BIOGRAPHICAL SKETCH

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NAME: Darst, Seth A.

eRA COMMONS USER NAME (credential, e.g., agency login): SDARST

POSITION TITLE: Professor, Head of Laboratory

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
University of Colorado, Boulder, CO	B.S.	06/1982	Chemical Engineering
Stanford University, Stanford, CA	M.S.	06/1984	Chemical Engineering
Stanford University, Stanford, CA	Ph.D.	06/1987	Chemical Engineering (Dr. Channing Robertson)
Stanford University, Stanford, CA	Postdocoral	06/1987 – 09/1993	Structural Biology (Dr. Roger Kornberg)

Please refer to the Biographical Sketch sample in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

I obtained rigorous training in quantitative approaches with a B.S. and Ph.D. in chemical engineering. In my Ph.D., I specialized in biochemical engineering but came to realize I was more interested in science rather than engineering. I therefore undertook a postdoc in the laboratory of Roger D. Kornberg, where I began my training as a biochemist and structural biologist and developed an obsession with the process of transcription. There, I contributed to the development of novel approaches to produce two-dimensional crystals of macromolecular complexes suitable for electron microscopy analysis (at the time, obtaining three-dimensional crystals of RNA polymerase suitable for X-ray crystallography seemed impossible), generated the first two-dimensional crystals of cellular RNA polymerases, and used electron microscopy methods to determine the first low-resolution structures of RNA polymerases (RNAPs; 1, 2). I also showed that RNAP could grow three-dimensional crystals, setting the stage for future advances in RNAP structural biology. I established my own group at The Rockefeller University in 1992, focusing on bacterial transcription due to the relative simplicity of the bacterial system despite high structural and functional homology with eukaryotic RNAPs. Initially, we continued to use electron microscopy, but in 1999 we established the first high-resolution structure of a cellular RNAP by X-ray crystallography (3). Since then, my group has made many key contributions, including determining the structural mechanism for rifampicin inhibition of RNAP, structures of RNAP holoenzyme alone and bound to promoter DNA, determining the structural mechanism for σ factor function, and determining the structural basis for the regulation of RNAP by accessory factors. Thus, I played a major role in establishing the field of RNAP structural biology, and my group has a long-standing record of commitment to this field and to solving challenging problems.

During my postdoc and initial years at The Rockefeller University, electron microscopy provided the only structural information on cellular RNAPs (low-resolution) until 1999 when X-ray crystallography became the primary method of choice. In 2010, my group completely stopped using electron microscopy. Nevertheless, the fact that X-ray crystallography requires the analysis of samples prepared as high-quality three-dimensional crystals imposes many challenges to producing structures as well as interpreting them (due to crystal packing effects). Advances in direct electron detectors and image processing have revolutionized structural biology,

opening up new avenues for high-resolution structural analysis of macromolecular complexes by electron microscopy that do not require crystallization. We are using these new approaches in combination with biochemical, biophysical, and genetic approaches, to provide unprecedented insight into the structure, function, and regulation of the bacterial transcription cycle (4).

At the onset of the COVID-19 pandemic in New York City, The Rockefeller University closed (on March 18, 2020) with the exception of research directed towards the SARS-CoV-2 virus or the disease COVID-19 itself. Co-PI Elizabeth Campbell, two Graduate Students and a Research Assistant in the laboratory, and I felt that, with our expertise in nucleic acid polymerases and large macromolecular assemblies, we could make an impact towards understanding the SARS-CoV-2 replication-transcription complex (RTC). Starting essentially from scratch, in four months we published our manuscript describing the SARS-CoV-2 RTC complexed with the essential nsp13 helicase. We are continuing this work to understand the functional implications of this structure, as well as to generate additional structures of larger macromolecular assemblies involved in SARS-CoV-2 replication-transcription.

Since establishing my laboratory at The Rockefeller University 30 years ago, I have mentored 19 Postdocs (17 currently in research-related careers) and 10 Graduate Students (9 currently in research-related careers). Through the Rockefeller Summer Undergraduate Research Program, we have hosted roughly on average two undergraduates in the laboratory every summer, for a total of more than 50 undergraduates – most of these have gone on to graduate school (not necessarily at Rockefeller) and further academic careers, and 7 have co-authored manuscripts from our laboratory. I have also mentored (often with members of my group) 7 SMART teams (Students Modeling A Research Topic), teams of high school students and their teachers who work with research scientists to design and construct 3D physical models of the proteins being investigated in their laboratories.

1. **Darst SA**, Kubalek EW, Kornberg RD (1989) Three-dimensional Structure of *Escherichia coli* RNA Polymerase Holoenzyme Determined by Electron Crystallography, *Nature* **340**, 730-732.
2. **Darst SA**, Edwards AM, Kubalek EW, Kornberg RD (1991) Three-dimensional Structure of Yeast RNA Polymerase II at 16 Å Resolution, *Cell* **66**, 121-128.
3. Zhang G, Campbell E, Minakhin L, Richter C, Severinov K, **Darst SA** (1999) Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution, *Cell* **98**, 811-824.
4. Boyaci H, Chen J, Jansen R, **Darst SA**, Campbell EA (2019) Structures of an RNA polymerase promoter melting intermediate elucidate DNA unwinding, *Nature* **565**, 382-385 [PMCID: PMC6399747].

B. Positions and Honors

2000-	Professor, The Rockefeller University, New York, NY
1997-2000	Associate Professor, Head of Laboratory, The Rockefeller University, New York, NY
1992-1997	Assistant Professor, Head of Laboratory, The Rockefeller University, New York, NY
1990-1992	Lucille P. Markey Postdoctoral Scholar with Roger D. Kornberg, Department of Cell Biology, Stanford University, Stanford, CA
1987-1990	American Cancer Society Postdoctoral Fellow with Roger D. Kornberg, Department of Cell Biology, Stanford University, Stanford, CA
1982-1987	Graduate Student with Channing R. Robertson, Department of Chemical Engineering, Stanford University, Stanford, CA

Honors (selected)

PI

2022	Gregori Aminoff Prize (The Royal Swedish Academy of Sciences, shared with Elena Conti and Patrick Cramer)
2008	American Academy of Microbiology Fellow National Academy of Sciences Fellow
1995-1999	Pew Scholar in the Biomedical Sciences
1994-1999	Career Scientist of the Irma T. Hirschl Charitable Trust

Postdoctoral & PI

1990-1996	Lucille P. Markey Award in Biomedical Science

Postdoctoral

1987-1990 American Cancer Society Postdoctoral Fellow

Undergraduate

1983-1987 Kodak Fellow
1982-1983 Chevron Fellow
1981 Giroux Fellow
1980 Dean's Club Fellow

Service (selected)

2025 ZGM1 TWD-B (KR) review study section for K99/R00 applications
2025 Blavatnik National Awards Jury
2021 Chan Zuckerberg Biohub Investigator Award review panel
2019 2019 Blavatnik National Awards Jury
2018 NIH ZRG1 BST-T(40) review panel
NIH ZRG1 CB B55 (MIRA) review panel
2017 NIH K99 review panel
NIH Review panel for Regional Consortia for High Resolution Cryoelectron Microscopy (U24) applications
2012–2016 MSFC study section, standing member
NSLS II AMX/FMX beamline advisory team
2011 NCI PO1 review panel
NIH MSFC study section, *ad hoc*
NIH ZRG1 MSFC-K(02)S panel
2010 NIH special review panel
2009 NIH BCMB study section, *ad hoc*
2008 NIH MSFC study section, *ad hoc*
2006 NIH NRSA study section, *ad hoc*
Chair, 2007 FASEB Summer Research Conference – Mechanisms and Regulation of Prokaryotic Transcription
2005-2018 APS SBC Scientific Advisory Committee
2005 NIH BBCA study section, *ad hoc*
Co-chair, 2005 FASEB Summer Research Conference – Mechanisms and Regulation of Prokaryotic Transcription
2004 APS reviewer
NIH transcription cluster review panel
NIH study section for members
2003 APS reviewer
NIH special review panel
NIH MBC2 study section, *ad hoc*
NIH BBCA study section, *ad hoc*
2002 NSLS PSP review panel, chair
NIH PB study section, *ad hoc*
NIH special review panel (served on four separate panels)
2001 NSLS PSP review panel
NIH special review panel
2000 NIH BBCA study section, *ad hoc*
NIH special review panel
1998 NIH special review panels (served on two separate panels)
1997 NIH BBCB study section, *ad hoc*

Frequent reviewer for: *Biophys. J., Cell*, *eLife*, *J. Mol. Biol., Mol. Cell*, *Nature*, *Nature Chem. Biol.*, *Nature Commun., Nature Struct. Mol. Biol.*, *Nucleic Acids Res.*, *Proc. Natl. Acad. Sci. U.S.A.*, *Science*.

C. Contribution to Science

1. As a postdoc and then as an independent investigator, I used electron microscopy to provide the first (low-resolution) reconstructions of cellular RNAPs. These studies revealed the ‘crab-claw’ shape of RNAP and provided the first structural framework used by the field to interpret decades of accumulated biochemical

data. We revolutionized the transcription field in 1999 with the first high-resolution X-ray crystal structure of a cellular RNAP (a), providing the first detailed structural framework for the interpretation of decades of functional and genetic data, and enabling the design of much more incisive experiments moving forward. Important among the new studies made possible by this advance, structural and mechanistic studies of bacterial RNAP inhibitors (such as rifamycins, a key component of tuberculosis treatment) revealed the molecular mechanism of inhibition (b-d).

a. Zhang G, Campbell E, Minakhin L, Richter C, Severinov K, **Darst SA** (1999) Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution, *Cell* **98**, 811-824.
b. Campbell EA, Korzheva N, Mustaev A, Murakami K, Goldfarb A, **Darst SA** (2001) Structural mechanism for rifampicin inhibition of bacterial RNA polymerase, *Cell* **104**, 901-912.
c. Bae B, Nayak D, Mustaev A, Landick R, **Darst SA** (2015) CBR antimicrobials inhibit RNA polymerase via at least two bridge-helix cap-mediated effects on nucleotide addition, *Proc. Natl. Acad. Sci. USA* **112**, E4178-87 [PMCID: 4534225].
d. Boyaci H, Chen J, Lilic M, Palka M, Mooney RA, Landick R, **Darst SA***, Campbell EA* (2018) Fidaxomicin jams *M. tuberculosis* RNA polymerase motions needed for initiation via RbpA contacts, *Elife* **7**, e34823 [PMCID: PMC5837556].

