BIOGRAPHICAL SKETCH

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NAME: Jason T Kaelber

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POSITION TITLE: Associate Research Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University	B.A.	05/2011	Chemistry; Biology
Baylor College of Medicine	Ph.D.	09/2017	Molecular Virology & Microbiology

A. Personal Statement

I am a structural virologist with experience in every aspect of the cryoelectron microscopy and tomography workflow. My lab is the first to discover a novel pathogen by means of cryoEM (preprint *a*) in work led by co-investigator Dr. Judit Pénzes. I launched my independent research program in 2019, focusing on two areas: on the structural biology of viruses—specifically viral gene therapy vectors—and on methods development for cryoEM. The first structure that we published from my lab—the AAVhu.37 capsid—was briefly a record-holder as it was of higher resolution than any other peer-reviewed SPR (single-particle reconstruction) structure from a 200-kilovolt instrument. My aim is to leverage structural virology to improve gene therapy capsids so that they can be employed to prevent or treat human diseases, particularly pancreatic diseases including malignancies of the pancreas.

I began my classical and molecular virology training at Cornell where I discovered that the global pandemic of parvovirus in dogs was derived from a virus whose ancestor infected ancient canids (paper *b*). After virology internships at Harvard and Pasteur, I moved to the Department of Molecular Virology & Microbiology at Baylor College of Medicine to focus on structural virology (paper *c*), where in addition to basic research on the structural biology of viral replication and surveillance for new mosquito-borne viruses, I always made time to contribute to countermeasures-development programs such as a vaccine against chikungunya virus that is safe and effective in primates.

Since 2017, I lead the Rutgers CryoEM & Nanoimaging Facility (RCNF) and serve as a field expert in cryoelectron microscopy technologies for our state. Participating in investigator-initiated projects in a service capacity has forced me to stay current on best practices for cryoEM and I am proud of the capabilities of our well-maintained hardware, optimized workflows, and well-trained personnel. I organized the workshop “Cryo-electron Tomography: State of the Art Methods” as part of the American Crystallographic Association annual meeting in 2022 at the invitation of Dr. Liz Kellogg. I co-taught the Rutgers course “Cryo-Electron Tomography” in 2018 and lectured on single-particle reconstruction each year since at Rutgers and Cornell Universities. I have also trained many scientists hands-on in vitrification, operation of cryoelectron microscopes, and image processing.

This application pairs my expertise in cryoelectron microscopy (papers *a,c,d*) and deep background in virology (papers *a,b,c*) with that of my senior lab member Dr. Pénzes—an internationally-recognized expert in parvovirology with significant experience in cryoEM—to enable breakthroughs in the mechanisms of gene delivery by AAV to the human nucleus.

a. Penzes JJ, Kaelber JT. Identification by cryoEM of a densovirus causing mass mortality in mass-reared larval darkling beetles (*Zophobas morio*). bioRxiv 491968 [Preprint]. 2022 May 15. Available from: https://www.biorxiv.org/content/10.1101/2022.05.14.491968v1 doi: 10.1101/2022.05.14.491968
b. Kaelber JT, Demogines A, Harbison CE, Allison AB, Goodman LB, Sawyer SL, Parrish CR. Evolutionary reconstructions of the transferrin receptor of Caniforms supports canine parvovirus being a re-emerged and not a novel pathogen in dogs. *PLoS Pathogens.* 2012 May;**8**(5):e1002666. PMID: 22570610 PMCID: PMC3342950
c. Kaelber JT*, Jiang W, Weaver SC, Auguste AJ, Chiu W. Arrangement of the Polymerase Complexes inside a Nine-Segmented dsRNA Virus. *Structure*. 2020 Feb 7. PMID: 32049031. PMCID: PMC7289189
d. Molodtsov V, Wang C, Firlar E, Kaelber JT, Ebright RH. Structural basis of Rho-dependent transcription termination. *Nature.* 2023 Jan 25. doi: 10.1038/s41586-022-05658-1. PMID: 36697824. PMCID: In process.

*= as corresponding author

B. Positions and Honors

Current Positions

2021- Associate Research Professor, Institute for Quantitative Biomedicine, Rutgers Univ
2019- Member, Cancer Institute of New Jersey
2017- Director, Rutgers CryoEM & Nanoimaging Facility

C. Contributions to Science

Providing structural insights into virion assembly and encapsidation architecture

After discovering Fako virus and establishing it as a useful system for understanding reovirus architecture by virtue of its streamlined architecture (paper *e*), in order to understand heterogeneity of intra-particle polymerase organizations within a population of virions I invented a computational method to do hypothesis-testing with a set of synthetic 3D maps (paper *f*). In brief, I treat the **cryo-EM dataset as a set of single-molecule observations**, compare each observation to a set of priors, and statistically assess the ensemble. Through this method, I discovered that (in contrast to what would be expected from standard hypotheses in the field), the number of polymerases inside the Fako virus capsid exceeds the number of RNA segments. As a spin-off from my work supporting alphavirus vaccine development, I discovered an alternate, smaller form of Eastern equine encephalitis virus capsids (paper *g*).

