BIOGRAPHICAL SKETCH

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NAME: **Gino Cingolani, Ph.D.**

eRA COMMONS USER NAME (agency login): **cingolag**

POSITION TITLE: **Professor**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Bari, Italy	B.S./M.S.	07/1995	Biochemistry
University Joseph Fourier, Grenoble, France	D.E.A.	06/1996	X-ray Crystallography
European Molecular Biology Laboratory	Ph.D.	07/1999	Structural Biology
The Scripps Research Institute, CA, USA	Post-doc	12/2003	Virus Crystallography/Cell Biology

A. PERSONAL STATEMENT

My research interests and expertise lie in the field of Structural Biology. I trained in Biological Chemistry and Macromolecular Crystallography at different European universities in the nineties. I moved to the US in 1999 to continue training in Structural Virology and Cell Biology at the Scripps Research Institute. I have been a principal investigator since 2004, first at SUNY Upstate Medical University, Syracuse, NY (2004-2009), then at Thomas Jefferson University, Philadelphia, PA (2009-2023). In October 2023, I relocated to the University of Alabama at Birmingham (UAB) to lead a Center for Integrative Structural Biology. My research employs biophysical, biochemical, and cellular techniques to study macromolecules' structure and function. We focus on medically-relevant problems that help decipher the most fundamental mechanisms of life and improve human health. Faithful to the idea that '*seeing is believing*,' we investigate the structure of signaling and viral macromolecules as a starting point to probe their function and engineer their activity using small-molecule compounds. We are particularly interested in solving the atomic structures of 'druggable' biological targets linked to human diseases. Multiple grants from NIH have continuously supported my laboratory for two decades.

Our current research focuses on understanding the molecular mechanisms of protein nuclear import and viral genome delivery into bacteria. We study how the importin α and β mediate the nuclear import of crucial signaling molecules implicated in human diseases (e.g., STATs, NF-κB, TDP-43). We also study the delivery of viral genomes in eukaryotes and prokaryotes, providing a framework to support a better understanding of phages as biomedicines for phage therapy and inform engineering opportunities. Since 2020, we have entirely replaced X-ray crystallography with cryogenic electron microscopy (cryo-EM) single particle analysis, which we use as the primary methodology in the lab to decipher biological structures. We have solved the complete architecture of several intact virions, e.g., Sf6 (Li et al., 2022), E217 (Li et al., 2023), HBV (Yang et al., 2024), as well as proteins in the 400-100 kDa range (Swanson et al., 2021; Yang et al., 2024) and deposited >25 PDBs built *de novo* in high-resolution cryo-EM densities.

Throughout my career, I have maintained a solid commitment to graduate education. I have mentored 13 Ph.D. and 6 Master's students and am currently mentoring four graduate students. I have developed a graduate course focused on biophysical methods in structural biology, and I taught graduate courses in Biochemistry for Pharmacy and Master students. My leadership experience includes serving as Vice-Chair for Research of the Dept of Biochemistry and Mol Biology at Thomas Jefferson for seven years; directing the X-ray Crystallography Facility at the Sidney Kimmel Cancer Center for a decade; founding the Jefferson Cryo-Electron Microscopy Core, operational since March 2021. At UAB, I direct a new Center for Integrative Structural Biology. I have been the contact PI on three shared instrumentation (S10) grants, all successfully funded, to acquire a hybrid diffractometer, a crystallization robot, a 200 kV Glacios cryo-electron microscope, and associated equipment (totaling ~$3ML in equipment). Lastly, I chaired the NIH Prokaryotic Cell and Molecular Biology (PCMB) study section panel between 2021-2023.