2. In bacteria, the initiation-specific σ factor combines with the RNAP catalytic core to form the holoenzyme, which is required to direct promoter-specific transcription initiation. A major mechanism through which bacteria regulate transcription initiation is through regulation of σ activity. Starting with the structure of a σ fragment (a), my group has determined high-resolution structures of σ factor domains (a, c). These studies provided the first insights into σ factor function in promoter recognition and melting, and greatly facilitated our understanding of the first holoenzyme structures. Our structural and functional studies of σ factor complexes with their cognate regulatory anti-σ factors (for example, b, d) have elucidated regulatory principles governing a major mechanism to control gene expression in bacteria.
   a. Malhotra A, Severinova E, **Darst SA** (1996) Crystal structure of an *Escherichia coli* RNA polymerase σ^{70} subunit fragment, *Cell* **87**, 127-136.
   b. Campbell EA, Masuda S, Sun JL, Olson CA, Wang S, Muzzin O, **Darst SA** (2002) Crystal structure of the *Bacillus stearothermophilus* anti-σ factor SpoIIAB with the sporulation σ factor σ^{F}, *Cell* **108**, 795-807.
   c. Campbell EA, Muzzin O, Chlenov M, Sun JL, Olson CA, Weinman O, Trester-Zedlitz ML, **Darst SA** (2002) Structure of the bacterial RNA polymerase promoter specificity σ subunit, *Mol. Cell* **9**, 527-539.
   d. Campbell EA, Greenwell R, Anthony JR, Wang S, Lim L, Das K, Sofia HJ, Donohue TJ, **Darst SA** (2007) A conserved structural module regulates transcriptional responses to diverse stress signals in eubacteria, *Mol. Cell* **27**, 793-805 [PMCID: PMC2390684].

3. RNAP holoenzyme & transcription initiation. Our studies of RNAP holoenzyme and complexes with promoters have revealed the organization of σ/RNAP interactions in the holoenzyme (a), the overall architecture of the holoenzyme/promoter complex (b), how σ initiates the formation of the transcription bubble (c), and how the holoenzyme stabilizes the final open promoter complex.
   a. Murakami KS, Masuda S, **Darst SA** (2002) Structural basis of transcription initiation: RNA polymerase holoenzyme at 4 Å resolution, *Science* **296**, 1280-1284.
   b. Murakami KS, Masuda S, Campbell EA, Muzzin O, **Darst SA** (2002) Structural basis of transcription initiation: An RNA polymerase holoenzyme/DNA complex, *Science* **296**, 1285-1290.
   c. Feklistov A, **Darst SA** (2011) Structural basis for promoter –10 element recognition by the bacterial RNA polymerase σ subunit, *Cell* **147**, 1257-1269 [PMCID: PMC3245737].
   d. Saecker RM*, Mueller AU*, Malone B, Chen J, Budell WC, Dandey VP, Maruthi K, Mendez JH, Molina N, Eng ET, Yen LY, Potter CS, Carragher B, **Darst SA** (2024) Early intermediates in bacterial RNA polymerase promoter melting visualized by time-resolved cryo-electron microscopy, *Nature Struct Mol Biol* **31**, 1778-1788 [PMCID: PMC11821292].

4. The bacterial transcription cycle is regulated by hundreds of accessory factors. In addition to regulation of σ factor activity, transcription initiation in bacteria is modulated by extrinsic factors (a, d). RNAP function can also be co-opted by extrinsic factors during bacteriophage infection (b, c). Structural and mechanistic analyses of such regulators elucidate the function and regulation of the RNAP itself.
    a. Jain D, Nickels BE, Sun L, Hochschild A, **Darst SA** (2004) Structure of a ternary transcription activation complex, *Mol. Cell* **13**, 45-53.
    b. Osmundson J, Montero-Diez C, Westblade LF, Hochschild A, **Darst SA** (2012) Promoter-specific transcription inhibition in *Staphylococcus aureus* by a phage protein, *Cell* **151**, 1005-1016 [PMCID: PMC3604623].
    c. Chen J, Wassarman KM, Feng S, Leon K, Feklistov A, Winkelman JT, Li Z, Walz T, Campbell EA, **Darst SA** (2017) 6S RNA mimics B-form DNA to regulate *Escherichia coli* RNA polymerase, *Mol. Cell* **68**, 388-397 [PMCID: PMC5683422].
    d. Kang JY, Mooney RA, Nedialkov Y, Saba J, Mishanina TV, Artsimovitch I, Landick R, **Darst SA** (2018) Structural basis for transcript elongation control by NusG/RfaH universal regulators, *Cell* **173**, 1650-1662.e14 [PMCID: PMC6003885].
5. At the beginning of the COVID-19 pandemic, we used our expertise in structural and functional analysis of bacterial transcription to study RNA synthesis and processing in SARS-CoV-2
    a. Chen* J, Malone* B, Llewellyn E, Grasso M, Shelton PMM, Olinares PDB, Maruthi K, Eng E, Vatandaslar H, Chait BT, Kapoor T, **Darst SA***, Campbell EA* (2020) Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex, *Cell* **182**, 1560-1573 [PMCID: PMC7386476].
    b. Chen J*, Wang Q*, Malone B, Llewellyn E, Pechersky Y, Maruthi K, Eng ET, Perry JK, Campbell EA, Shaw DE*, **Darst SA*** (2022) Ensemble cryo-electron microscopy reveals conformational states of the nsp13 helicase in the SARS-CoV-2 helicase replication-transcription complex, *Nature Struct. Mol. Biol.* **29**, 250-260 [PMCID: PMC8935131].
    c. Small G, Fedorova O, Olinares PDB, Chandanani J, Banerjee A, Choi YJ, Molina H, Chait B, Pyle AM, **Darst SA**, Campbell EA (2023) Structural and functional insights into the enzymatic plasticity of the SARS-CoV-2 NiRAN domain, *Mol Cell* **83**, 3921-3930 [PMCID: PMC10843261].
    d. Malone B, Perry JK, Olinares PDB, Chen J, Appelby TK, Feng JY, Bilello JP, Ng H, Sotiris J, Ebrahim M, Chua EYD, Mendez JH, Eng ET, Landick R, Chait BT, Campbell EA, **Darst SA** (2023) Structural basis for substrate selection by the SARS-CoV-2 replicase, *Nature* **614**, 781-787 [PMCID: PMC9891196].

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/seth.darst.1/bibliography/41155375/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

NAME: **Montserrat Bárcena**

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Assistant Professor, Leiden University Medical Center, the Netherlands

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Complutense University of Madrid (UCM), Spain	B.Sc.	07/1993	Chemistry/Biochemistry
Complutense University of Madrid (UCM), Spain	M.Sc.	06/1994	Biochemistry
Autónoma University of Madrid (UAM), Spain	Ph.D.	07/2000	Structural Biology

A. Personal Statement

Since my PhD, my research has centered on electron microscopy as a tool to understand macromolecular and cellular structure. I started my career using single-particle analysis to study helicases and glycolytic enzymes during my PhD research and first postdoc project, then progressively expanded into tomography and cell-focused EM. Over the years, I have applied nearly the full spectrum of EM techniques –from conventional EM and immunolabeling to advanced methods such as CLEM, serial block-face SEM and in situ cryo-ET. This breadth of technical experience has been central to my group's approach, allowing biological questions to drive methodological choices.

Since 2005, I have worked at the interface of virology and cell biology, particularly on positive-strand RNA viruses. After generating the first cryo-tomographic structure of a coronavirus particle in 2009 I shifted my focus to the cell biology of viral infection. Viral replication organelles have been a major research theme in my group for more than a decade. We have made key contributions to elucidating the structure, biogenesis and function of the replication organelles induced by coronaviruses, arteriviruses and picornaviruses. My group pioneered the application of in situ cryo-ET to study virus-infected cells. This work led to the discovery of molecular pores in the coronavirus replication organelles in 2020 –a breakthrough that has changed our understanding of nidoviral replication and that is central to this proposal. More broadly, I am deeply committed to integrating structural and cellular virology, and to developing and refining imaging workflows that bridge spatial scales and enhance interpretability, with potential implications for antiviral strategy development.

I have a strong record of national and international collaboration, including a long-standing partnership with LUMC co-PI Prof. Snijder, and have co-authored multiple studies and reviews across groups and disciplines. I have secured competitive funding from the Netherlands Organization for Scientific Research (NWO), including the MEERVOUD and Open Competition Domain Science programs, and have participated in transnational research consortia such as the EU-funded EUVIRNA and CARE projects. I have organized international training courses, and I actively contribute to the scientific community through peer review, grant evaluation, and institutional committee work (see section B).

Mentorship statement:

In addition to my research activities, I place high value on mentorship and scientific training. I have co-supervised five PhD students (three currently ongoing) and twelve MSc/BSc, students, helping them navigate interdisciplinary projects combining microscopy and virology. My mentoring approach emphasizes critical and independent thinking and methodological rigor. I work closely with trainees on experimental design, data interpretation and scientific writing, and encourage collaborative exchange within and beyond our lab.

Additionally, I encourage active participation in conferences, training courses and publication efforts to prepare trainees for successful scientific careers. I view mentorship not only as a core responsibility, but also as a key opportunity to shape future scientists and foster a collaborative, curiosity-driven research culture.

B. Positions, Scientific Appointments, and Honors

2013- Assistant professor, Leiden University Medical Center (LUMC), The Netherlands
2009-2013 Senior researcher, Leiden Univ. Medical Center (LUMC), The Netherlands
2005-2009 Postdoctoral associate, Leiden University Medical Center (LUMC), The Netherlands
2004-2005 Postdoctoral associate, Utrecht University, The Netherlands
2003 Postdoctoral associate, University of Vermont, USA.
2000-2002 Postdoctoral associate, National Center for Biotechnology, UAM, Madrid, Spain.
1996-2000 Graduate fellow, National Center for Biotechnology, UAM, Madrid, Spain.