e. Auguste JA,* Kaelber JT,* Fokam E,* Guzman H, Carrington C, Erasmus JH, Kamgang B, Popov VL, Jakana J, Liu X, Wood TG, Widen SG, Vasilakis N, Tesh RB, Chiu W, Weaver SC. A newly-isolated reovirus has the simplest genomic and structural organization of any reovirus. *J Virol*. 2015 Jan;89(1):676-687 PMID: 25355879. PMCID: PMC4301156 (* denotes co-first authors)
f. Kaelber JT, Jiang W, Weaver SC, Auguste AJ, Chiu W. Arrangement of the Polymerase Complexes inside a Nine-Segmented dsRNA Virus. *Structure*, 2020 Feb 7. PMID: 32049031. PMCID: PMC7533842.
g. Kaelber JT, Chmielewski D, Chiu W, Auguste AJ. Alphavirus Particles Can Assemble with an Alternate Triangulation Number. *Viruses*. 2022 Nov 27;14(12):2650. PMID: 36560655. PMCID: PMC9780915.

h. Kaelber JT, Hryc CF, Chiu W. Electron Cryomicroscopy of Viruses at Near-Atomic Resolutions. *Annu Rev Virol*. 2017 Sep 29;4(1):287-308. PMID: 28715974.

Reconstructing ancient events in viral evolution

The rapid evolution of viruses and lack of universal genes make sequence comparison insufficient to decipher their ultimate origin. To query the history of a non-endogenized virus, I used the signatures of selective pressure it left in host genomes. Integrating reverse genetics, bioinformatics, and cell biology, I reconstructed ancient host-cell binding events to reconstruct the history of canine parvovirus. I showed its ancestor infected ancient canids until they evolved a defense, but the virus was maintained in other Carnivora until a 20th-century spillover (paper *i*). Because this technique cannot reconstruct the most ancient events, I moved to structure-based inference of deep evolutionary events. After establishing the Fako virus system and considering the architecture of the last universal ancestor of *Spinareovirinae* (paper *j*), I solved the atomic structure of Fako virus and am using this to shed light on the evolutionary mechanisms of architectural variation in this family. I've collaborated with George Fox (co-discoverer of Archaea) to solve structures of ribosomal insertions and use these to better understand ribosomal evolution (paper *k*). My long-term goal is to determine how many times viruses originated and where they came from (paper *l*).

i. Kaelber JT, Demogines A, Harbison CE, Allison AB, Goodman LB, Sawyer SL, Parrish CR. Evolutionary reconstructions of the transferrin receptor of Caniforms supports canine parvovirus being a re-emerged and not a novel pathogen in dogs. *PLoS Pathogens* 2012 May;**8**(5):e1002666. PMID: 22570610. PMCID: PMC3342950
j. Auguste JA,* Kaelber JT,* Fokam E,* Guzman H, Carrington C, Erasmus JH, Kamgang B, Popov VL, Jakana J, Liu X, Wood TG, Widen SG, Vasilakis N, Tesh RB, Chiu W, Weaver SC. A newly-isolated reovirus has the simplest genomic and structural organization of any reovirus. *J Virol*. 2015 Jan;89(1):676-687 PMID: 25355879. PMCID: PMC4301156 (* denotes co-first authors)
k. Tirumalai MR, Kaelber JT, Park DR, Tran Q, Fox GE. Cryo-Electron Microscopy Visualization of a Large Insertion in the 5S ribosomal RNA of the Extremely Halophilic Archaeon *Halococcus morrhuae*. *FEBS Open Bio*. 2020 Aug 31. PMID: 32865340. PMCID: PMC7530397.
l. Trubl G, Stedman K, Bywaters K, Boston PJ, Kaelber JT, Roux S, Emerson JB, Breitbart M, Yin J, Janjic A, Sommers P, Rodríguez-Román E. Astrovirology: Expanding the Search for Life. *Bulletin of the American Astronomical Society*. 2021 March 18; 53(4). Available from: https://baas.aas.org/pub/2021n4i516

Leveraging cutting-edge cryoEM methods to reconstruct biochemical mechanisms

Single-particle reconstruction has revolutionized structural biology and made possible the determination of many structures that were otherwise intractable. I like to find interesting biological systems that require some **methodological novelty** and then come up with a new way to solve the structure. For instance, I modified my Talos Arctica cryoTEM with a custom condenser aperture to allow 64-target parallel-beam aberration-free image shift, breaking the usual limits caused by that microscope's lack of a three-condenser system; this modification was key to solving the structure of an antimicrobial peptide inside multidrug efflux pump TolC at 3Å (paper *m*). I was part of a multi-institutional team that solved 39 cryoEM structures from 20 cryoEM datasets to crack the mechanism of transcription-translation coupling in bacteria (paper *n*). I continue to collaborate with Prof. Ebright on biochemistry of bacterial transcription and, after intensive workflow optimization, the resolutions we attain with our 200 kV instrument match those obtained by 300 kV instruments at national centers (paper *o*). I was the first person to solve a helical structure using the software cryoSPARC (paper *p*), which was necessary to get an especially tricky structure of a ribonucleotide reductase filament where one component was disordered.