Li, F., David, Hou C-F, Yang, R., Whitehead R., Teschke, M.C., and **Cingolani, G**. (2022) High-resolution cryo-EM structure of the Shigella-virus Sf6 genome-delivery tail machine at 2.7 Å resolution, *Science Adv*, 8(49):eadc9641. PMCID: PMC9728967

Li, F., Hou D.C-F., Lokareddy, K.R., Yang, R., Forti, F., Briani, F., and **Cingolani, G.** (2023) High-resolution cryo-EM structure of the Pseudomonas bacteriophage E217, *Nat Commun*, 14(1):4052; PMCID: PMC10329688

Yang, R., Ko, Y-H., Li, F., Lokareddy, K.R., Hou D.C-F., Kim, C., Klein, S., Antolínez, S., Marín, F.J., Pérez-Segura, C., Jarrold, M.F., Zlotnick, A., Hadden-Perilla, A.J. and **Cingolani, G.** (2024) Structural basis for nuclear import of Hepatitis B Virus (HBV) nucleocapsid core. *Science Adv*. 10(2):eadi7606. PMCID: PMC10780889

Swanson, N.A., Lokareddy, R.K., Li, F., David, Hou C-F, Leptihn, S., Pavlenok, M., Niederweis, M., Pumroy, R.A., Moiseenkova-Bell, V.Y., **Cingolani, G.** (2021) Cryo-EM Structure of the Periplasmic Tunnel of T7 DNA-Ejectosome at 2.7 Å resolution. *Molecular Cell.* 81(15):3145-3159. PMCID: PMC8349896

Ongoing and recently completed projects that I would like to highlight include:

R35 GM140733
Cingolani (PI)
06/01/2021 – 05/31/2026
Mechanism of Viral Genome Delivery into Cells

R01GM122844
Cingolani (PI)
03/01/2018 – 12/31/2021
Regulation of Nuclear Import through Importin Alpha Isoforms

R01 AI137338
Niederweis, Cingolani (mPI)
04/01/2019 – 03/31/2024
Hemoglobin utilization by Mycobacterium tuberculosis

1S10 OD030457-01
Cingolani (PI)
07/1/2022 – 06/30/2023
A new Cryo-Transmission Electron Microscope at Thomas Jefferson University

B. POSITIONS, SCIENTIFIC APPOINTMENTS AND HONORS

Positions

2023-	Professor, Dept of Biochemistry and Mol. Genetics, University of Alabama at Birmingham, AL
2017-2023	Vice-Chair, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
2015-2023	Director, X-ray Crystallography Facility, Sidney Kimmel Cancer Center
2015-2023	Professor, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
2015	Visiting Professor, Dept. of Biochemistry and Mol. Biology, University of Bari, Italy
2009-2014	Associate Professor, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
2004-2009	Assistant Professor, Dept. of Biochemistry and Mol. Biology, SUNY Upstate, NY

Scientific Appointments

2021-2023	National Institute of Health (NIH) PCMB study section, chair
2022	Antiviral Drug Discovery (AViDD) Centers for Pathogens of Pandemic (U19), reviewer
2018-2023	National Institute of Health (NIH) PCMB study section, permanent member
2018-present	European Union, Horizon 2020, reviewer
2017	National Institute of Health (NIH) ZRG1 F04B-D (20) L study section, reviewer
2016	National Institute of Health (NIH) ZRG1 F04B-D (20) L study section, reviewer
2015	Israel Science Foundation (ISF), ad hoc reviewer
2014	National Institute of Health (NIH) ZRG1 F04B-D (20) L study section, reviewer
2013	Italian Ministry of Health (MoH), Research Proposals, reviewer

2013	National Institute of Health (NIH) ZRG1 F04-W(20) L study section, reviewer
2013	Advanced Photon Source (APS) beamlines NE-CAT's 24-ID-C and 24-ID-E, reviewer
2013	National Institute of Health (NIH) MFSC study section, ad hoc reviewer
2012	Italian Ministry of Health (MoH), Research Proposals, reviewer
2012	National Institute of Health (NIH) ZRG1 F04-D (20)/F04-K (09) L study section, reviewer
2012-present	Italian Scientists and Scholars in North America Foundation, member
2011	National Institute of Health (NIH) MFSC study section, ad hoc reviewer
2011	Research Grants Council (RGC) of Hong Kong, reviewer
2010	National Institute of Health (NIH) ZRG1 F04B-B(20) study section, reviewer
2010	National Institute of Health (NIH) ZGM1 CBB-0 (BC) U01 for Structural Biology, reviewer
2010	National Institute of Health (NIH) ZRG1 F05-C(20) L study section, reviewer
2009-2014	National Synchrotron Light Source (NSLS) Users' Committee beamline X6a, member
2008	National Science Foundation (NSF), ad hoc grant reviewer
2007-2009	American Society for Cell Biology (ASCB), reviewer
2007-2008	Cornell High Energy Synchrotron Source (CHESS) Executive Committee, member
2007	National Science Foundation (NSF), panelist
2006-2007	Cornell High Energy Synchrotron Source (CHESS) Executive Committee, member
2006	Medical Research Council (MRC), UK, ad hoc reviewer
2004-2005	Cornell High Energy Synchrotron Source (CHESS) Express Mode proposal, reviewer

