Daniel Kirchhofer

Bob Lazarus

Ingrid Wertz

Patrick Lupardus

Erin Dueber

Andrea Cochran

Jeff Blaney

Dan Sutherlin

Steven Magnuson

Ben Sellers

Dan Ortwine

Steven McKerral

Matthew Volgraf

Huifen Chen

Raymond Schrijver

Anke Mulder

Bryan Majkrzak

Glenn Gilbert

Mark Cruz

Yung Ho,

Hui Xu

Thomas Clairfeuille

Kuan Lin

Christine Jao

Robin Aglietti

Ryan Ferrao

Henry Maun

Michael Holliday

Dawei Sun

Joshua Webster

Mike Reichelt

Heather Jutila

Emi Savage

Dave Sterling

Amanda Hansen

Jessica Foster

Leah Esturas-Pierson

Suzanne Jillo

Dana Tolari

Christopher Wu

Christopher Stewart

Pak Yim

Bob Crisciuolo

Jesse Swafford