

**HIV**

# Coexistence of potent HIV-1 broadly neutralizing antibodies and antibody-sensitive viruses in a viremic controller

Natalia T. Freund,<sup>1</sup> Haoqing Wang,<sup>2\*</sup> Louise Scharf,<sup>2,\*†</sup> Lilian Nogueira,<sup>1</sup> Joshua A. Horwitz,<sup>1</sup> Yotam Bar-On,<sup>1</sup> Jovana Golijanin,<sup>1</sup> Stuart A. Sievers,<sup>2‡</sup> Devin Sok,<sup>3</sup> Hui Cai,<sup>4</sup> Julio C. Cesar Lorenzi,<sup>1</sup> Ariel Halper-Stromberg,<sup>1</sup> Ildiko Toth,<sup>5</sup> Alicja Piechocka-Trocha,<sup>5</sup> Harry B. Gristick,<sup>2</sup> Marit J. van Gils,<sup>6</sup> Rogier W. Sanders,<sup>6</sup> Lai-Xi Wang,<sup>4</sup> Michael S. Seaman,<sup>7</sup> Dennis R. Burton,<sup>3,5</sup> Anna Gazumyan,<sup>1</sup> Bruce D. Walker,<sup>5,8</sup> Anthony P. West Jr.,<sup>2</sup> Pamela J. Bjorkman,<sup>2</sup> Michel C. Nussenzweig<sup>1,8§</sup>

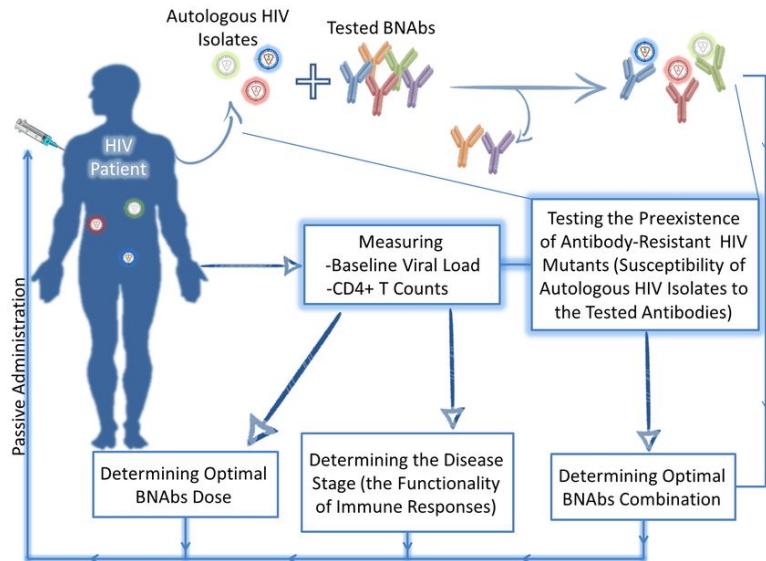
## Presentation By:

Shea Mowry, Meghan Pinter, Maya Bartels, Felipe Munoz & Han Kahvecioglu

# Introduction

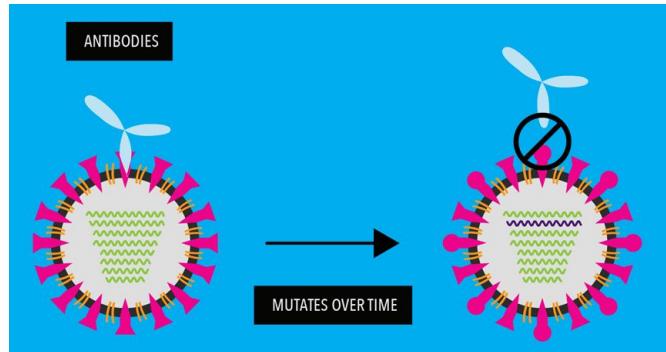
# The Problem:

## broadly neutralizing antibodies (bNAbs)

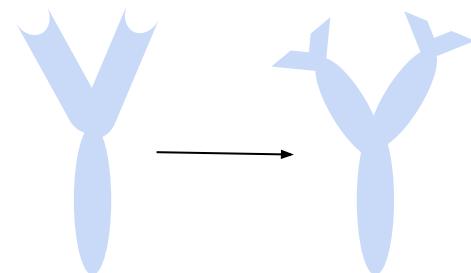


Yaseen, M. M., Yaseen, M. M., & Alqudah, M. A. (2016).  
*International Reviews of Immunology*

## HIV-virus and bNAb coevolution



Pfizer, 2024



→ bNAbs not be effective in controlling chronic HIV-1 infection for the individual who developed them

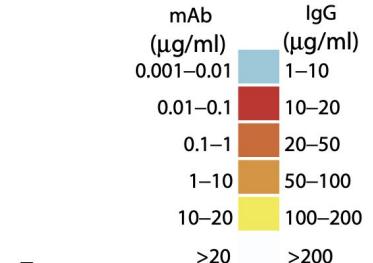
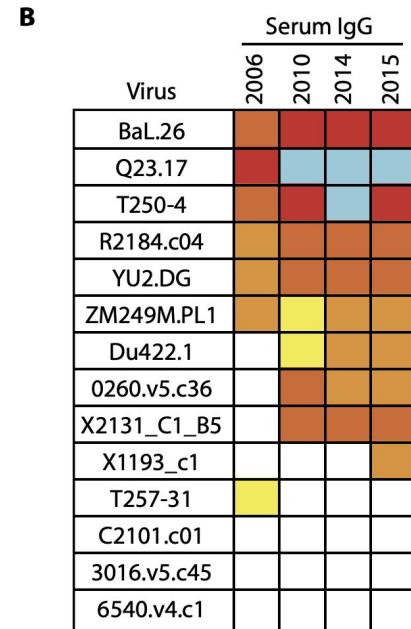
## Research Question:

Is it possible to have “autologous bNAbs [that] can coexist with bNAb-sensitive viruses and contribute to HIV-1 control?”

# Methodology

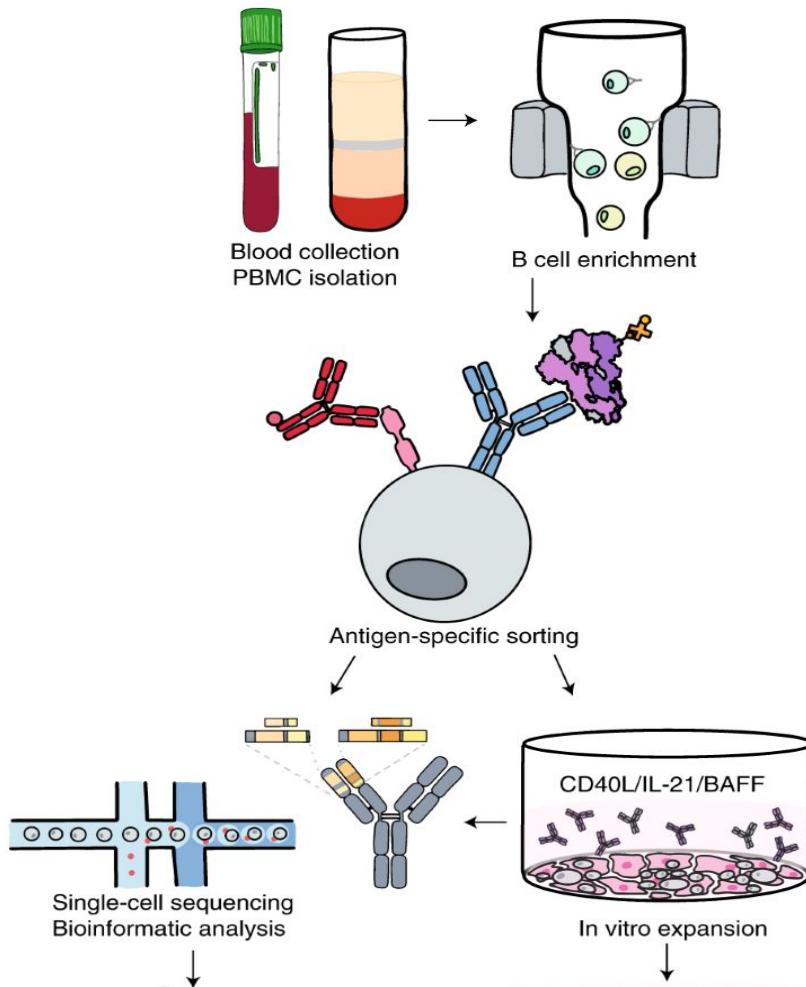
# Donor Selection

- Long term control** → unique immune response to HIV-1 without therapy
- Broadly Neutralizing Antibodies** → multiple bNAbs targeting different HIV-1 sites
- Coexistence of sensitive viruses** → presence of both potent bNAbs and sensitive HIV-1 strains



