BIOGRAPHICAL SKETCH

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

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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 United States in 1999 to continue my training at The Scripps Research Institute in the laboratories of Dr. Larry Gerace (Cell Biology) and Dr. Jack Johnson (Structural Virology). I have been a principal investigator since 2004, first at SUNY Upstate Medical University, and since 2009, at Thomas Jefferson University (TJU). For the last fifteen years, my laboratory has been continuously supported by multiple grants from NIH. Consistent with my multi-disciplinary training, my lab uses biophysical, biochemical, and cellular techniques to study macromolecules' structure and function. Our long-term goal is to employ rigorous chemical and physical methodologies to study 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.

Currently, the main focus of my laboratory's research is to understand the molecular mechanisms of protein nuclear import and viral genome-delivery into gram-negative bacteria. Much of the work on nuclear import spearheaded from the pioneered structure of human importin β solved in complex with a classical (Cingolani et al, *Nature*, 1999) and non-classical (Cingolani et al., *Mol Cell*, 2002) import cargo, which I determined as a trainee. Over the last two decades, we have expanded the analysis of the structure/function of importins to importin α isoforms, with emphasis on the aberrant nuclear translocation of disease-associated transcription factors (STATS, NF-kB, RCC1, etc) and viral proteins. As far as the second research area, we study the mechanisms used by bacterial viruses to package genomes into empty capsid as well as eject genomes into Gram-negative. Both events occur through a dodecameric protein channel known as the portal protein (Olia et al., *NSMB* 2011; Lokareddy et al., *Nat Comms* 2017), which occupies a unique vertex of the icosahedral capsid breaking the icosahedral symmetry. We are particularly interested in bacteriophages used in phage therapy that eradicates human pathogens such as *Pseudomonas aeruginosa* and *Salmonella enterica*.

Throughout my career, I have maintained a strong commitment to graduate education. I have mentored eight Ph.D. students and four MS students, and I am currently mentoring four Ph.D. students in addition to four post-docs. I have developed and a graduate course (PR613) focused on biophysical methods in structural biology, and I teach a full graduate course in Biochemistry (PHRM 510) for Pharmacy students (a class of ~65 students). My service to the community includes serving as director of the X-ray Crystallography & Molecular Characterization Facility, one of the eight NCI-supported shared resources in the Sidney Kimmel Cancer Center at TJU. I also founded and developed the Jefferson Cryo-Electron Microscopy Core (JEMiCo), which is operational since March 2021 under my leadership. This state-of-the-art cryo-EM core boasts a new Glacios 200 kV cryo-transmission electron microscope equipped with a Falcon 4 direct electron detector, a Vitrobot, a glow discharger, and three multi-GPUs workstations for advanced cryo-EM image-processing. I also serve as Vice-Chair for Research of the Dept of Biochemistry and Mol Biology, and chair of the NIH Prokaryotic Cell and Molecular Biology (PCMB) study section panel. I have been the main PI on three shared instrumentation (S10) grants, all successfully funded, to acquire a hybrid diffractometer for macromolecular crystallography and bioSAXS, a crystallization robot, and associated equipment.

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.
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.
Olia, A.S, Prevelige Jr., P.E., Johnson, J.E. and **Cingolani, G.** (2011) Three-dimensional structure of a viral genome-delivery portal vertex. *Nature Struc Mol Biol.* 18(5):597-603. PMCID: PMC3087855.
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.