Select Service and Professional Memberships

2022-2024 Board member, LUMC research profile area Infection. Co-chair of the Technlogy subcommittee.
2019-2022 Organizing and chair of the monthly Leiden EM seminar series.
2019 Session co-organizer and co-chair in the 7th European Congress of Virology. Rotterdam, The Netherlands.
2009 & 2010 Co-organizer EMBO Practical Course on Electron Tomography in Life Science. Leiden University Medical Center, The Netherlands.
2006-2012 Co-organizer of the M&M pre-conference course “Electron Tomography in Life and material Science”.
2003 Co-organizer UVM international practical course on 3D cryo-EM and SPA

- Regular reviewer of research grant proposals for funding agencies such as NWO (Netherlands Organization for Scientific Research), ANR (Agence Nationale de la Reserche, French Research Agency), Biotechnology and Biological Sciences Research Council (BBSRC, UK) and MRC (Medical Research Council, UK).
- Regularly serve as peer reviewer for a range of both high-impact and specialized journals, including *Nature, Nature Communications, mBio, ACS nano, Cell Reports, Journal of General Virology, Journal of Virology, Viruses, Scientific Reports, Cellular Microbiology, Future Virology, Micron,* and *FEBS letters.*
- Member of the European Microscopy Society and the American Society for Virology.

Select Honors

2021 Outstanding Paper Award, category Life Science 2020. European Microscopy Society.
2005 Marie Curie postdoctoral fellowship
2004 EMBO postdoctoral fellowship
2003 Fulbright postdoctoral fellowship
2000 UAM award to the best PhD thesis (section Chemistry)

C. Contributions to Science

My research focuses on the structural and cell biology of positive-strand RNA viruses –a large group of viruses that includes many important human pathogens---, with particular emphasis on viral replication organelles. We have contributed key insights into their architecture and function, by combining a broad range of imaging techniques with molecular virology approaches. With a strong background in EM and imaging, my group also actively contributes to method developments, tailoring imaging workflows to the biological questions at hand.

Topic 1: Early EM studies in structural biology

During my PhD research and early postdoctoral work, I focused on single-particle electron microscopy (SPA) to study macromolecular complexes. At a time when SPA was still emerging as a structural biology technique, I used it to characterize helicases and glycolytic enzymes. Highlights of this period include the cryo-EM structure of the *E. coli* helicase DnaB in complex with its loader DnaC [1], and the structure of yeast phosphofructokinase [2]. My interest then shifted toward virology and cryo-ET, an incipient technique at the

time, which I applied to resolve the molecular architecture of the pleomorphic coronavirus particle [3]. This work broadened my EM expertise and sparked a deeper interest in viruses and complex biological systems, leading my transition into the cell biology of infection.

1. **Bárcena M**, Ruiz T, Donate LE, Brown SE, Dixon NE, Radermacher M, Carazo JM. (2001). The DnaB·DnaC complex: a structure formed by asymmetric dimers around an occluded channel. EMBO J, 20(6):1462-8.
2. **Bárcena M**, Radermacher M, Bär J, Kopperschläger G, Ruiz T. (2007). The structure of the ATP-bound state of S. cerevisiae phosphofructokinase determined by cryo-electron microscopy. J Struct Biol, 159(1):135-43.
3. **Bárcena M**, Oostergetel GT, Bartelink W, Faas FG, Verkleij A, Rottier PJ, Koster AJ, Bosch BJ. (2009). Cryo-electron tomography of mouse hepatitis virus: insights into the structure of the coronavirion. Proc Natl Acad Sci USA,106(2):582-7.

Topic 2: Nidovirus replication organelles.

Nidoviruses, including coronaviruses and arteriviruses, induce extensive remodeling of ER membranes to generate a network of interconnected membrane structures collectively termed the viral replication organelle. This includes double-membrane vesicles (DMVs), convoluted membranes, zippered ER and double-membrane spherules. Our group has made seminal contributions to elucidating the complex architecture of these replication organelles [1], established that the DMVs are the central hubs for viral RNA synthesis [2], and identified key viral factors driving their biogenesis [3]. We pioneered the application of in situ cryo-ET to study viral replication organelles in their native cellular context at macromolecular resolution [4]. This work led to the de novo discovery of DMV-spanning complexes – the focus of the current proposal –, which are postulated to serve as viral RNA export channels and represent promising targets for future antiviral drug development.

1. van der Hoeven B, Oudshoorn D, Koster AJ, Snijder EJ, Kikkert M, **Bárcena M**. (2016) Biogenesis and architectures or arterivirus replication organelles. Virus Res, 220:70-90.
2. Snijder EJ, Limpens RWAL, de Wilde AH, de Jong AWM, Zevenhoven-Dobbe JC, Maier HJ, Faas `FFGA, Koster AJ, **Bárcena M.** (2020). A unifying structural and functional model of the coronavirus replication organelle: Tracking down RNA synthesis. PLoS Biol, 18(6): e3000715.
3. Oudshoorn D, Rijs K, Limpens RWAL, Groen K, Koster AJ, Snijder EJ, Kikkert M, **Barcena M.** (2017). Expression and cleavage of MERS-coronavirus nsp3-4 polyprotein induces the formation of double-membrane vesicles that mimic those associated with coronaviral RNA replication. mBio, 8(6): e01658-17.
4. Wolff G, Limpens RWAL, Zevenhoven-Dobbe JC, Laugks U, Zheng S, de Jong AWM, Koning RI, Agard DA, Grünewald K, Koster AJ, Snijder EJ, **Bárcena M.** (2020). A molecular pore spans the double-membrane of the coronavirus replication organelle. Science, 69(6509):1395-1398.

Topic 3: Picornavirus replication organelles.

Picornaviruses are a family of viruses that includes important human pathogens like poliovirus, coxsackievirus, and rhinoviruses. Like nidoviruses, they induce extensive remodeling of cytoplasmic membranes, which are transformed upon infection into single, double- or multi-membrane structures. Our group has been pivotal in establishing the architecture of these replication organelles and unraveling their dynamic morphological transformations throughout the course of infection [1,2] as well as investigating their interplay with host factors [2,3]. In addition, using advanced CLEM techniques, we established the previously unclear origin of the viral replication organelles, showing that sequentially originate from the ER and Golgi apparatus.

1. Limpens RW, van der Schaar HM, Kumar D, Koster AJ, Snijder EJ, van Kuppeveld FJ, **Bárcena M**. (2011). The transformation of enterovirus replication structures: a three-dimensional study of single- and double-membrane compartments. mBio, 2(5):e00166-11.
2. Melia CE, van der Schaar HM, de Jong AWM, Lyoo HR, Snijder EJ, Koster AJ, van Kuppeveld FJM*, **Bárcena M***. (2018). The origin, dynamic morphology, and PI4P-Independent formation of encephalomyocarditis virus replication organelles. mBio, 9(2):e00420-18. doi: 10.1128/mBio.00420-18
3. Melia CE*, van der Schaar HM*, Lyoo H, Limpens RWAL, Feng Q, Wahedi M, Overheul GJ, van Rij RP, Snijder EJ, Koster AJ, **Bárcena M***, van Kuppeveld F.J.M.* (2017). Escaping host factor PI4KB inhibition: enterovirus genomic RNA replication in the absence of replication organelles. Cell Rep, 21(3):587-599.

4. Melia CE, Peddie CJ, de Jong AWM, Snijder EJ, Collinson LM, Koster AJ, van der Schaar HM, van Kuppeveld FJM, **Bárcena M**. (2019). Origins of enterovirus replication organelles established by whole-cell electron microscopy. mBio, 10(3):e00951-19.

Topic 4: Contributions to imaging method development

Throughout my career, I have actively contributed to the advancement and optimization of microscopy techniques, driven by the strong belief that methodological innovation is essential to unlock new biological insights. For example, in close collaboration with technology developers, I have contributed to the implementation of an integrated light and electron microscope [1], the development of fluorescent tools to study viral infections [2], and the creation of AreTomo, a widely used software for automated cryo-ET alignment and reconstruction [3]. My group also introduced the micro-expansion joints approach (also known as stress-relief cuts) [4], which significantly improved the success rate in the preparation of FIB-milled lamellae for cryo-ET and has been widely adopted in the field.

1. Faas FG, **Bárcena M**, Agronskaia AV, Gerritsen HC, Moscicka KB, Diebolder CA, van Driel LF, Limpens RW, Bos E, Ravelli RB, Koning RI, Koster AJ. (2013). Localization of fluorescently labeled structures in frozen-hydrated samples using integrated light electron microscopy. J Struct Biol, 181(3):283-90.
2. van der Schaar HM*, Melia CE*, van Bruggen JAC, Strating JRPM, van Geenen MED, Koster AJ, **Bárcena M***, van Kuppeveld FJM*. (2016). Illuminating the sites of enterovirus replication in living cells by using a split-GFP-tagged viral protein. mSphere, 1(4):e00104-16. doi: 10.1128/mSphere.00104-16
3. Zheng S, Wolff G, Greenan G, Chen Z, Faas FGA, **Bárcena M**, Koster AJ, Cheng Y, Agard DA. AreTomo: An integrated software package for automated marker-free, motion-corrected cryo-electron tomographic alignment and reconstruction. (2022). J Struct Biol X, 6:100068.
4. Wolff G, Limpens RWAL, Zheng S, Snijder EJ, Agard DA, Koster AJ, **Bárcena M**. (2019). Mind the gap: Micro-expansion joints drastically decrease the bending of FIB-milled cryo-lamellae J Struct Biol, 208(3):107389.

For a full list of peer-reviewed publications, see: https://orcid.org/0000-0002-7719-4443

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: CAMPBELL, ELIZABETH

eRA COMMONS USER NAME (credential, e.g., agency login): elizcamp

POSITION TITLE: Professor, Head of Laboratory

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
Swarthmore College, Swarthmore, PA	BA	06/1992	Biology
The Rockefeller University, New York, NY	PhD	06/1998	Microbial Pathogenesis
The Rockefeller University, New York, NY	Postdoctoral	07/2003	Structural Biology

A. Personal Statement

• **Ph.D. Training:** Studied natural transformation in *Streptococcus pneumoniae* at The Rockefeller University under Drs. Elaine Tuomonen and Robert Masure**.**

• **Postdoctoral Studies:** Focused on structural and biophysical aspects of bacterial transcription with Dr. Seth Darst.