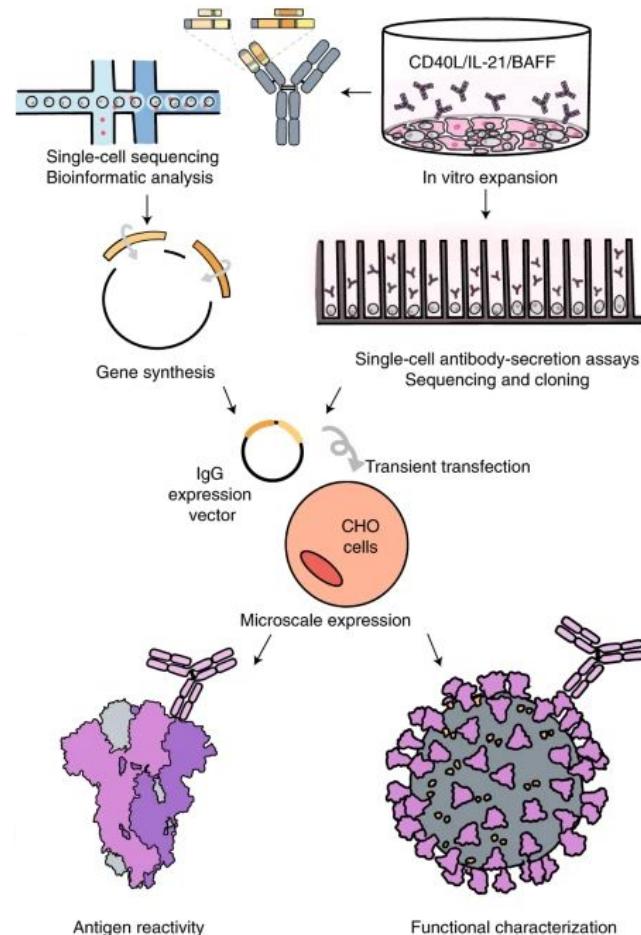
# B Cell Sorting

- Human donor B cells sorted and pre-enriched with  $\alpha$ -CD19 magnetic beads
- B Cells stained with 4 different HIV-1 baits, covering all epitopes



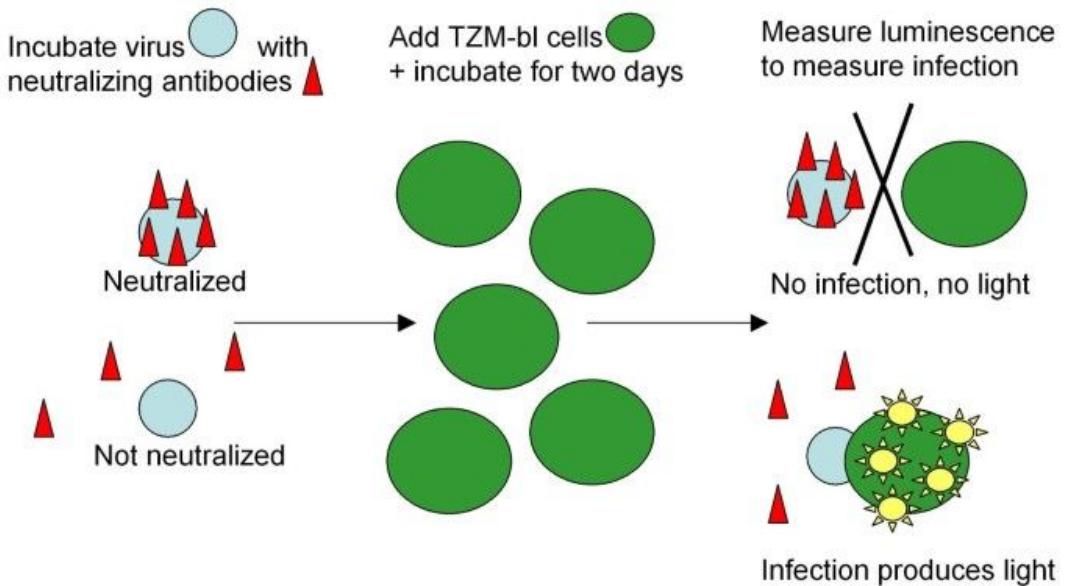
# Antibody Isolation

- Igλ & Igκ chains amplified using primers
- PCR products cloned into vector
- Antibodies produced using human embryonic kidney cells



# HIV-1 Neutralization

- Virus is neutralized using luciferase-based TZM-bl cell assay
- Env pseudoviruses were incubated with fivefold serial dilutions of single antibodies
- Applied to TZM-bl cells
- Recorded luminescence reduction of 50% and 80%