Honors

2024	Anderson Family Endowed Chair in Medical Education, Research & Patient Care
2024-	Director, UAB Center for Integrative Structural Biology
2021-2023	Director, Jefferson Cryo-Electron Microscopy Core, Thomas Jefferson University
2021	Vice-Chair, Jefferson Committee on Research (JCoR)
2019	Provost Award for Basic Research
2018	Fredric Rieders Faculty Prize in Graduate Education for Academic Year 2018-19
2017-present	*Acta Crystallographica D*, co-Editor
2013	Jefferson Medical College Early Career Investigator Award
2004	Leukemia Research Foundation Young Investigator Award
2000	Human Frontier Science Program (HFSP) post-doctoral fellowship
2000	Award for the best presentation at the TSRI Society of Fellows 4th Symposium
1999	Award for the best presentation at the TSRI Society of Fellows 3rd Symposium
1996	European Molecular Biology Laboratory (EMBL) pre-doctoral fellowship

C. CONTRIBUTIONS TO SCIENCE

1. **Mechanisms and regulation of nucleocytoplasmic transport.** Nucleocytoplasmic transport is central to the functioning of eukaryotic cells and is an integral part of the processes that lead to most human diseases. The nuclear availability of essential molecules such as transcription factors, DNA replication factors, and oncogenes is emerging as a powerful way to control gene expression, cellular differentiation, and transformation and a novel and promising target *for pharmacological intervention.* In the first part of my career, I elucidated the molecular basis for the recognition of *classical* (Cingolani et al., *Nature,* 1999) and *non-classical* (Cingolani et al., *Mol Cell,* 2002) import substrates by the transport factor importin β. As an independent investigator (2004-present), I studied the molecular basis for nuclear import of critical signaling molecules such as the phospholipid scramblase 1 (Chen et al., *J Biol Chem*, 2005) and 4 (Lott et al., *J Biol Chem*, 2010), the small nuclear ribonucleoprotein transporter snurportin (Mitrousis et al., *J Biol Chem*, 2008), the transcription factor STAT1 (Nardozzi et al., *J Mol Biol*, 2011) and the mechanisms of membrane protein translocation to the Inner Nuclear Membrane (Lokareddy et al., *structure*, 2015). More recently, my lab became interested in understanding the regulation of nuclear transport by importin α isoforms. We delineated the molecular basis for nuclear import of Influenza polymerase subunit Pb2 (Pumroy et al., *structure*, 2015) and RCC1 (Sankhala et al., *Nature Commun*, 2017) by the isoform importin α3. We recently determined the heterodimeric transcription factor NF-κB (p65:p50) is imported by the isoform importin α3 (Florio et al., *submitted*), and we are pursuing basic and translational studies on the ALS-related RNA-binding protein TDP-43. Overall, the long-term goal of our work is to decipher the mechanisms governing the nuclear entry of critical signaling molecules and to devise new small molecule inhibitors that could reduce aberrant nuclear

translocation of signaling factors linked to cancer (Liao et al., *Mol Cancer Ther*, 2015; De Dominici et al., *Blood*, 2020). Key publications include:

a. **Cingolani, G.**, Petosa, C., Weis, K., and Mueller, C.W. (1999) Structure of importin β bound to the IBB domain of importin α. *Nature*, 399(6733): 221-229. PMID: 10353244.
b. **Cingolani, G.**, Bednenko, J., Gillespie, M., and Gerace, L. (2002) Molecular basis for the recognition of a *non-classical* nuclear localization signal by importin β. *Molecular Cell*, 10: 1345-1353. PMID: 12504010.
c. Florio, T.J., Lokareddy, R.K., Yeggoni, D.P., Sankhala, R.S., Ott, C.A., Gillilan, E.R. and **Cingolani, G.** (2022) Differential recognition of canonical NF-κB dimers by Importin α3. *Nature Commun.*13(1):1207. PMCID: PMC8904830
d. Doll, G.S., Meshkin, H., Bryer, J.A., Li, F., Ko, YH., Lokareddy K.R., Gillilan, E.R., Gupta K., Perilla, R.J. and **Cingolani, G.** (2022) Recognition of the TDP-43 Nuclear Localization Signal by importin α1/β. *Cell Reports*, 39(13):111007. PMCID: PMC9290431