•Current Research

Transcriptional Mechanisms in Bacterial Pathogens

My current focus is on structure-function studies of transcription mechanisms in phylogenetically distinct bacteria, with an emphasis on pathogens. Since establishing my independent lab as a Full Professor (following appointments as Senior Research Associate and Research Associate Professor), I have focused on the structure and function of RNAP and the factors that regulate this enzyme in the pathogen, *Mycobacterium tuberculosis*, throughout the transcription cycle.

My group has found that, unlike *Escherichia coli* RNAP, which has been the focus of most biochemical characterizations, *Mycobacterium* sp. RNAPs differ substantially at various kinetic steps of initiation. Several essential transcription factors (absent in *E. coli*) are critical for boosting the activity of the mycobacterial enzyme. Our success in achieving the first high-resolution structures of mycobacterial RNAP revealed new mechanisms of initiation. It facilitated our focus on characterizing inhibitor mechanisms to guide the development of treatments for tuberculosis. One of the significant outcomes of our research was the first X-ray crystal structure of a mycobacterial RNAP, followed by the first cryo-EM structures of *M. tuberculosis* RNAP, positioning us for further studies.

We then expanded our research to study RNAP from another deadly pathogen, *Clostridioides difficile*, the causative agent of intestinal disease. *C. difficile* infections are treated with the antibiotic fidaxomicin (Fdx), which targets RNAP. Additionally, we expanded our study from the general transcription factors RbpA and CarD to the specialized and uncharacterized WhiB family of transcription factors, which are found exclusively in Actinobacteria.

The Transcription Cycle in Mycobacteria

Although my focus for the last eight years has been on transcription initiation in the deadliest human pathogen, *M. tuberculosis*, I am now including studies of post-initiation mechanisms such as pausing, termination, and transcription-translation coupling. We recently published a paper describing the pro-pausing activity of *M. tuberculosis* NusG and the structural basis for this activity in contrast to the anti-pausing activity of *E. coli* NusG. We then published a follow-up study describing the significance of pausing as a compensatory mechanism in rifampicin-resistant clinical isolates of *M. tuberculosis*.

Structural and Functional Characterization of the SARS-CoV-2 Replication and Transcription Processes

In March 2020, during the COVID-19 pandemic, I recognized that many of the approaches we used for mechanistic studies of bacterial transcription could be applied to studying SARS-CoV-2 replication and transcription. Upon reviewing the literature on the regulation of the SARS-CoV-2 RNA-dependent polymerase (RdRp), I realized that it was relatively underexplored compared to bacterial transcription. Therefore, I began working with Dr. Seth Darst on the replication-transcription complex of SARS-CoV-2 to structurally and biochemically elucidate higher-order complexes involved in different steps of viral transcription and replication. My training in structurally and functionally characterizing antimicrobials also positioned me to collaborate in the discovery of potential new antivirals. This work proved fruitful, as outlined in my contributions, and led to a new research direction for my group.

• **Mentorship Statement:** My lab is committed to training the next generation of scientific leaders through rigorous, interdisciplinary research on the molecular mechanisms of the central dogma in pathogens, biochemistry, and biophysics. I strive to foster an environment where all students can thrive. Our training program offers hands-on experience in cutting-edge research, with a focus on biochemistry, microbiology, and structural biology. Students engage in scientific methodology, data analysis, presentation, and manuscript writing, with opportunities to present at conferences. My mentoring style is highly individualized, featuring an open-door policy for regular meetings and support from senior lab members. My institutional service includes teaching in the biophysics course, interviewing and hosting prospective students, mentoring rotation students, serving on thesis committees, and acting as a thesis advisor. I also nominate students for fellowships and awards, while encouraging them to participate in conferences. Each year, I meet with students to discuss their career paths and provide opportunities for them to mentor high school, undergraduate, and graduate students. My ultimate goal is to prepare students for successful and impactful careers in science.

• **Expertise:** My expertise in bacterial and viral transcription and pathogenesis is reflected in my leadership roles and invited presentations. I have chaired multiple sessions at FASEB meetings on "*Mechanisms of Prokaryotic Transcription"* (2009–2017) and was later elected co-chair and subsequently chair. I have also served as a convener and speaker at ASM Microbe meetings (2017, 2019, 2021), in symposia covering antibiotic mechanisms, small molecule regulators of gene expression, and regulation of RNA synthesis. Additionally, I have contributed to the scientific advisory board of the European ERASynBio initiative. Recognition of my expertise includes election to the American Academy of Microbiology (2018) and receipt of the Emil von Behring Prize (2023). I was also invited as a panelist at the November NIH Antiviral Summit (*Targeting Viral Replication Machinery*) and as a keynote speaker at the 2023 Gordon Research Conference on Three-Dimensional Electron Microscopy. My expertise in gene expression in bacterial and pathogenic viruses is also evidenced by the following peer-reviewed articles (references **a** and **b**).

Reference key: (* corresponding authors)

a. Chen, J, Boyaci, H, and **Campbell, EA*.** (2021). Diverse and unified mechanisms of transcription initiation in bacteria. Nature Reviews Microbiology *19*, 95–109. PMCID: PMC7855538.

b. Malone B, Urakova N, Snijder EJ*, **Campbell EA***. Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design. Nat Rev Mol Cell Biol. 2022 Jan;23(1):21-39. PMCID: PMC8613731.

B. Scientific Appointments, Service, and Honors

Scientific Appointments

2024-current	Professor and Head of Laboratory, The Rockefeller University, New York, NY
2018-2024	Research Associate Professor, The Rockefeller University, New York, NY
2011–2018	Senior Research Associate, The Rockefeller University, New York, NY
2003–2011	Research Associate, The Rockefeller University, New York, NY

Select Service and Professional Memberships

2025	Scientific Advisory Board (SAB) of the Max Planck Institute of Biochemistry
2025	NIH Molecular and Cellular Biology of Virus Infection panel reviewer, ad hoc
Ongoing-	Peer Reviewer: mBio, Molecular Microbiology, Proceedings of the National Academy of Sciences, Journal of Bacteriology, Journal of Biochemistry, Nucleic Acids Research and Acta Crystallographica, PLOS Genetics, Genes and Development, Frontiers in Molecular Biosciences, Journal of Molecular Biology, ACS Chemical Biology, Nature Structure and

Molecular Biology, Nature Communications, Nature Microbiology, Nature, Science, Molecular Cell.

2020 Panelist, NIH Antiviral Summit (Targeting Viral Replication Machinery)

2017-2022 Convener and speaker at the American Society of Microbiology

2017, 2022 NIH PCMB panel reviewer, ad hoc

2017-2019 Chair, Gordon Research Conference on the Mechanism and Regulation of Microbial Transcription

2013-2018 Member of the scientific advisory board of a translational research consortium under the umbrella of the European ERASynBio initiative

2009-2017 Session Chair, Federation of American Societies for Experimental Biology Conference on the Mechanism and Regulation of Prokaryotic Transcription

2015-2017 Co-chair organizer, Federation of American Societies for Experimental Biology Conference on the Mechanism and Regulation of Prokaryotic Transcription

Select Honors

2024 Rockefeller University's 2024 Mentoring Excellence Award in Faculty

2023 Emil von Behring Prize 2023

2019 Keystone Symposia Early Career Investigator Travel Award

2018 Elected to Fellowship in the American Academy of Microbiology

2000-2003 Individual National Research Service Award, National Institutes of Health

1993-1996 NSF Graduate Fellow, National Science Foundation

1993 John Kluge Graduate Fellow, The Rockefeller University

1992 Pew Undergraduate Fellow, Swarthmore College

1992 Distinction in Biology in Graduation, Swarthmore College

1992 Sigma Xi Fellow, Swarthmore College

C. Contributions to Science Transcription is a universal process and, in bacteria, is performed by the enzyme RNAP. We have found that studying phylogenetically distinct clades of bacteria increases our understanding of the general principles of transcription. I study Actinobacteria because the biophysical principles underlying the molecular mechanisms of transcription in this clade are relatively understudied, and it includes deadly pathogens. Our work has now contributed to the general and regulated transcription paradigms previously established in model organisms such as *E. coli* and *Bacillus subtilis*. As of 2020, I have now expanded my program to study another deadly pathogen, SARS-CoV-2, uncovering new mechanisms of viral replication and gene expression.

1. Structural and functional characterization of mycobacterial RNAP with basic transcription factors. One of my current research foci is to characterize the basic transcriptional properties of RNAP from *M. tuberculosis*. *M. tuberculosis* contains two essential transcription factors, CarD and RbpA, which are not found in *E. coli*. We first studied how CarD functions by crystallizing Thermus RNAP with Thermus CarD on a promoter initiation complex (**a**). Our biochemical and structural studies revealed that the paradigm of transcription initiation, previously defined in *E. coli*, does not apply to other bacteria, including mycobacteria (**a, b, c**). Our work has provided the first crystal structure of RNAP from the Actinobacteria clade, *M. smegmatis*, in complex with promoter DNA and RbpA (**b, c**), as well as the first cryo-EM structure of the *M. tuberculosis* RNAP initiation complex with RbpA and CarD (**d**). In addition, we compared the kinetics of transcription between *M. tuberculosis* RNAP and *E. coli* and found critical differences in their function (**b**). Finally, we applied the unique kinetic properties of *M. tuberculosis* RNAP to capture a promoter melting intermediate, revealing the pathway of DNA unwinding (**d**). This work is significant for understanding how RNAPs generally work and for understanding the specific structural and kinetic properties of RNAP from this pathogen.

a. Bae B, Chen J, Davis E, Leon K, Darst SA*, **Campbell EA***. CarD uses a minor groove wedge mechanism to stabilize the RNA polymerase open promoter complex. eLife. 2015 Sep 8;4. PMCID: PMC4593161.

b. Hubin EA, Fay A, Xu C, Bean JM, Saecker RM, Glickman MS, Darst SA*, **Campbell EA***. Structure and function of the mycobacterial transcription initiation complex with the essential regulator RbpA. eLife. 2017 Jan 9;6. PMCID: PMC5302886.

c. Hubin EA, Lilic M, Darst SA, **Campbell EA***. Structural insights into the mycobacteria transcription initiation complex from analysis of X-ray crystal structures. Nat. Commun. 2017 Jul 13;8:16072. PMCID: PMC5511352.

d. Boyaci H, Chen J, Jansen R, Darst SA, **Campbell EA***. Structures of an RNA polymerase promoter melting intermediate elucidate DNA unwinding. Nature. 2019 Jan; 565 (7739):382-385. PMCID: PMC6399747.