# X-ray Crystallography

- Explored structure of Fab fragments & gp120 protein complex

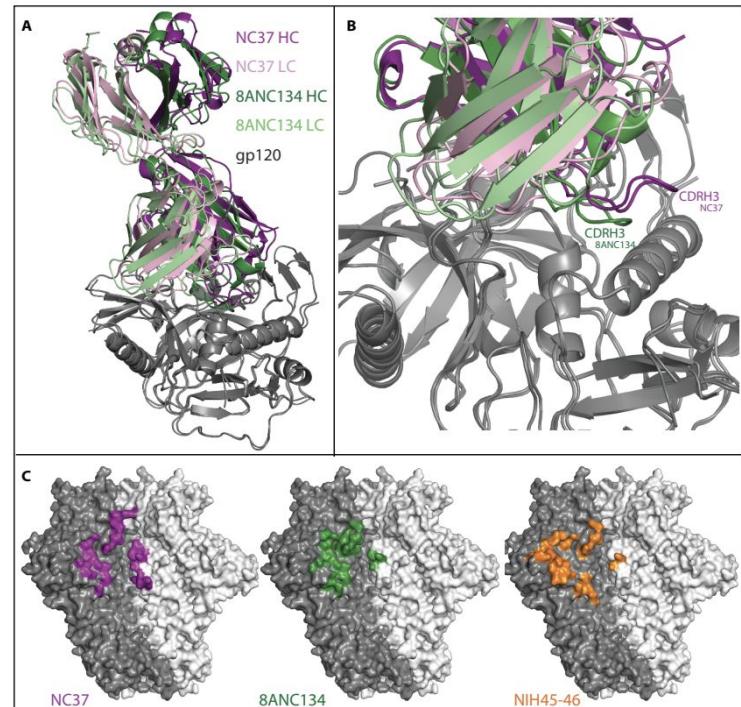
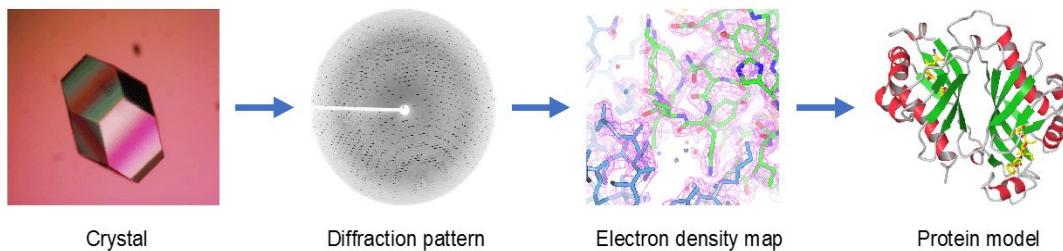
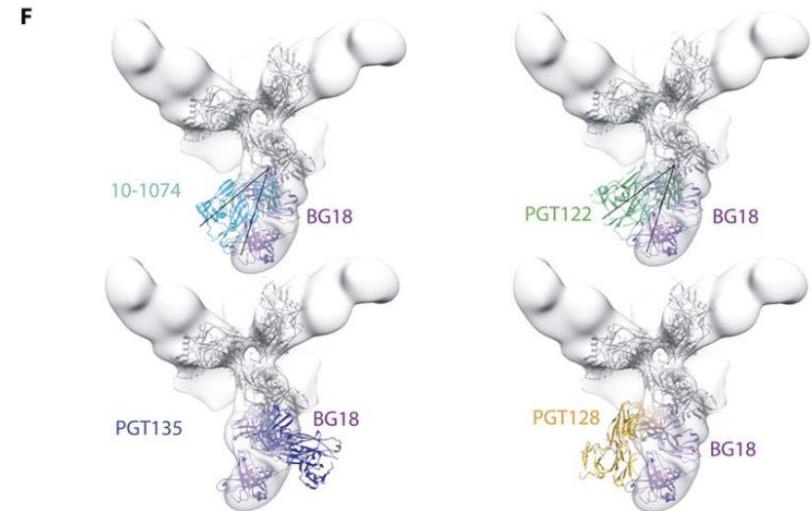
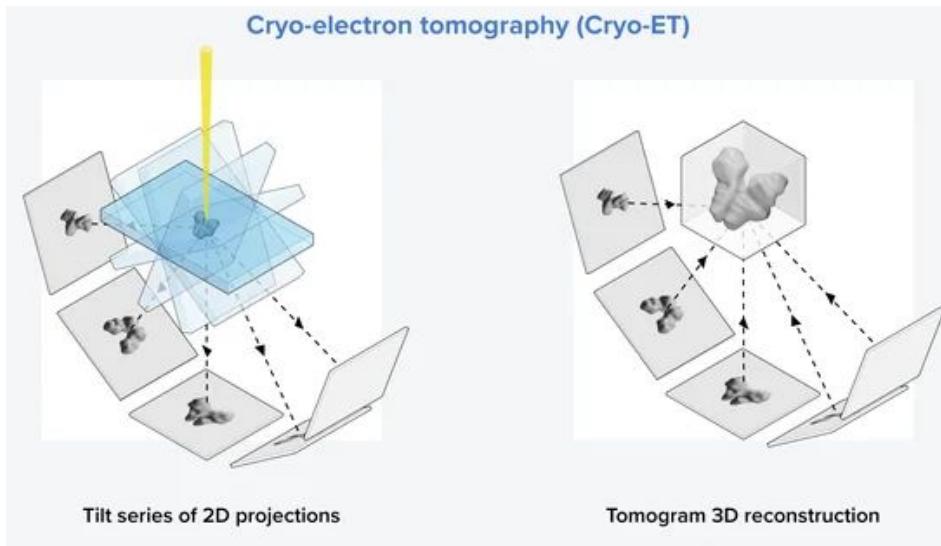


Figure S3: NC37 Fab-gp120 complex structure.

# Cryo-Electron Tomography/Subtomogram

- Provides high resolution structure using single-particle EM
  - BG18 bNAb



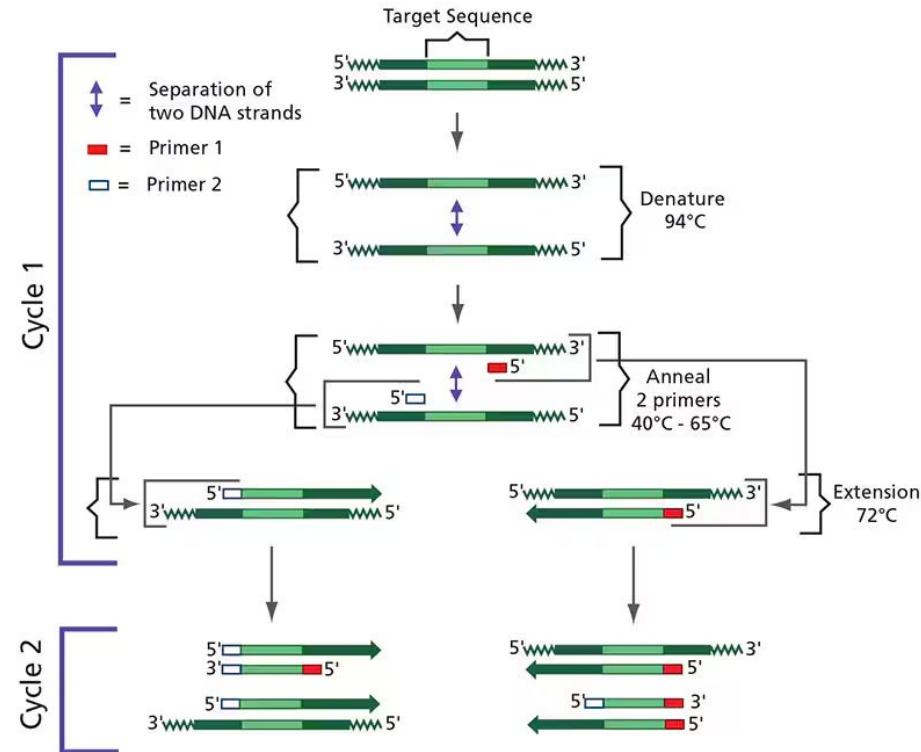
**Fig. 2.** Sequence and structural analysis of BG18 bNAb

# Autologous Virus

HIV-1 RNA extracted from donor plasma.

2 rounds of PCR performed to amplify the HIV-1 RNA extracted from the donor.

CMV-Env expression cassettes were generated and then transfected into 293T cells.



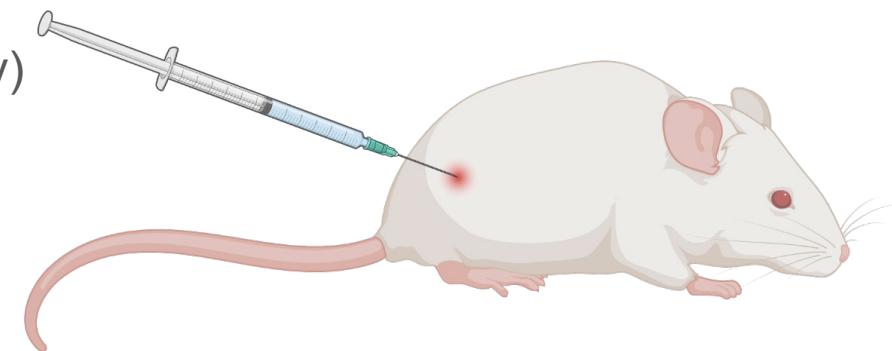
# *In vivo* Mouse Model

Using humanized nonobese diabetic mice - IACUC approved protocols

Mice treated subcutaneously with 1 mg dose of each antibody twice per week for 3 weeks (6 total antibody injections).