2. **Mechanisms of viral genome delivery.** Our work aims to understand how double-stranded DNA viruses such as herpesviruses and tailed bacteriophages package their large genomes (~40-250 kb) inside empty precursor capsids ('procapsids'), as well as eject the genomes inside infected cells. Both processes are mediated by complex, multi-subunit machines that assemble transiently during the virus life cycle. Using hybrid structural methods, we have investigated the architecture, composition, and assembly of bacterial virus packaging motors for nearly two decades. We determined the portal protein changes conformation during genome-packaging and determined the structure of the mature (Olia et al., *NSMB*, 2011) and immature portal assembly (Lokareddy et al., *Nature Commun*, 2017) from the *Salmonella*-phage P22. We also dissected the architecture of the small (TerS) and large (TerL) terminase subunits implicated in viral genome recognition and packaging (Niazi et al., *Nucleic Acid Res* 2020). Our more recent work focuses on the DNA-ejectosome and *Pseudomonas* phages used in phage therapy. Using cryo-EM single-particle analysis, we provide high-resolution architectural insights into the machines used by *Pseudomonas* phages to attach the bacterium cell envelope, eject their genome into the host, and package newly replicated genomes into precursor capsids. Key publications in this field include:

a. Lokareddy, R.K., Sankhala, R.S., Roy, A., Afonine, P.V., Motwani, T., Teschke, C.M., Parent, K.N. and **Cingolani, G.** (2017) Portal protein functions akin to a DNA-sensor that couples genome-packaging to icosahedral capsid maturation. *Nature Commun*. 8:14310. PMCID: PMC5290284.
b. Niazi, M., Florio, T.J., Yang, R., Lokareddy, R.K., Swanson, N.A. Gillilan, R.E. and **Cingolani, G.** (2020) Biophysical analysis of Pseudomonas-phage PaP3 small terminase suggests a mechanism for sequence-specific DNA-binding by lateral interdigitation. *Nucleic Acid Res*. 48(20):11721-11736. PMCID: PMC7672466
c. Swanson, N.A., Lokareddy, R.K., Li, F., David, Hou C-F, Leptihn, S., Pavlenok, M., Niederweis, M., Pumroy, R.A., Moiseenkova-Bell, V.Y., **Cingolani, G.** (2021) Cryo-EM Structure of the Periplasmic Tunnel of T7 DNA-Ejectosome at 2.7 Å resolution. *Molecular Cell*. 81(15):3145-3159. PMCID: PMC8349896
d. Li, F., David, Hou C-F, Yang, R., Whitehead R., Teschke, M.C., and **Cingolani, G.** (2022) High-resolution cryo-EM structure of the Shigella virus Sf6 genome delivery tail machine, *Science Advances*. 8(49):eadc9641. PMCID: PMC9728967.

3. **Structure and inhibition of disease-related dual-specificity phosphatases.** Dual specificity phosphatases (DSPs) are essential signaling enzymes whose misregulation is intimately linked to human diseases such as cancer, diabetes, inflammation, and Alzheimer's disease. The human genome encodes 38 DSPs (also known as VH1-like phosphatases), which regulate critical aspects of the cell cycle. In my laboratory, we study the structure and function of disease-related DSPs. We have determined the atomic structure of the prototypical Vaccinia virus VH1 (Koksal et al., *J Biol Chem*, 2009; Koksal and Cingolani, *J Biol Chem*, 2011), the p53-phosphatase DUSP26 (Lokareddy et al., *Biochemistry*, 2013), the 5'-RNA-phosphatase PIR1 (Sankhala et al., *Biochemistry*, 2014) and the glycogen phosphatase laforin (Sankhala et al., *J Biol Chem*, 2015). The long-term work of this work is to decipher the molecular determinants that make DSPs substrate-specific *in vivo*. In the case of multi-domain phosphatases like laforin, we seek to understand how phosphatase activity is regulated in the context of the full-length enzyme. This is essential to develop