2. Structural basis of antibiotic inhibition of RNAP. During my postdoctoral tenure, I became interested in how antibiotics target RNAP function. Consequently, I solved the first structure of RNAP bound to the inhibitor Rif, an antibiotic used for treating TB, elucidating the mechanisms of inhibition and understanding the molecular nature of resistance to this essential antibiotic. I subsequently solved the structure of RNAP bound to another inhibitor, Sorangicin (Sor). This work showed that Sor, although it occupies the Rif pocket, inhibits Rif-resistant RNAP due to its flexible nature, suggesting a desirable feature for future antibiotics. This work was done using the only then-crystallizable RNAP from *Thermus aquaticus*. I then directed a research program to characterize RNAP from two pathogens. One of the goals of this program is to investigate the mechanisms of inhibitors of RNAPs from *M. tuberculosis*, the causative agent of TB. First, we crystallized RNAP from *M.smegmatis*, a bacterium from the same clade as *M. tuberculosis*. Because of advances in cryo-EM, we have switched to working directly on RNAP from *M. tuberculosis*, addressing the structural and biochemical basis of how rifamycin analogs (in collaboration with Dr. Sean Brady) and Sor inhibit rifamycin-resistant RNAP from mycobacterial RNAPs (**a, b, c**). In collaboration with Dr. Robert Landick, we have extended our studies of RNAP to the deadly opportunistic pathogen *C. difficile*, with a focus on the clinical inhibitor Fdx. Our cryo-EM structures of *M. tuberculosis* RNAP with Fdx reveal how the Actinobacteria-specific transcription factor RbpA interacts with the antibiotic, conferring the clade-specific sensitivity to this antibiotic (**c**). Our work on determining the structural and biochemical basis of Fdx's narrow-spectrum activity on *C. difficile* revealed a sensitizing residue in RNAP in gram-positive bacteria (**d**).

a. Peek J, Lilic M, Montiel D, Milshteyn A, Woodworth I, Biggins JB, Ternei MA, Calle PY, Danziger M, Warrier T, Saito K, Braffman N, Fay A, Glickman MS, Darst SA, **Campbell EA***, Brady SF*. Rifamycin congeners kanglemycins are active against rifampicin-resistant bacteria via a distinct mechanism. Nat Commun. 2018 Oct 8;9(1):4147. PMCID: PMC6175910.

b. Lilic M, Chen J, Boyaci H, Braffman N, Hubin EA, Herrmann J, Müller R, Mooney R, Landick R, Darst SA, **Campbell EA***. The antibiotic sorangicin A inhibits promoter DNA unwinding in a *Mycobacterium tuberculosis* rifampicin-resistant RNA polymerase. Proc Natl Acad Sci USA. 2020 Dec 1;117(48):30423-30432. PMCID: PMC7720108.

c. Boyaci H, Chen J, Lilic M, Palka M, Mooney RA, Landick R, Darst SA*, **Campbell EA*.** Fidaxomicin jams *Mycobacterium tuberculosis* RNA polymerase motions needed for initiation via RbpA contacts. eLife. 2018 Feb 26;7:e34823. PMCID: PMC5837556.

d. Cao X, Boyaci H, Chen J, Bao Y, Landick R*, **Campbell EA***. Basis of narrow-spectrum activity of fidaxomicin on *Clostridioides difficile*. Nature. 2022 Apr;604(7906):541-545. PMCID: PMC9635844.

3. Functional and structural characterization of *M. tuberculosis* specialized transcription factors. Our work focuses on metal-binding transcription factors that regulate RNAP activity in *M. tuberculosis*, with distinct efforts in initiation and elongation.

Initiation: We structurally and biochemically characterized the WhiB7–RNAP complex, showing how WhiB7 engages AT-rich promoter DNA via an AT-hook (**a)**. In collaboration with Dr. Buttner, we solved the structure of Streptomyces RNAP with WhiA and WhiB, revealing dual activation of division gene expression by iron- and zinc-binding factors (**b**).

Elongation: In collaboration with the Landick lab, we demonstrated that *M. tuberculosis* NusG is a pro-pausing factor, stabilizing a swiveled elongation complex via interactions with RNAP and the non-template strand. Our cryo-EM structures explain how NusG orthologs control pausing through gate loop contacts (**c**). In work with Jeremy Rock, we uncovered that loss-of-function mutations in NusG can compensate for the fitness cost of Rifampicin resistance (S450L) by suppressing hyper-pausing and premature termination (**d**).

a. M. Lilic, S. A. Darst, **E. A. Campbell***. Structural basis of transcriptional activation by the *Mycobacterium tuberculosis* intrinsic antibiotic-resistance transcription factor WhiB7. Mol Cell 2021 July 15;81(14): 2875-2886.e5. PMCID: PMC8311663.

b. Lilic M, Holmes NA, Bush MJ, Marti AK, Widdick DA, Findlay KC, Choi YJ, Froom R, Koh S, Buttner MJ*, **Campbell EA*.** Structural basis of dual activation of cell division by the actinobacterial transcription factors WhiA and WhiB**.** Proc Natl Acad Sci U S A. 2023 Mar 14;120(11):e2220785120. PMCID: PMC10243135.

c. Delbeau M, Omollo EO, Froom R, Koh S, Mooney RA, Lilic M, Brewer JJ, Rock J, Darst SA*, **Campbell EA***, Landick R*. Structural and functional basis of the universal transcription factor NusG pro-pausing activity in *Mycobacterium tuberculosis*. Mol Cell. 2023 May 4;83(9):1474-1488.e8. PMCID: PMC10231689.

d. Eckartt KA, Delbeau M, Munsamy-Govender V, DeJesus MA, Azadian ZA, Reddy AK, Chandanani J, Poulton NC, Quiñones-Garcia S, Bosch B, Landick R, **Campbell EA**[*], Rock JM[*]. Compensatory evolution in NusG improves fitness of drug-resistant *M. tuberculosis*. Nature. 2024 Apr; 628(8006): 188-194. PMCID: PMC10990936.

4. Replication and Transcription Mechanisms of SARS-CoV-2

At the onset of the COVID-19 pandemic, relatively little was known about the structure and regulation of the SARS-CoV-2 replication-transcription machinery. To identify regulators of SARS-CoV-2 gene expression and replication, we used gel shift assays and native mass spectrometry to show that the helicase nsp13 forms a stable complex with the holo-RdRp (nsp12). We then resolved the structure of this complex, which led to structure-based models of backtracking, template switching, and replication (**a**). We followed up on the backtracking model by providing structural and biochemical evidence of the product RNA path during backtracking (**b**).

Our expertise in purifying and characterizing the SARS-CoV-2 transcription/replication enzymes and complexes led to a collaboration with Gilead Sciences to characterize the molecular basis of the pre-incorporation binding of natural and synthetic clinical antivirals (**c**). To achieve this, we determined the structures of each of the four natural nucleosides and the antiviral nucleoside analog remdesivir triphosphate in the active site of the RdRp.

In addition to containing a conserved RNA-dependent RNA polymerase (RdRp), nsp12 has a second and separate active site that mediates nucleotidyltransferase activity within an N-terminal domain called NiRAN (Nidovirus RdRp-Associated Nucleotidyltransferase). This domain is essential for viral replication and for capping mRNA. We recently captured several intermediate structures of this domain bound to substrate nucleotides and RNA, offering a platform for guiding drug design and development (**d**).

a. Chen J, Malone B, Llewellyn E, Grasso M, Shelton PMM, Olinares PDB, Maruthi K, Eng E, Vatandaslar H, Chait BT, Kapoor T, Darst SA*, **Campbell EA*** (2020) Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex, Cell. 2020 Sep 17;182(6):1560-1573. PMCID: PMC7386476

b. Malone BF, Perry JK, Olinares PDB, Lee HW, Chen J, Appleby TC, Feng JY, Bilello JP, Ng H, Sotiris J, Ebrahim M, Chua EYD, Mendez JH, Eng ET, Landick R, Götte M, Chait BT, Darst SA*, **Campbell EA***. Structural basis for backtracking by the SARS-CoV-2 replication-transcription complex. Proc Natl Acad Sci USA. 2021 May 11;118(19). PMCID: PMC8126829.

c. Malone BF, Perry JK, Olinares PDB, Lee HW, Chen J, Appleby TC, Feng JY, Bilello JP, Ng H, Sotiris J, Ebrahim M, Chua EYD, Mendez JH, Eng ET, Landick R, Götte M, Chait BT, **Campbell EA**, Darst SA. "Structural Basis for Substrate Selection by the SARS-CoV-2 Replicase." Nature. 2023 Feb23: 614 (7949):781–87. PMCID: PMC9891196

d. Small GI, Fedorova O, Olinares PDB, Chandanani J, Banerjee A, Choi YJ, Molina H, Chait BT, Darst SA[*], **Campbell EA**[*]. Structural and functional insights into the enzymatic plasticity of the SARS-CoV-2 NiRAN domain. Mol Cell. 2023 Nov 2;83(21):3921-3930.e7. PMCID: PMC10843261

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/sites/myncbi/1n_ekkuzXr25P/bibliography/47915347/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

(June 27, 2025)

NAME: **Eric J. Snijder**

eRA COMMONS USERNAME (credential, e.g., agency login): SNIJDER

POSITION TITLE: Professor Molecular Virology, PI Nidovirus research group, Leiden Univ. Medical Center, NL

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion date	FIELD OF STUDY
Utrecht University, the Netherlands	B.Sc.	10/1983	Biology/Biochemistry
Utrecht University, the Netherlands	M.Sc.	08/1986	Molecular Biology
Utrecht University, the Netherlands	Ph.D.	01/1991	Molecular Virology

A. Personal Statement

Since 1991, my research team has been investigating the replication, virus–host interactions, and evolution of positive-strand RNA (+RNA) viruses, with a particular focus on nidoviruses such as corona- and arteriviruses. Our work includes the study of zoonotic coronaviruses capable of causing severe respiratory disease in humans—namely SARS, MERS, and COVID-19—which emerged in 2003, 2012, and 2020, respectively.