Control humanized mice: reconstituted with donor cells and infected with HIV but not treated with antibodies

Measurement: Plasma viral loads (weekly)



# **Results**

# Measuring bNAb affinity

Introduce epitope-specific point mutations into HIV-1 YU2 (well-characterized clade B strain)

IgG - polyclonal antibodies (positive control)

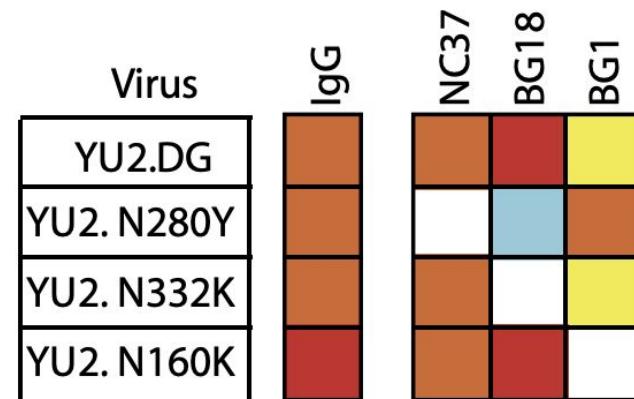
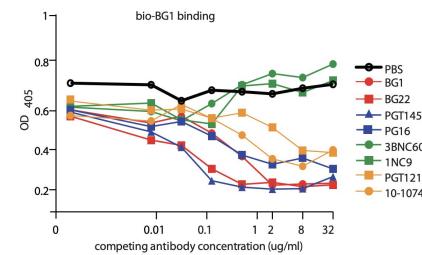
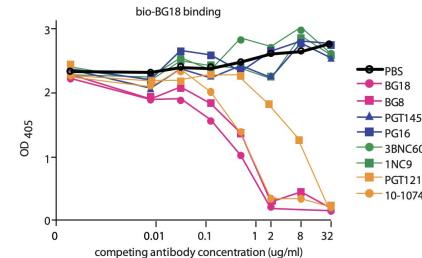
N280Y - CD4 binding site

N332K - glycan-V3

N160K - V1V2

Competitive ELISA (enzyme-linked immunosorbent assay) for validation

mAb ( $\mu\text{g/ml}$ )	IgG ( $\mu\text{g/ml}$ )
0.001–0.01	1–10
0.01–0.1	10–20
0.1–1	20–50
1–10	50–100
10–20	100–200
>20	>200

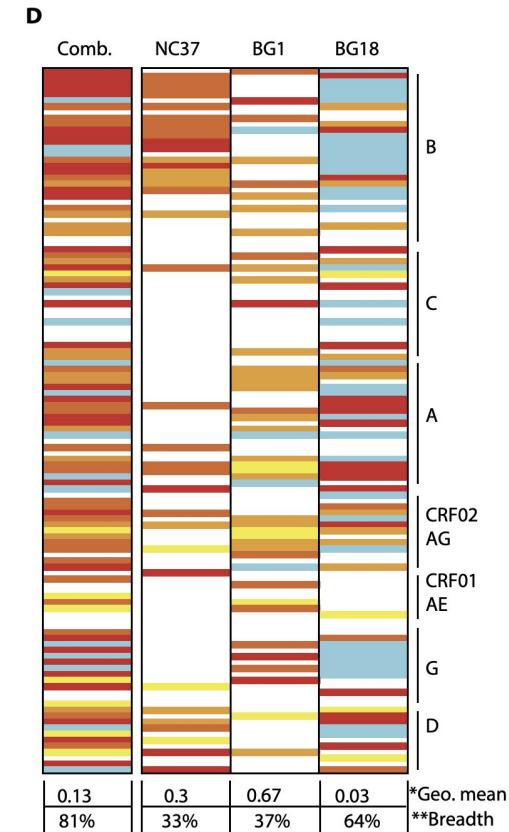
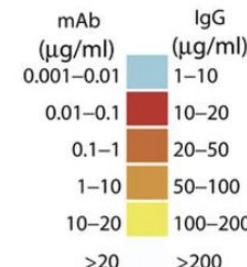


# Measuring bNAb affinity

Neutralization potency of NC37, BG1, and BG18 tested against HIV-1 pseudovirus.

BG18 neutralized more than NC37 and BG1 when not mixed, but a combination produced  
**81% neutralization of HIV-1.**

BG18 emerged between 2010 and 2013, NC37 and BG1 emerged earlier.



\*Geometric mean of the viruses neutralized at  $\text{IC}_{50}$  of  $<30 \mu\text{g/ml}$

\*\*Precent of viruses neutralized at  $\text{IC}_{50}$  of  $<30 \mu\text{g/ml}$