new 'smart' drugs that selectively interfere with substrate recognition instead of catalytic activity. Key publications in this field include:

a. Koksal, A., Nardozzi, J., and **Cingolani, G.** (2009) Dimeric quaternary structure of the prototypical dual-specificity phosphatase VH1. *J. Biol. Chem.* 284(15):10129-37. PMCID: PMC2665067.
b. Lokareddy, K.R., Bhardwaj, A. and **Cingolani, G.** (2013) Atomic structure of DUSP26, a novel p53 phosphatase. *Biochemistry*, 52(5):938-48. PMCID: PMC3619938.
c. Sankhala, S.R., Koksal, C.A., Ho, L., Nitschke, F., Minassian, A.B. and **Cingolani, G.** (2015) Dimeric quaternary structure of human laforin. *J Biol. Chem*. 290(8):4552-559. PMCID: PMC4335197.
d. Florio T., Lokareddy R.K., Gillilan R. and **Cingolani, G.** (2019) Molecular architecture of the inositol phosphatase Siw14. *Biochemistry*. 58(6):534-545. PMCID: PMC6526948

4. **Structure and inhibition of bacterial virulence factors.** We are interested in pathogenesis-related bacterial proteins' structure and enzymatic mechanisms as novel antibacterial targets. We determined the structure of the Escherichia coli F_1 ATPase core auto-inhibited by the epsilon subunit (Cingolani and Duncan, Nature Struc Mol Biol. 2011), which revealed a novel mode of intramolecular regulation of rotary catalysis. In collaboration with Dr. Michael Niederweis at the University of Alabama, we determined the crystal structure of the Mycobacterium tuberculosis Necrotizing Toxin (TNT) in complex with the Immunity factor IFT, at 1.1 Å resolution (Sun et al. Nature Struc Mol Biol. 2011). We also demonstrated that TNT hydrolyzes the essential co-enzyme nicotinamide adenine dinucleotide (NAD^+) in the cytosol of Mtb-infected macrophages. In collaboration with Dr. Paumet at Thomas Jefferson University, we have studied the Chlamydia trachomatis inclusion protein IncA, which is the first example of a bacterial SNARE-like protein mediating homotypic fusion of intracellular inclusions (Cingolani et al., Nature Commun 2019). Our current work focuses on the structural analysis of Mycobacterium tuberculosis outer membrane proteins that harbor catalytic domains and that represent virulence factors. Key publications in this field include:

a. **Cingolani, G.*** and Duncan, T.M. (2011) Structure of the ATP synthase catalytic complex (F1) from Escherichia coli in an auto-inhibited conformation. *Nature Struc Mol Biol.* 18(6):701-7. (* *corresponding authors*) PMCID: PMC3109198
b. Sun, J., Siroy, A., Lokareddy, K.R., Speer, A., Doornbos, K.S., **Cingolani, G.*** and Niederweis, M.* (2015) The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD+. *Nature Struc Mol Biol.* 22(9):672-8. (* *corresponding authors*) PMCID: PMC4560639
c. **Cingolani, G.***, McCauley, M., Lobley, A., Bryer, A.J., Wesolowski, J., Greco, D.L., Lokareddy, R.K., Ronzone, E., Perilla, J.R. and Paumet, F.* (2019) Structural basis for the homotypic fusion of chlamydial inclusions by the SNARE-like protein IncA. *Nature Commun.* 10(1):2747. (* *corresponding authors*) PMCID: PMC6588587
d. Mitra, A., Ko, YH., **Cingolani, G.*** and Niederweis, M.* (2019) Heme and hemoglobin utilization by Mycobacterium tuberculosis. *Nature Commun.* 10(1):4260. (* *corresponding authors*) PMCID: PMC6751184

List of Published Work in MyBibliography (105 pubs, >7,425 citations, h-index=42; i10-index=85):
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