By integrating technologies from bioinformatics, molecular biology, biochemistry, and cell biology, we employ a multidisciplinary approach that ranges from detailed analyses of viral genomes and their expression to the investigation of immune responses and pathogenesis in infected hosts. A cornerstone of our research has been the development and application of reverse genetics systems, which enable the generation and functional analysis of defined viral mutants—a task particularly challenging for coronaviruses due to their unusually large genomes. Within this experimental framework, our efforts to identify coronavirus replication inhibitors and elucidate their mechanisms of action have served a dual purpose: they have significantly advanced our understanding of the complex molecular biology of coronaviruses, while also uncovering both viral and host targets for novel antiviral strategies. Key achievements of our research include: (i) the dissection of the intricate RNA replication and transcription mechanisms of nidoviruses; (ii) in-depth characterization of nidoviral proteases and the extensive proteolytic processing of their replicase polyproteins; (iii) the identification and detailed study of various nidoviral enzymes involved in RNA synthesis and metabolism (e.g., RNA polymerases, exoribonucleases, capping enzymes, and helicases); (iv) biochemical and ultrastructural characterization of nidoviral replication organelles within infected cells; and (v) the discovery and mechanistic investigation of small-molecule inhibitors of (corona)virus replication.

Alongside several biosafe nidovirus models, many of these projects have involved studies with live SARS-CoV, MERS-CoV, or SARS-CoV-2, conducted within our state-of-the-art BSL-3 facility at LUMC. This infrastructure and our coronavirus expertise formed the foundation for over a dozen basic and translational research projects launched during the 2020–2021 pandemic response. Currently, one of our key goals is the identification and development of potent broad-spectrum inhibitors against coronaviruses and other high-risk RNA virus families—a critical first line of defence in case of future outbreaks or epidemics.

In addition to a long-standing track record in pioneering nidovirus research, I maintain an extensive international network in the +RNA virology field and have led a range of national and international research initiatives. Most recently, I served as the coordinator of the EU-funded consortia SCORE (2020–2022) and PANVIPREP (2024–2028), and as a task leader in the European IMI project CARE (Corona Accelerated R&D in Europe; 2020–2025), all aimed at developing broad-spectrum antiviral therapeutics against coronaviruses. My research has also received substantial funding from the Netherlands Organization for Scientific Research (NWO), where I also served as a group leader in the Nucleic Acids and Proteins study section. Moreover, I was a member of the LUMC Science Committee (2007–2018) and a board member of the LUMC research profile areas "Infection, Immunity and Tolerance" (2016–2020) and "Infection" (2020–2024). As a professor of molecular virology, I have mentored or am currently supervising approximately 60 PhD students and post-docs, and numerous undergraduate students, many of whom have continued to successful careers in science.

Websites:

- https://www.universiteitleiden.nl/en/staffmembers/eric-snijder#tab-1
- https://www.lumc.nl/afdelingen/lucid/ej-snijder2/
- https://www.panviprep.eu/Article/Home
- https://www.imi-care.eu/
- https://cordis.europa.eu/project/id/101003627

Complete publication lists of Eric J. Snijder:

- https://orcid.org/0000-0003-3297-2309
- https://www.webofscience.com/wos/author/record/E-6073-2018
- https://pubmed.ncbi.nlm.nih.gov/?term=Snijder+EJ&sort=date

B. Positions

2020-2024	Board member, LUMC research profile area Infection
2016-2020	Board member, LUMC research profile area Infection, Immunity and Tolerance
2012-present	Elected Member of the American Academy of Microbiology
2009-2023	Head Section Research and Vice-Chair, Dept. of Medical Microbiology, Leiden University Medical Center, The Netherlands
2007-2018	Member of LUMC Science Committee (leading science policy advisory committee)
2007-present	Full Professor and PI of the Nidovirus research group, Dept. Medical Microbiology / Leiden Univ. Center for Infectious Diseases (LUCID), Leiden Univ. Medical Center, The Netherlands
2003-present	Member of grant review panels for Netherlands Organization for Scientific Research (NWO)
1999-present	Member/chair of multiple study groups, International Committee on Taxonomy of Viruses
1995	Visiting Scientist, European Molecular Biology Laboratory, Heidelberg, Germany
1994-2007	Associate Professor, Dept. Medical Microbiology, Leiden Univ. Medical Center, Netherlands
1990-1993	Assistant Professor, Dept. Medical Microbiology, Leiden Univ. Medical Center, Netherlands
1986-1990	Research Associate/graduate student, Inst. Veterinary Virology, Utrecht Univ., Netherlands

Selection of other Experience, Professional Memberships, Honors and Awards

2024-2028	Coordinator PANVIPREP project; Developing broad-spectrum antiviral drugs for pandemic preparedness; European Commission (Horizon Europe)
2022	Co-organized meeting (SCORE project and European Commission) “Broad-spectrum antiviral therapeutics: a key tool for pandemic preparedness and response”; Brussels, Belgium)
2020-2022	Coordinator SCORE project; Swift Coronavirus therapeutics Response; Eur. Comm. (H2020)
2020-2025	Task leader CARE project; Corona Accelerated R&D in Europe; IMI EFPIA & European Commission (H2020)
2020-2022	Frequently consulted in the context of coverage of the coronavirus pandemic (national and international TV, newspapers, websites, University outreach programs)
2020-2021	Successful crowdfunding campaign Wake-up-to-corona, initiated by the Leiden University Fund (over 1 million euro raised for LUMC coronavirus research)
2017-2024	Highly cited researcher (Web of Science) in 2017, 2021, 2022, 2023, and 2024
2015-2020	Steering Committee member & work package leader of ZAPI - Zoonoses Anticipation and Preparedness Initiative; IMI EFPI & European Commission (FP7)
2015-2019	Steering Committee member & Deputy Coordinator ANTIVIRALS; Marie Curie European Training Network on Antiviral Drug Development; European Commission (H2020)
2013	Co-organizer, Xth International Symposium on Positive Strand RNA viruses, Boston, Ma, U.S.A.; organized by Keystone Symposia
2010-2015	Steering Committee & work package leader SILVER; Small-molecule inhibitors against neglected and emerging RNA viruses; European Commission (FP7)
2010-2015	Steering Committee & Deputy Coordinator EUVIRNA; Marie Curie European Training Network on (+)RNA Virus Replication and Antiviral Drug Development; Eur. Comm. (FP7)

2008-present	Editorial Board Member Journal of Virology
2004-2008	Coordinator SARS-DTV; Research action to support SARS-related diagnostic tests, therapeutic interventions and vaccine development. European Commission (FP6)
2004-2008	Editor Journal of General Virology
2003/08/10	TOP grant recipient from Netherlands Organization for Scientific Research (NWO)
2003	Organizer IXth International Symposium on Nidoviruses, The Netherlands
2000	LUMC Price for Biomedical Research
1997-2015	Group leader Council for Chemical Sciences, Netherlands Organization for Scientific Research
1995-1999	Editorial Board Member, Journal of General Virology

- Long-term member (>25 years) of American Society for Virology, Netherlands Society of Medical Microbiology, American Society of Microbiology, Society for General Microbiology UK
- Frequent reviewer for all leading virology journals, Nature, Nucleic Acids Res., PNAS USA, and others.
- Frequent grant reviewers for national (NWO) and foreign/European funding agencies

C. Contribution to Science and Publications (236 in peer-reviewed journals included in PubMed):

My team studies the molecular biology and evolution of +RNA viruses and has become one of the leading groups in this field in Europe. Our research has made significant contributions to understanding RNA synthesis, non-structural protein function, and virus–host interactions in +RNA viruses in general, with a particular focus on nidoviruses (coronaviruses and arteriviruses). Our position in the field was further solidified following the emergence of three zoonotic coronaviruses associated with high fatality rates in humans: the SARS outbreak in 2003, the discovery of MERS-CoV in 2012, and the COVID-19 pandemic caused by SARS-CoV-2 (2020–2022).

We have also been at the forefront of developing reverse genetics systems and viral vectors for multiple +RNA virus families. In addition, we operate a certified BSL-3 laboratory for research on human pathogenic viruses—including SARS-CoV, MERS-CoV, SARS-CoV-2, chikungunya virus, and Zika virus—which serves as a key infrastructure in numerous national and international collaborations. Several of our current research themes and recent discoveries have broad relevance across the field of +RNA virology, such as the study of viral replication organelles and antiviral strategies that target host rather than viral functions. Some topics even extend beyond virology, including innate immune responses, viral immune evasion, and non-canonical translation mechanisms. These are often explored in collaboration with leading international research groups. To date, I have published 236 peer-reviewed articles in journals indexed in PubMed (h-index 86, Web of Science). For a full list of peer-reviewed publications by Eric J. Snijder, see https://pubmed.ncbi.nlm.nih.gov/?term=Snijder+EJ&sort=date.

Topic 1: Molecular biology and evolution of +RNA viruses

+RNA viruses, the largest group of RNA viruses, are important pathogens of both humans and animals. Their molecular biology is marked by several unconventional features, including an RNA genome that functions directly as mRNA for the synthesis of viral replicative enzymes, and the often rapid and extensive replication of this genome in the host cell cytoplasm. Due to the low fidelity of their RNA-dependent RNA polymerase, +RNA viruses display high genetic variability and rapid evolution. This enables them to adapt quickly and, for instance, to emerge as novel human pathogens. Notable examples of (re)emerging +RNA viruses with major impacts on human health and society include MERS-CoV, SARS-CoV, and SARS-CoV-2 (coronaviruses), chikungunya virus (an alphavirus), and Zika virus (a flavivirus). Our research focuses on the in-depth molecular characterization and evolutionary dynamics of these +RNA viruses.