# BG18

Emerged between 2010 and 2013

Comparable to PGT121 and 10-1074 - previously discovered bNAbs targeting V3

BG18 more potent

## Heavy Chain

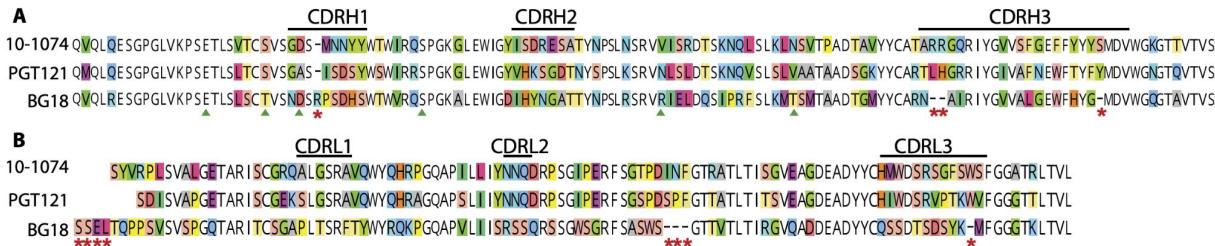
90% identity of germline genes

Less than 62% identity of mature heavy chain

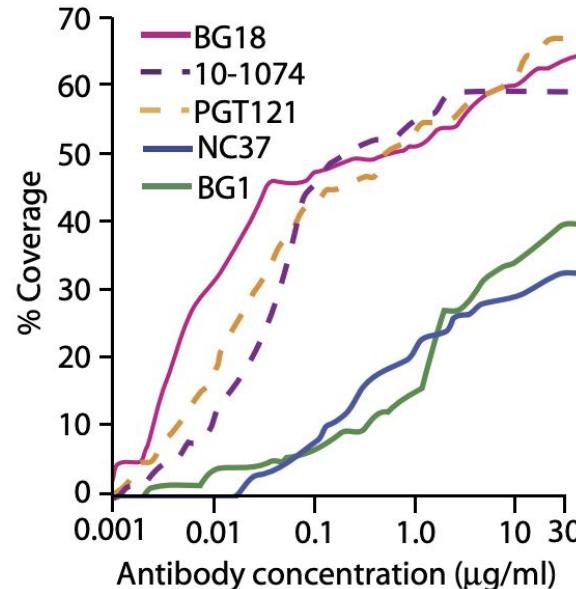
## Light Chain

70% germline

48% mature



**E** >20 >200



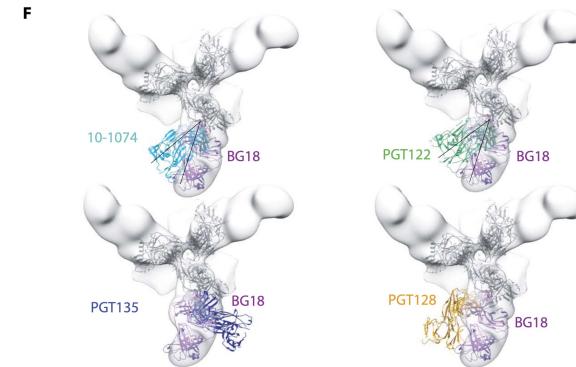
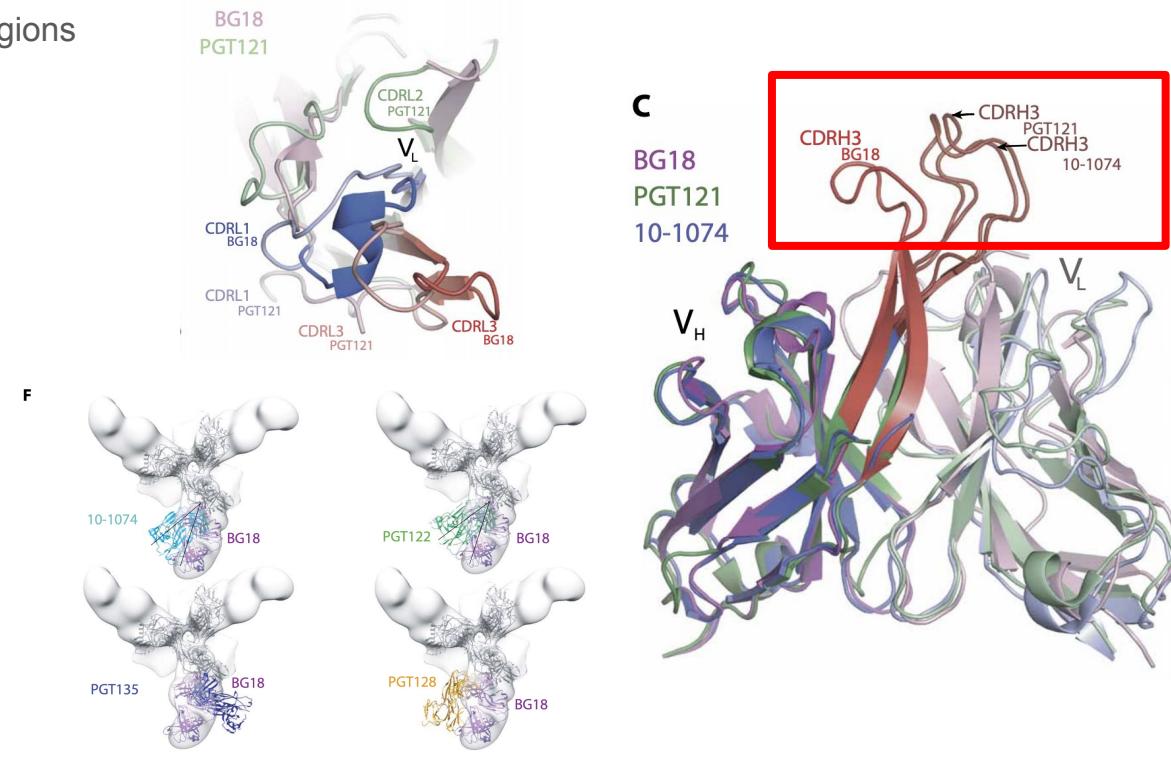
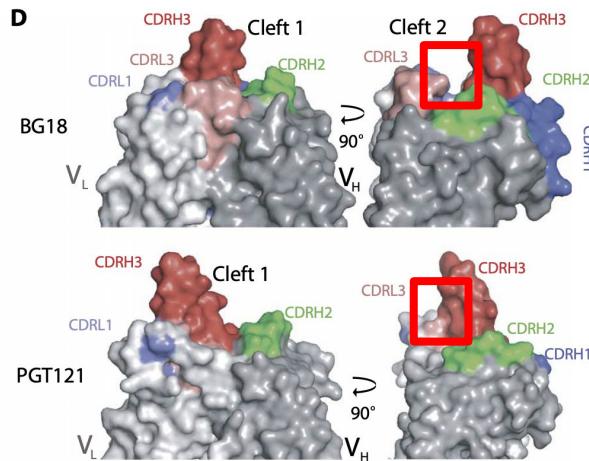
# BG18 Fab (Fragment antigen-binding) structure

CDRs - complementarity determining-regions

Part of variable region of antibody

3 CDRs on heavy and light chains

CDR3 most diverse

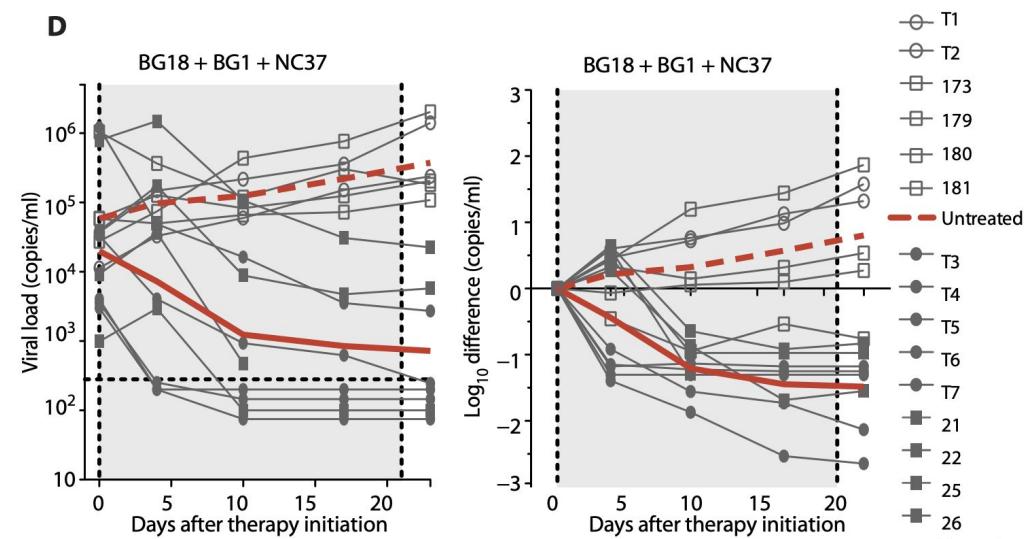
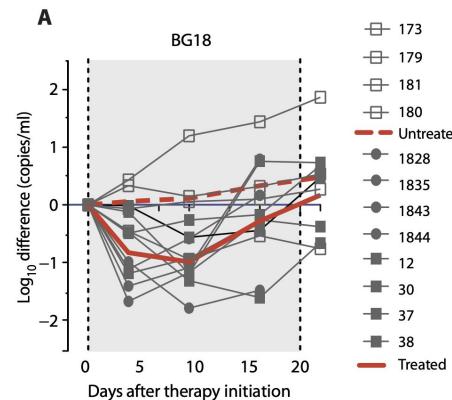


# Combination-bNAb Therapy Controls HIV-1 in Mice

Nine HIV-1YU2-infected humanized mice treated with 1:1:1 combination of new bNAbs

Effect: prolonged drop in viremia

6 of 9 mice showed undetectable viral load after 3 weeks



# Results - Outline

Isolated three new bNAbs that account for serologic neutralizing activity and circulating viruses

- **BG18 (the most potent)**
  - Structural findings
  - targets Asn332 gp120-centered glycan patch (frequent target for anti-HIV-1 bNAbs, because contains key carbohydrates bNAbs bind to)
  - BG18 approaches HIV-1 envelope protein from a different angle (vs. other well known antibodies)
  - BG18 Fab has distinct orientation and a shorter length of CDRH3 region (compared to other members of the same class)
    - Lack of insertions or deletions in the BG18 CDRH3 suggests that BG18-like antibodies may be easier to elicit by vaccination.