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
NIH/NIGMS
Regulation of Nuclear Import through Importin Alpha Isoforms

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

P01 NS097197
Minassian (PI), Role co-project lead
06/01/2016 – 05/31/2021 NCE
Genome Editing, mRNA suppression, and Glycogen Chain Termination to Inhibit Glycogen Storage as Therapy for Lafora Disease

P30 CA056036
Knudsen (PI), Role: director of the X-ray Crystallography Shared Resource
06/01/2018 – 5/31/2021
Translational Research in Cancer

R01 CA237398
Rodriguez-Bravo (PI), Role: co-investigator
12/01/2019 – 11/30/2024
Role of Nuclear Pore-Regulated Mechanisms in Prostate Cancer Aggressiveness

B. Positions and Honors

Positions

04/2017-present	Vice-Chair, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
06/2015-present	Director, X-ray Crystallography Facility, Sidney Kimmel Cancer Center
09/2015-12/2015	Visiting Professor, Dept. of Biochemistry and Mol. Biology, University of Bari, Italy
01/2015-present	Professor, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
06/2009-12/2014	Associate Professor with Tenure, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA, USA
01/2004-05/2009	Assistant Professor, Dept. of Biochemistry and Mol. Biology, SUNY Upstate, NY, USA

Scientific Appointments

2021-2023	National Institute of Health (NIH) PCMB study section, chair
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, study section member
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, study section member
2012	National Institute of Health (NIH) ZRG1 F04-D (20) L study section, reviewer
2012	National Institute of Health (NIH) ZRG1 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), member
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 grant reviewer
2004-2005	Cornell High Energy Synchrotron Source (CHESS) Express Mode proposal reviewer

Honors

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. Contribution to Science

1. The architecture of multisubunit ATPases involved in viral genome packaging. The main focus of my lab is to understand how double-stranded DNA viruses such as herpesviruses, adenoviruses, and tailed bacteriophages package their large genomes (~40-250 kb) inside empty precursor capsids (known as 'procapsids'). For over a decade, we have used the *Salmonella*-phage P22 as a model system for viral genome packaging. Using hybrid structural methods, we investigate the architecture, composition, and assembly of P22 packaging motor. 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). We also dissected the architecture of P22 small (TerS) and large (TerL) terminase subunit (Roy et al., *Structure,* 2012; Roy and Cingolani, *J Biol Chem*, 2012) and a complex of the two proteins (McNulty et al., *J Mol Biol*, 2015). Our current work focuses on the terminase subunits from Pseudomonas-phages that have direct applicability to phage therapy. We recently solved the structure of Pseudomonas PaP3

TerS, which led us to postulate a mechanism of sequence-specific DNA recognition by lateral interdigitation (Niazi et al., *submitted*). Key publications in this field include:

a. Olia, A.S, Prevelige Jr., P.E., Johnson, J.E. and **Cingolani, G.** (2011) Three-dimensional structure of a viral genome-delivery portal vertex. *Nature Struc Mol Biol.* 18(5):597-603. PMCID: PMC3087855.

b. 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.

c. 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

d. 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

2. 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. 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, as well as a novel and promising target *for pharmacological intervention.* In the first part of my carrier, I elucidated the molecular basis for 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. Pumroy, A.R., Ke, S., Hart, D.J., Zachariae, U. and **Cingolani, G.** (2015) Molecular determinants for nuclear import of Influenza A PB2 by importin α isoforms 3 and 7. *Structure.* 23(2): 374-384. PMCID: PMC4346194.

d. Sankhala, R.S., Lokareddy, R.K., Begum S., Pumroy, A.R., Gillilan, E.R. and **Cingolani, G.** (2017) Three-dimensional context rather than NLS amino acid sequence determines importin α subtype specificity for RCC1. *Nature Commun.* 8(1):979. PMCID: PMC5645467

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 (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, as opposed to catalytic activity. Key publications in this field include:

e. 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.

f. Lokareddy, K.R., Bhardwaj, A. and **Cingolani, G.** (2013) Atomic structure of DUSP26, a novel p53 phosphatase. *Biochemistry*, 52(5):938-48. PMCID: PMC3619938.

g. 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.

h. 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 have a general interest in the structure and enzymatic mechanisms of pathogenesis-related bacterial proteins as novel antibacterial targets. We determined the structure of the Escherichia coli F_1 ATPase core auto-inhibited by 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:

i. **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

j. 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

k. **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

l. 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

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