Some key publications:

1. Soultsioti M, de Jong AWM, Blomberg N, Tas A, Giera M, **Snijder EJ**, Bárcena M.J Perturbation of de novo lipogenesis hinders MERS-CoV assembly and release, but not the biogenesis of viral replication organelles. J Virol. 2025 Mar 18;99(3):e0228224. *doi: 10.1128/jvi.02282-24*.
2. Ogando NS, Zevenhoven-Dobbe JC, van der Meer Y, Bredenbeek PJ, Posthuma CC, **Snijder EJ**. The Enzymatic Activity of the nsp14 Exoribonuclease Is Critical for Replication of MERS-CoV and SARS-CoV-2. J Virol. 2020 Nov 9;94(23):e01246-20. *doi: 10.1128/JVI.01246-20.*

3. Li Y, Treffers EE, Napthine S, Tas A, Zhu L, Sun Z, Bell S, Mark BL, van Veelen PA, van Hemert MJ, Firth AE, Brierley I, **Snijder EJ**, Fang Y. Transactivation of programmed ribosomal frameshifting by a viral protein. Proc Natl Acad Sci USA. 2014 May 27;111(21):E2172-81. *doi: 10.1073/pnas.1321930111*
4. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbalenya AE, **Snijder EJ**, Fouchier RA. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. mBio. 2012 Nov 20;3(6). pii: e00473-12. *doi: 10.1128/mBio.00473-12.*

Topic 2: Functional characterization of nidovirus/coronavirus enzymes

+RNA virus replication relies directly on a set of virus-encoded enzymes that mediate genome replication and gene expression. These enzymes, which assemble into a multi-enzyme replication complex, include RNA-dependent RNA polymerases, helicases, capping enzymes, and proteases—all of which represent major targets for antiviral drug development. Coronaviruses are unique among RNA viruses due to their exceptionally large genome, ranging from 27 to 34 kilobases. This genome encodes an unusually large replicase/transcriptase polyprotein of approximately 7,000 amino acids, which is autoproteolytically processed into at least 16 mature nonstructural proteins (nsp1–16). In addition to the canonical replicative enzymes, coronaviruses encode several accessory functions that are largely unique to this virus family and its close relatives. Many of these nonstructural proteins have now been implicated in RNA synthesis or in specific virus–host interactions, offering further promising targets for antiviral intervention.

Some key publications:

1. Malone B, Urakova N, **Snijder EJ**, Campbell EA. Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design. Nat Rev Mol Cell Biol. 2022 Jan;23(1):21-39. *doi: 10.1038/s41580-021-00432-z*.
2. Ogando NS, El Kazzi P, Zevenhoven-Dobbe JC, Bontes BW, Decombe A, Posthuma CC, Thiel V, Canard B, Ferron F, Decroly E, **Snijder EJ**. Structure-function analysis of the nsp14 N7-guanine methyltransferase reveals an essential role in Betacoronavirus replication. Proc Natl Acad Sci USA. 2021 Dec 7;118(49):e2108709118. *doi: 10.1073/pnas.2108709118.*
3. Lehmann KC, Gulyaeva A, Zevenhoven-Dobbe JC, Janssen GM, Ruben M, Overkleeft HS, van Veelen PA, Samborskiy DV, Kravchenko AA, Leontovich AM, Sidorov IA, **Snijder EJ**, Posthuma CC, Gorbalenya AE. Discovery of an essential nucleotidylating activity associated with a newly delineated conserved domain in the RNA polymerase-containing protein of all nidoviruses. Nucleic Acids Res. 2015 Sep 30;43(17):8416-34. *doi: 10.1093/nar/gkv838.*
4. **Snijder EJ**, Bredenbeek PJ, Dobbe JC, Thiel V, Ziebuhr J, Poon LL, Guan Y, Rozanov M, Spaan WJ, Gorbalenya AE. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol Biol. 2003 Aug 29;331(5):991-1004. *doi: 10.1016/S0022-2836(03)00865-9*.

Topic 3: Ultrastructure and function of +RNA virus replication complexes

Viruses depend on the host cell's infrastructure and metabolism at every stage of their replication cycle. A now widely recognized hallmark of animal +RNA viruses is the close association of their RNA-synthesizing machinery with virus-induced membrane structures. Through the action of transmembrane subunits within the viral replication complex, host membranes are remodeled into novel structures that serve as specialized platforms for viral RNA synthesis. This conserved feature offers a promising target for the development of broad-spectrum antiviral therapies. In cells infected with the +RNA viruses that we study—such as SARS-CoV—viral replication is supported by an extensive network of modified host membranes. These include unique double-membrane vesicles (DMVs), believed to originate from the endoplasmic reticulum, carrying oligomeric pore complexes that span their double membrane and are believed to serve as export channel for newly made viral RNA, which needs to be translocated to the cytosol for translation and packaging. Using an integrated approach that combines biochemistry, structural and molecular biology, and advanced electron microscopy, we aim to dissect the architecture and function of these "headquarters of viral replication." This research will deepen our understanding of the early stages of +RNA virus infection and will provide essential knowledge for the long-term development of strategies to block +RNA virus replication at its earliest and most vulnerable stages.

Some key publications:

1. Wolff G, Limpens RWAL, Zevenhoven-Dobbe JC, Laugks U, Zheng S, de Jong AWM, Koning RI, Agard DA, Grünewald K, Koster AJ, **Snijder EJ**, Bárcena M. A molecular pore spans the double membrane of the coronavirus replication organelle. Science. 2020 Sep 11;369(6509):1395-1398. *doi: 10.1126/science.abd3629.*

2. **Snijder EJ**, Limpens RWAL, de Wilde AH, de Jong AWM, Zevenhoven-Dobbe JC, Maier HJ, Faas FFGA, Koster AJ, Bárcena M.A unifying structural and functional model of the coronavirus replication organelle: Tracking down RNA synthesis. PLoS Biol. 2020 Jun 8;18(6):e3000715. *doi: 10.1371/journal.pbio.3000715.*
3. Knoops K, Bárcena M, Limpens RW, Koster AJ, Mommaas AM, **Snijder EJ**. Ultrastructural characterization of arterivirus replication structures: reshaping the endoplasmic reticulum to accommodate viral RNA synthesis. J Virol. 2012 Mar;86(5):2474-87. *doi: 10.1128/JVI.06677-11*.
4. Knoops K, Kikkert M, Worm SH, Zevenhoven-Dobbe JC, van der Meer Y, Koster AJ, Mommaas AM, **Snijder EJ**. SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol. 2008 Sep 16;6(9):e226. *doi: 10.1371/journal.pbio.0060226*.

Topic 4: Antiviral drug discovery

For most virus families—including coronaviruses—approved antiviral drugs remain scarce. However, the success stories of herpesviruses, HIV, and hepatitis B and C viruses demonstrate that decades of fundamental research into viral replication mechanisms and protein functions can ultimately lead to major therapeutic breakthroughs. The development and clinical implementation of antiviral drugs is inherently complex and requires sustained, multidisciplinary collaboration. This process typically begins with the identification of suitable viral or host targets, followed by high-throughput screening and cell-based assays to identify candidate compounds and elucidate their mechanisms of action. Our research, in collaboration with partners across several European consortia, focuses on the discovery of antiviral agents against emerging and neglected RNA viruses—a particularly challenging task due to their high mutation rates and propensity to develop resistance. We specifically target coronaviruses, alphaviruses, and flaviviruses. For these pathogens, we have developed a comprehensive molecular biology toolkit that enables in-depth analysis of their replication cycles and facilitates the identification and mechanistic characterization of antiviral hits. Recent advances—such as structure-guided drug design, high-resolution imaging of replication complexes, and the exploration of host-directed antiviral strategies—have further expanded our ability to identify novel therapeutic targets and counteract viral escape mechanisms. Our work contributes to the broader goal of developing broad-spectrum antivirals capable of responding swiftly to current and future viral threats.

Some key publications:

1. Zhang L, Lin D, Kusov Y, Nian Y, Ma Q, Wang J, von Brunn A, Leyssen P, Lanko K, Neyts J, de Wilde A, **Snijder EJ**, Liu H, Hilgenfeld R.J α-Ketoamides as Broad-Spectrum Inhibitors of Coronavirus and Enterovirus Replication: Structure-Based Design, Synthesis, and Activity Assessment. Med Chem. 2020 May 14;63(9):4562-4578. *doi: 10.1021/acs.jmedchem.9b01828.*
2. Laporte M, Jochmans D, Bardiot D, Desmarets L, Debski-Antoniak OJ, Mizzon G, Abdelnabi R, Leyssen P, Chiu W, Zhang Z, Nomura N, Boland S, Ohto U, Stahl Y, Wuyts J, De Jonghe S, Stevaert A, van Hemert MJ, Bontes BW, Wanningen P, Groenewold GJM, Zegar A, Owczarek K, Joshi S, Koukni M, Arzel P, Klaassen H, Vanherck JC, Vandecaetsbeek I, Cremers N, Donckers K, Francken T, Van Buyten T, Rymenants J, Schepers J, Pyrc K, Hilgenfeld R, Dubuisson J, Bosch BJ, Van Kuppeveld F, Eydoux C, Decroly E, Canard B, Naesens L, Weynand B, **Snijder EJ**, Belouzard S, Shimizu T, Bartenschlager R, Hurdiss DL, Marchand A, Chaltin P, Neyts J. A coronavirus assembly inhibitor that targets the viral membrane protein. Nature. 2025 Apr;640(8058):514-523. *doi: 10.1038/s41586-025-08773-x.*
3. Van Damme E, Abeywickrema P, Yin Y, Xie J, Jacobs S, Mann MK, Doijen J, Miller R, Piassek M, Marsili S, Subramanian M, Gottlieb L, Abdelnabi R, Van Gool M, Van den Broeck N, De Pauw I, Diels A, Vermeulen P, Temmerman K, Scobey T, Mattocks M, Schäfer A, Jochmans D, De Jonghe S, Leyssen P, Chiu W, Diosa Toro M, Zwaagstra M, Leijs AA, De Gruyter HLM, Buyck C, Van Den Heede K, Jacobs F, Van den Eynde C, Thijs L, Raeymaekers V, Miller S, Del Rosario A, Neyts J, Peeters D, Baric RS, van Kuppeveld FJM, **Snijder EJ**, van Hemert MJ, Monshouwer M, Sharma S, Draghia-Akli R, Koul A, Van Loock M. A small-molecule SARS-CoV-2 inhibitor targeting the membrane protein. Nature. 2025 Apr;640(8058):506-513. *doi: 10.1038/s41586-025-08651-6.*
4. de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, Nieuwkoop S, Bestebroer TM, van den Hoogen BG, Neyts J, **Snijder EJ**. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob Agents Chemother. 2014 aug;58(8):4875-84. *doi: 10.1128/AAC.03011-14*.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Small, Gabriel

eRA COMMONS USER NAME (credential, e.g., agency login): GABESMALL

POSITION TITLE: Graduate Fellow

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
Washington University, St. Louis	AB	05/2020	Biochemistry and Molecular Biology
Rockefeller University, New York	PhD	09/2025 (Expected)	Molecular Biophysics