# Results - Outline

The other two neutralizing specificities:

- BG1
  - binds to V1V2 region of Env (frequent target of bNAbs arising in natural infection)
  - first antibody in this class to be isolated from a clade B–infected donor
  - is potent, but not as strong as other known bNAbs
- NC37
  - uniquely, NC37 recognizes a quaternary trimer epitope, the core of which overlaps with the CD4bs (important region for viral entry into cells)

## Key outcomes

- EB354 developed multiple bNAbs recognizing different parts of the HIV-1 Env protein. Non overlapping targets provides broader protection
- Mouse trials confirmed BG1, BG18, and NC37 combined were able to suppress HIV-1 infection, indicating potential therapeutic use: **combination antibody therapy**

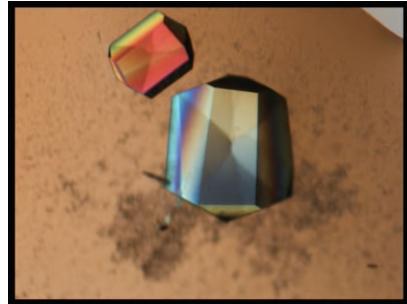
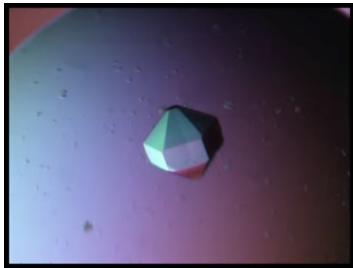
# **Highlighting Structural Analysis Techniques**

# X-Ray Crystallography

## Target is crystallized

Purified and crystallized

Highly-ordered, solid state



From Royal Institute: "Understanding Crystallography"

## Target is irradiated with x-ray beams

X-rays interact with electrons in molecule

Produce diffraction pattern

Reveals density map at angstrom resolution

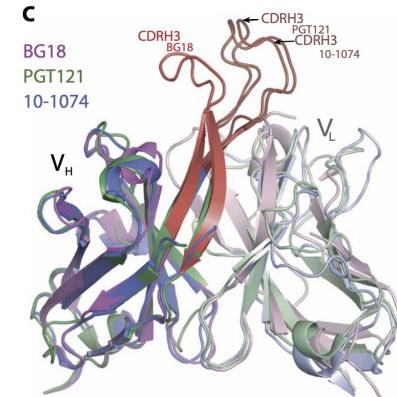


Iowa State University Chemical Instrumentation Facility

## Target shape reconstruction

Target structure deduced from diffraction data

Use computational techniques like molecular replacement (MR)



# Cryo-electron tomography

## Sample is flash frozen

Frozen, then sectioned

This keeps tissue close to its native state

High pressure freezing avoids formation of ice

## Sample is placed under electron microscope

Use of EM means no staining is needed

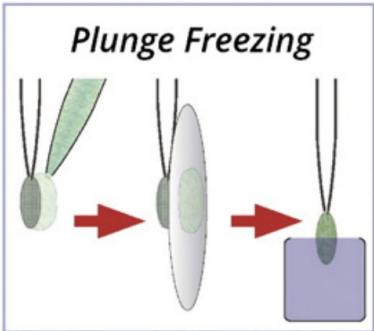
Allows nanometer resolution

Not a traditional electron microscope, specialized equipment to tilt the sample

## Sample is rotated and imaged

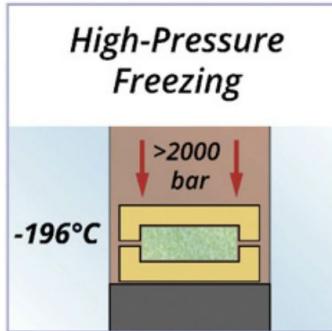
2D images used to understand 3D structure, just like a tomography scan

Images collected from many angles, computationally merged to obtain 3D structure

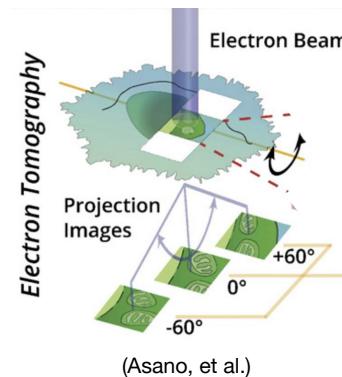


(Asano, et al.)

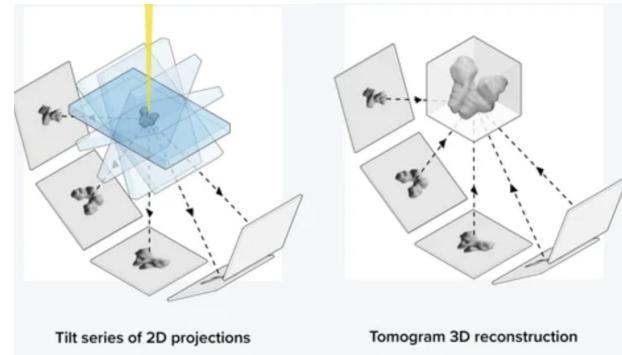
OR



(Asano, et al.)



(Asano, et al.)

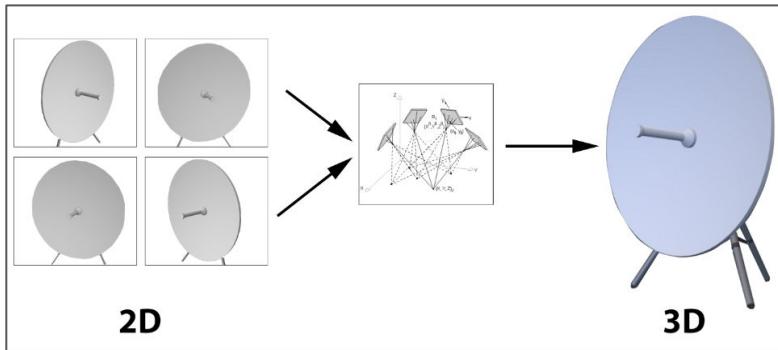


Tilt series of 2D projections

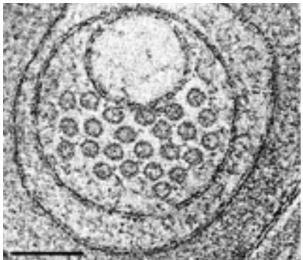
Tomogram 3D reconstruction

("What Is the Difference between Cryo-EM and Cryo-Et?")

# Cryo-electron tomography



If you have images of an object from **multiple angles**, and figure out the points in space you are looking at the object from, you can figure out the **3D shape of the given object**.



(David H., et al.)

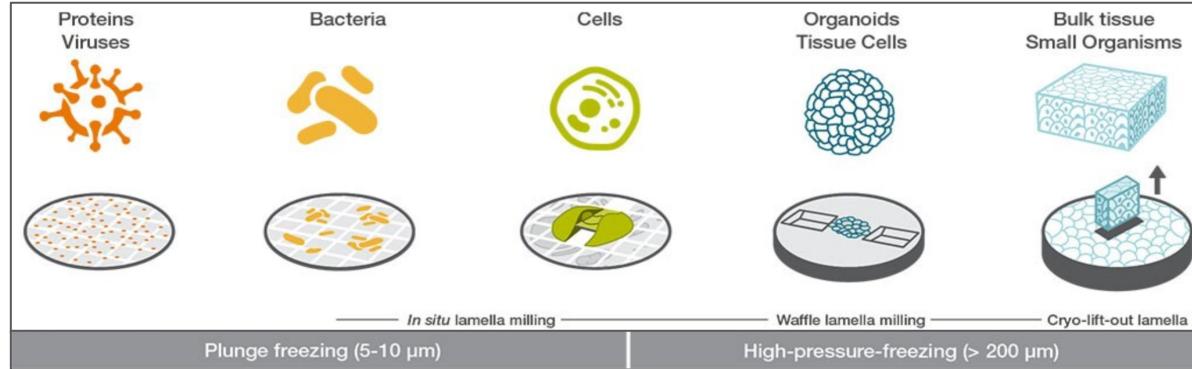
Cryo-ET does this on a **nanometer-scale**, using electron beams, meaning instead of an images we have **tomograms**



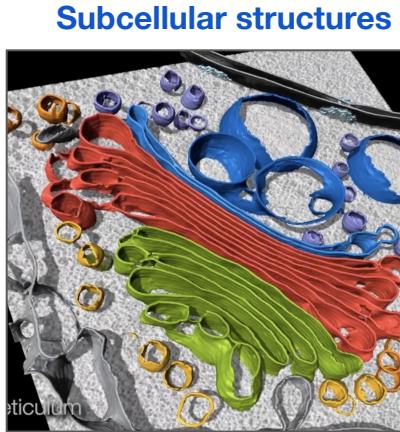
(“*Cryo EM Tomography*.”)