m. Budiardjo SJ, Stevens JJ, Calkins AL, Ikujuni AP, Wimalasena VK, Firlar E, Case DA, Biteen JS, Kaelber JT, Slusky JSG. Colicin E1 opens its hinge to plug TolC. *Elife*. 2022 Feb 24;11:e73297. doi: 10.7554/eLife.73297. PMID: 35199644. PMCID: PMC9020818

n. Wang C, Molodtsov V, Firlar E, Kaelber JT, Blaha G, Su M, Ebright RH. Structural basis of transcription-translation coupling. *Science*. Epub 2020 Aug 20. PMID: 32820061. PMCID: PMC7566311.
o. Molodtsov V, Wang C, Firlar E, Kaelber JT, Ebright RH. Structural basis of Rho-dependent transcription termination. *Nature.* 2023 Jan 25. Online ahead of print. PMID: 36697824. PMCID: In process.
p. Thomas WC, Brooks FP 3rd, Burnim AA, Bacik JP, Stubbe J, Kaelber JT, Chen JZ, Ando N. Convergent allostery in ribonucleotide reductase. *Nat Commun*. 2019 Jun 14;10(1):2653. doi: 10.1038/s41467-019-10568-4. PMID: 31201319. PMCID: PMC6572854.

Elucidating the basis of cellular phenotypes through cryoelectron tomography and EM ultrastructure

Cryoelectron tomography can provide an untargeted ultrastructural census of whole cells. This makes it ideal to pursue known phenotypes with unknown mechanisms. For example, it was known that ovarian cancer causes a change in platelets but the nature of that change was unknown. I showed that based only on tomograms of platelets I can predict whether the person whence they were derived has an ovarian malignancy (paper *q*). To understand why lateral attachment of the *Trypanosoma* flagellum is required for directional motion, I imaged mutants by cryoET, wrote new code to analyze inter-microtubule relationships and cytoskeletal bending, proposed a model for force transduction in this organism, and collaborated closely with several colleagues to find that beating of the laterally-attached *Trypanosoma* flagellum contorts the cell body in a way necessary for directional motion (paper *r*). I also employ classical TEM ultrastructure techniques to characterize organellar features—for example, using thin-section resin-embedded TEM I recently analyzed the response of plankton photosynthetic structures to heat stress such as occurs in our warming oceans (paper *s*). For that project, all my undergraduates performed independent, double-blinded scoring of photomicrographs for qualitative evaluation of features that are difficult to quantify by automated image segmentation. Currently, my lab is applying cryoelectron tomography to phage-infected cells to understand host range determinants. Since 2021 I am a reviewer for the National Service Network for Cryo-Electron Tomography.

q. Wang R, Stone RL, Kaelber JT, Rochat RH, Nick AM, Vijayan KV, Afshar-Kharghan V, Schmid MF, Dong JF, Sood AK, Chiu W. Electron cryotomography reveals ultrastructure alterations in platelets from patients with ovarian cancer. *PNAS* 2015 Nov 17;112(46):14266-71 PMID: 26578771. PMCID: PMC4655568
r. Sun SY, Kaelber JT, Chen M, Dong X, Nematbakhsh Y, Shi J, Dougherty M, Lim CT, Schmid MF, Chiu W, He CY. Flagellum couples cell shape to motility in *Trypanosoma brucei*. *PNAS* 2018 Jun 11; PMID: 29891682. PMCID: PMC6042131
s. Cheong KY, Firlar E, Ficaro L, Gorbunov MY, Kaelber JT, Falkowski PG. Saturation of thylakoid-associated fatty acids facilitates bioenergetic coupling in a marine diatom allowing for thermal acclimation. *Global Change Biol*. 2021 Jul;27(13):3133-3144. PMID: 33749034

Building technology for cryoEM collaboration

I collaborate with the Ecosystem for Research Networking to build tools for inter-institutional cryoEM access and remote work. Our technology pilot (paper *t*) was awarded Best Short Paper in the Systems and System Software track at PEARC22.

t. Dougherty M, Zink M, von Oehsen B, Dalenberg K, Desinghu B, Kaelber JT, Schafer J, Goodhue J, Hey W, Ludwig M, Wilson B, McKnight C. The ERN Cryo-EM Federated Instrument Pilot Project. *PEARC '22: Practice and Experience in Advanced Research Computing*. 2022 Jul 52:1-4.

List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/jason.kaelber.1/bibliography/public/