# Cryo-electron tomography

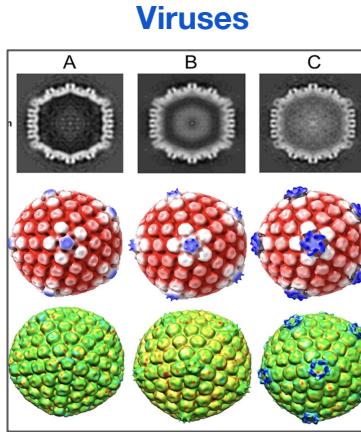
(“Cryo-Electron Tomography Technology.”)



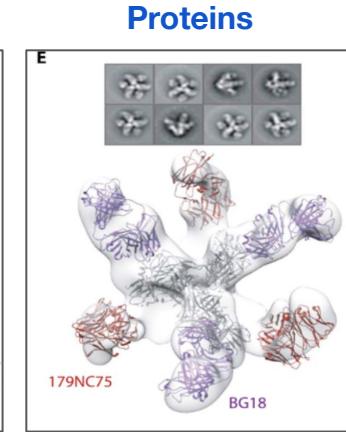
Many types of samples can be investigated. Tissue preparation changes slightly based on sample; for smaller samples like proteins, placed on an **“electron microscopy grid”**.



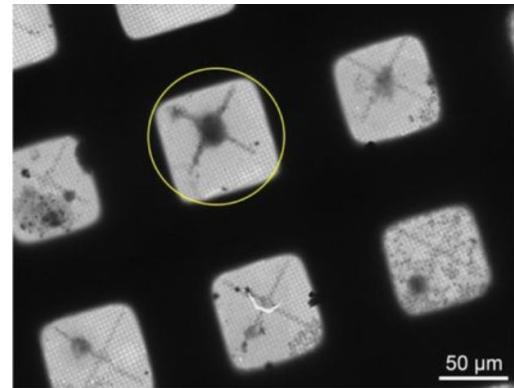
(“Cryo-Electron Tomography Sample Data.”)



(Vijayakrishnan, Swetha, et al.)



(Freund, Natalia T., et al.)



(Sibert, et al.)

50 µm

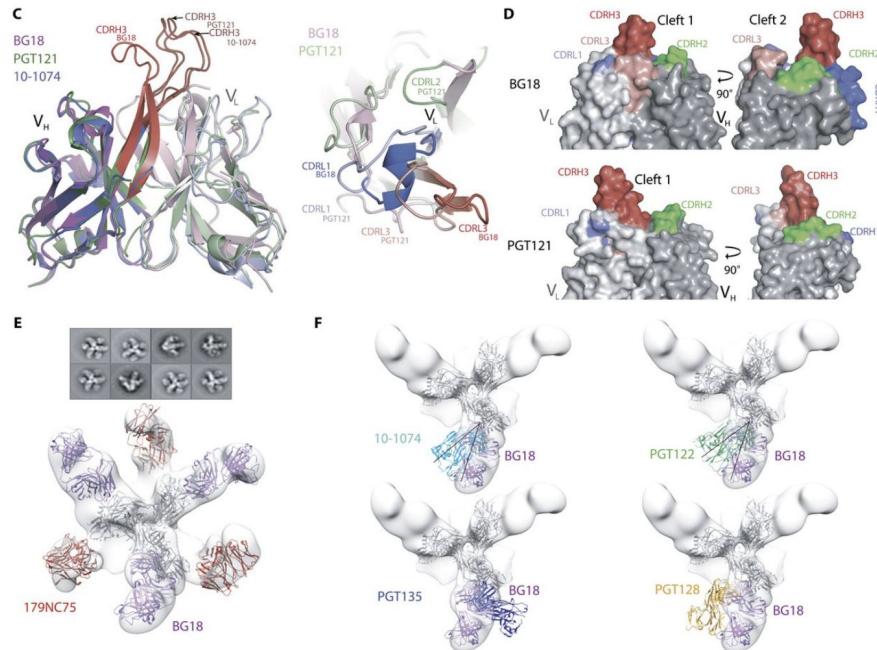
# Cryoelectron tomography/subtomogram: Analysis Tools

## RELION - Unsupervised 3D Classification

- Determine the structure of BG18, PGT121, 10-1074 using cryo-EM data
- Uses Bayesian approach → infers parameters for statistical modeling of data. Parameters are usually something that researchers need to know how to tune.
- Yields reconstruction of biological molecules

## UCSF Chimera

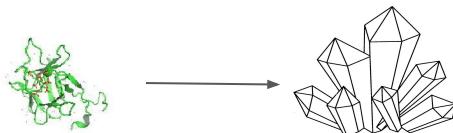
- Program used to visualize and interact with molecular models
- Used to generate structural images of BG18, PGT121, 10-1074



# X-Ray Crystallography

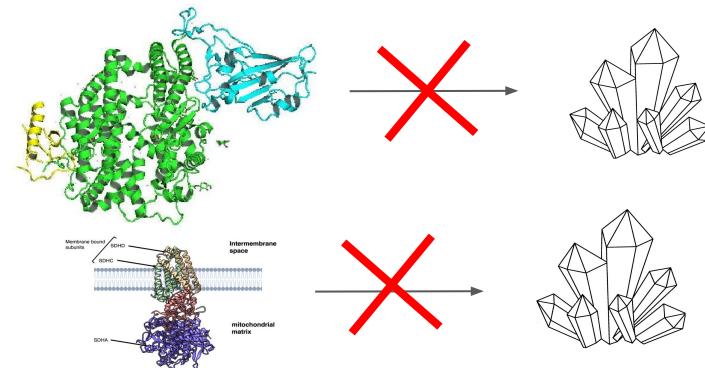
## Advantages:

- Not limited by sample molecular weight
- **High resolution**



## Disadvantages:

- **Requires sample to be crystallized** (time consuming + challenging)
  - Especially for large, complex or membrane-embedded proteins
- Represents static form of protein
- Phase problem
  - Mediated by Molecular Replacement



# Cryoelectron Tomography/ Subtomogram

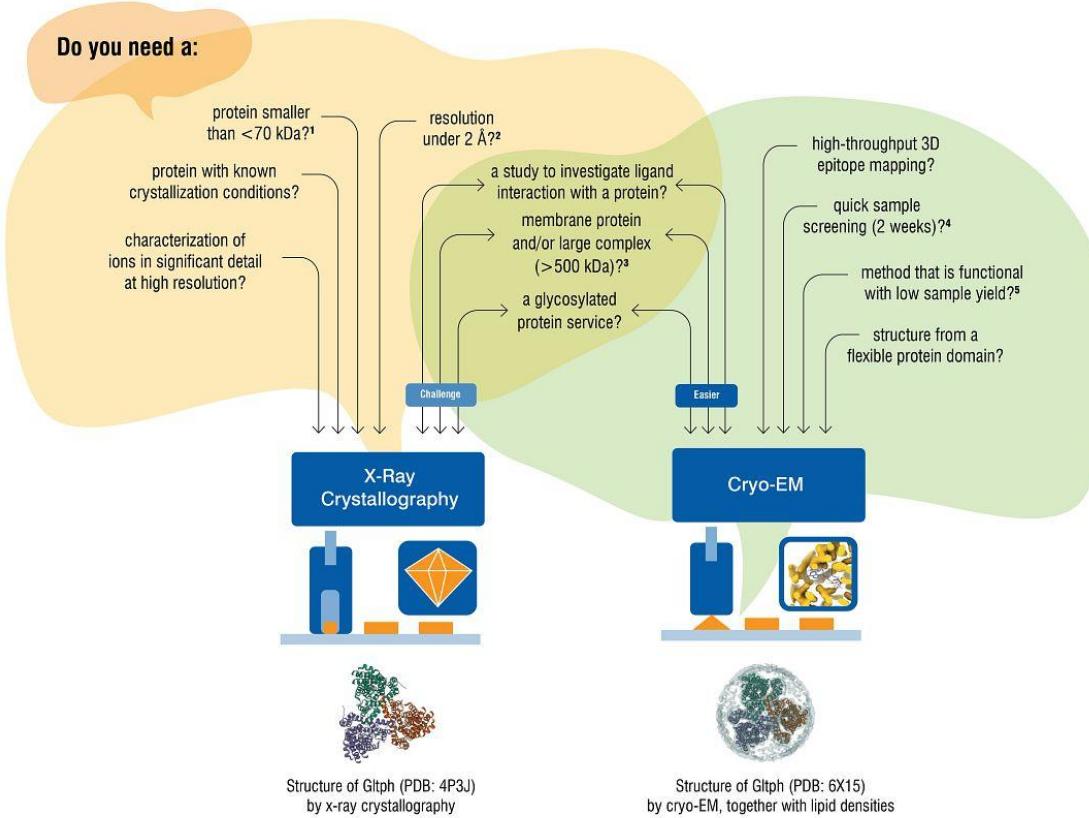
## Advantages:

- **Closer to native state**  
(fast-freezing means native structures are well preserved)
- More dynamic view of the structure
  - allows for time-resolved imaging that capture structural changes over time
- Allows for study of large, flexible, membrane associated complexes

## Disadvantages:

- Large datasets that are computationally demanding
- Generally **lower resolution**
  - although improvements have been made towards near-atomic-level resolution (new technology and averaging → 2-5 Å )

# When to use these techniques



→ using both can illuminate areas of low resolution while maintaining information on more native-like states of the structure

# More X-ray Crystallography Applications

- Chemistry
- Pharmacology
- Materials Science
- Geology and Mineralogy
- Forensic Science

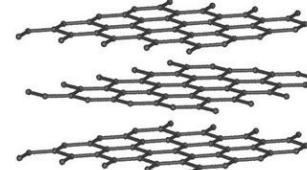
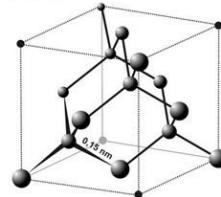
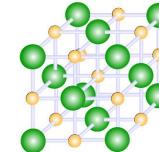
**Quartz**

● Silicon  
● Oxygen



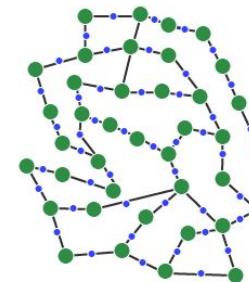
**Salt Crystal**

● Chlorine (Cl)  
● Sodium ( $\text{Na}^+$ )

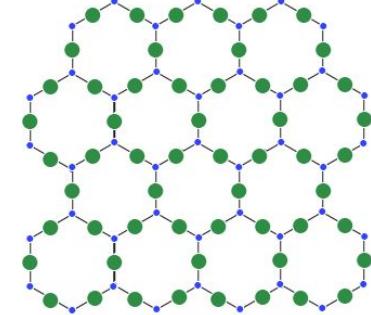


# More Cryo-electron Tomography/Subtomogram Applications

- Nanotechnology and Nanomaterials
- Synthetic Biology
- Materials Science
- Environmental Science
- Food Science



Amorphous Solids



Crystalline Solids

**Thank you!**

# Citations

"Basics of Photogrammetry." *Geodetic Systems, Inc*, [www.geodetic.com/basics-of-photogrammetry/](http://www.geodetic.com/basics-of-photogrammetry/).

Hall, David H., et al. "Modern electron microscopy methods for *C. elegans*." *Methods in Cell Biology*, 2012, pp. 93–149, <https://doi.org/10.1016/b978-0-12-394620-1.00004-7>.

Vijayakrishnan, Swetha, et al. "In situ structure of virus capsids within cell nuclei by correlative light and cryo-electron tomography." *Scientific Reports*, vol. 10, no. 1, 19 Oct. 2020, <https://doi.org/10.1038/s41598-020-74104-x>.

Freund, Natalia T., et al. "Coexistence of potent HIV-1 broadly neutralizing antibodies and antibody-sensitive viruses in a viremic controller." *Science Translational Medicine*, vol. 9, no. 373, 18 Jan. 2017, <https://doi.org/10.1126/scitranslmed.aal2144>.

"Cryo-Electron Tomography Sample Data." *Thermo Fisher Scientific - US*, [www.thermofisher.com/us/en/home/electron-microscopy/life-sciences/cryo-tomography/sample-data.html](http://www.thermofisher.com/us/en/home/electron-microscopy/life-sciences/cryo-tomography/sample-data.html). Accessed 19 Sept. 2024.

"Cryo-Electron Tomography Technology." *Thermo Fisher Scientific - US*, [www.thermofisher.com/us/en/home/electron-microscopy/life-sciences/cryo-tomography/workflow.html#:~:text=Cryo%2Delectron%20tomography%20has%20been,cells%20and%20large%20tissue%20samples](http://www.thermofisher.com/us/en/home/electron-microscopy/life-sciences/cryo-tomography/workflow.html#:~:text=Cryo%2Delectron%20tomography%20has%20been,cells%20and%20large%20tissue%20samples). Accessed 19 Sept. 2024.

Sibert, Bryan S., et al. "Micropatterning transmission electron microscopy grids to direct cell positioning within whole-cell cryo-electron tomography workflows." *Journal of Visualized Experiments*, no. 175, 13 Sept. 2021, <https://doi.org/10.3791/62992>.

"Cryo EM Tomography." *YouTube*, [www.youtube.com/watch?v=frgjc-ZNhOY](http://www.youtube.com/watch?v=frgjc-ZNhOY). Accessed 19 Sept. 2024.

Regents of the University of California. "UCSF Chimera an Extensible Molecular Modeling System." UCSF Chimera Home Page, 2018, [www.cgl.ucsf.edu/chimera/](http://www.cgl.ucsf.edu/chimera/).

# Citations cont.

RELION developer team. "Unsupervised 3D Classification." Unsupervised 3D Classification - RELION Documentation, [relion.readthedocs.io/en/release-3.1/SPA\\_tutorial/Class3D.html](https://relion.readthedocs.io/en/release-3.1/SPA_tutorial/Class3D.html). Accessed 30 Sept. 2024.

Zost, S.J., Gilchuk, P., Chen, R.E. et al. Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* 26, 1422–1427 (2020). <https://doi.org/10.1038/s41591-020-0998-x>

Asano, Shoh, et al. "In situ cryo-electron tomography: A post-reductionist approach to structural biology." *Journal of Molecular Biology*, vol. 428, no. 2, Jan. 2016, pp. 332–343, <https://doi.org/10.1016/j.jmb.2015.09.030>.

"What Is the Difference between Cryo-EM and Cryo-Et?" *Delmic Microscopy Blog*, [blog.delmic.com/what-is-the-difference-between-cryo-em-and-cryo-et](https://blog.delmic.com/what-is-the-difference-between-cryo-em-and-cryo-et). Accessed 19 Sept. 2024.

Sébastien Pillet; Spin-crossover materials: Getting the most from x-ray crystallography. *J. Appl. Phys.* 14 May 2021; 129 (18): 181101. <https://doi.org/10.1063/5.0047681>

Gong, Y., Gu, T., Ling, L., Qiu, R., & Zhang, W. (2022). Visualizing hazardous solids with cryogenic electron microscopy (Cryo-EM). *Journal of Hazardous Materials*, 436, 129192–129192. <https://doi.org/10.1016/j.jhazmat.2022.129192>

Kupikowska-Stobba, Barbara, Jacek Domagała, and Mirosław M. Kasprzak. 2024. "Critical Review of Techniques for Food Emulsion Characterization" *Applied Sciences* 14, no. 3: 1069. <https://doi.org/10.3390/app14031069>

Do, G., Sase, S., Kobayashi, R., Sato, M., Bae, Y., Maeda, T., Ueno, S., & Araki, T. (2020). Determining the internal structure of ice cream using cryogenic microtome imaging and x-ray computed tomography. *Japan Journal of Food Engineering*, 21(3), 113–121.