A. Personal Statement

I am a doctoral student with a background in the structural biology of viral proteins. Previously, as an undergraduate, I was able to quickly adapt to the laboratory environment and used X-ray crystallography to obtain a high-resolution structure of an Ebola protein in complex with a human derived peptide. In my graduate studies I have had the opportunity to gain significant expertise in single particle cryo-electron microscopy (cryo-EM) from sample preparation, through data processing, and into model building and analysis. I have produced three published cryo-EM structures of the SARS-CoV-2 replication-transcription complex from start to finish as well as an additional published cryo-EM structure that was reprocessed and built from already published data. My interests continue to lie in determining the structures of viral replication machinery to inform on viral biology and accelerate drug discovery efforts that target these complexes.

B. Positions, Scientific Appointments, and Honors

2022 – 2025	National Science Foundation Graduate Research Fellowship
2024	37th International Conference on Antiviral Research 3rd Place Poster Award
2024	37th International Conference on Antiviral Research Travel Award
2021 – 2022	Genetics and Cell Biology NIH predoctoral training grant
2020	American Society for Virology student travel award (meeting cancelled due to COVID-19)
2019	Rockefeller University Summer Undergraduate Research Fellowship
2018	Washington University – HHMI Summer Undergraduate Research Fellowship
2017	Washington University Biology Summer Undergraduate Research Fellowship

C. Contributions to Science

I began my research career as an undergraduate studying the interaction between an Ebola viral transcription regulator, VP30, and a human ubiquitin ligase RBBP6. The interaction was identified as a high confidence hit in a protein-protein interaction (PPI) map generated by affinity-purification mass spectrometry. After validation of the PPI from a Co-IP, I, along with my mentor, designed a series of truncation mutants of RBBP6 and VP30 and performed a series of binding assays to determine the binding sites of the interaction. I identified a 23-residue minimal binding peptide of RBBP6 that binds to the C terminus of VP30 with nanomolar affinity and whose sequence resembles that of the Ebola nucleoprotein (NP) which also binds to the C terminus of VP30 in a critical interaction that regulates viral transcription. We next characterized the

thermodynamics of the binding interaction using isothermal titration calorimetry and crystallized VP30 in complex with the RBBP6 peptide and solved the structure.

Beyond just a sequence similarity, the binding mode, including a non-canonical polyproline secondary structure, of the RBBP6 peptide closely mimicked the NP's interaction with VP30. Both peptides, from RBBP6 and NP, contain the core PPxPxY motif that mediates binding and utilize a competitive binding assay, we demonstrated that RBBP6 can outcompete the NP for binding to a C terminal cleft on VP30. These findings were then validated by collaborators in a live cell infection system revealing an inhibitory effect of the RBBP6 peptide on Ebola virus replication, showcasing the therapeutic potential of PPI interfaces and peptide mimics. A further analysis of the PPI map generated for VP30 identified other interactions with proteins containing the PPxPxY motif. To further probe the interaction, I performed a series of binding assays on mutants of the RBBP6 peptide to determine the importance of different residues in the interaction and identifying additional residues that confer the superior binding affinity of RBBP6 that NP lacks. Showing that a primary sequence containing the PPxPxY motif is not sufficient for binding and that there are requisite structural elements needed for VP30 to recognize a proline rich motif and allow for modulation of viral transcription.

During my graduate studies I have been involved in several studies of coronavirus replicase proteins, with my primary focus regarding the SARS-CoV-2 NiRAN domain. The NiRAN domain has been identified as an essential enzyme conserved across the order Nidovirales. In coronaviruses it is encoded in the same protein, nsp12, as the RNA polymerase and thus is a constitutive member of the replication-transcription complex (RTC) formed by the polymerase with its essential cofactors, nsp7 and nsp8, as well as a duplex RNA scaffold. The NiRAN domain has been implicated in vitro in catalyzing three distinctive reactions involving the modification of a separate coronaviral protein, nsp9, that contains no analog in other Nidoviruses. These include 1) NMPylation: the formation of a phosphoramidate bond between a nucleoside monophosphate and the N-terminal amine of nsp9, 2) RNAylation: the formation of a phosphoramidate bond between a 5' monophosphorylated RNA and the N-terminal amine of nsp9, and 3) mRNA capping: the transfer of the 5' monophosphorylated RNA to a GDP moiety, restoring the nsp9 N-terminus and forming the core GpppA cap structure on the 5' end of the RNA. While RNAylation and mRNA capping have clear roles in viral replication, NMPylation's role is unclear and has been suggested to involve protein priming or improving nsp9 stability.

After determining the preferred substrates for these reactions, we proceeded to structural analysis with cryo-electron microscopy (cryo-EM). I trapped the RTC in complex with nsp9 and a UTP analog containing a non-hydrolyzable α-β-phosphate bond as well as in complex with a poorly reactive GDP analog and RNA-nsp9 and analyzed both complexes with cryo-EM. I determined high-resolution structures of these complexes, allowing for the elucidation of the chemical mechanism of NMPylation and mRNA capping and identifying a novel nucleotide binding mode. Previously, two distinctive nucleotide poses had been observed 1) 'base in': guanosine specific and buried deep in the active site, this was predicted to be relevant to mRNA capping and confirmed by my structure and 2) 'base out': a phosphate mediated non-specific pose with no biological relevance. Through my structural efforts I discovered a new pose, 'base up', that was nucleobase promiscuous and positioned the α-phosphate for the formation of a phosphoramidate bond. This new nucleotide pose was crucial in understanding the promiscuity of NMPylation and the positioning of the phosphoramidate in mRNA capping. Jointly, these structures allowed me to identify how the NiRAN domain positions its substrates and informs on the basic principles of how the NiRAN domain functions, how it choreographs multiple distinct activities, and provides scaffolding for any structure-based platform for therapeutic development against this essential enzyme found in all coronaviruses.

1. **Small, G.I.**, Darst, S.A., Campbell, E.A., 2025 The mechanism for GTP-mediated RNA capping by the SARS-CoV-2 NiRAN domain remains unresolved. *Cell.* Available online and in press for August 7th, 2025. https://doi.org/10.1016/j.cell.2025.05.044
2. **Small, G.I.**, Fedorova, O., Olinares, P.D.B., Chandanani, J., Banerjee, A., Choi, Y.J., Molina, H., Chait, B.T., Darst, S.A. and Campbell, E.A., 2023. Structural and functional insights into the enzymatic plasticity of the SARS-CoV-2 NiRAN Domain. *Molecular Cell*, 83(21), pp.3921-3930. https://doi.org/10.1016/j.molcel.2023.10.001
3. Grimes, S.L., Choi, Y.J., Banerjee, A., **Small, G.**, Anderson-Daniels, J., Gribble, J., Pruijssers, A.J., Agostini, M.L., Abu-Shmais, A., Lu, X., Darst, S.A., Campbell, E., and Denison, M.R. (2023). A mutation in the coronavirus nsp13-helicase impairs enzymatic activity and confers partial remdesivir resistance. *Mbio*, pp.e01060-23. https://doi.org/10.1128/mbio.01060-23
4. Piscotta, F.J., Hoffmann, H.H., Choi, Y.J., **Small, G.I.**, Ashbrook, A.W., Koirala, B., Campbell, E.A., Darst, S.A., Rice, C.M. and Brady, S.F. (2021). Metabolites with SARS-CoV-2 inhibitory activity identified from human microbiome commensals. *Msphere*, 6(6), pp.e00711-21. https://doi.org/10.1128/mSphere.00711-21
5. Batra, J., Mori, H., **Small, G. I.**, Anantpadma, M., Shtanko, O., Mishra, N., Zhang, M., Liu, D., Williams, C. G., Biedenkopf, N., Becker, S., Gross, M. L., Leung, D. W., Davey, R. A., Amarasinghe, G. K., Krogan, N. J., & Basler, C. F. (2021). Non-canonical proline-tyrosine interactions with multiple host proteins regulate Ebola virus infection. *The EMBO journal*, 40(18), e105658. https://doi.org/10.15252/embj.2020105658

6. Batra J., Hultquist J.F., Liu D., Shtanko O., VonDollen J., Satkamp L., Jang G.M., Luthra P., Schwarz T.M., **Small G.I.**, Arnett E., Anantpadma M., Reyes A., Leung D.W., Kaake R., Haas P., Schmidt C.B., Schlesinger L.S., LaCount D.J., Davey R.A., Amarasinghe G.K., Basler C.F., & Krogan N.J. (2018). Protein interaction mapping identifies RBBP6 as a negative regulator of Ebola virus replication. *Cell*, 175(7), 1917-1930. https://doi.org/10.1016/j.cell.2018.08.044

BIOGRAPHICAL SKETCH

NAME: Fronik, Stanley Wilhelmus Johannes Hendricus

eRA COMMONS USERNAME (credential, e.g., agency login):

POSITION TITLE: PhD candidate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Applied Sciences Utrecht	BSc	05/2019	Biology and Medical Laboratory Research
Utrecht University	MSc	02/2023	Molecular and Cellular Life Sciences
Leiden University Medical Center	PhD	02/2028 (expected)	Structural biology

A. Personal Statement

I am a second-year PhD candidate in the group of Dr. Montserrat Barcena at Leiden University Medical Center, the Netherlands, with a background in cell biology and structural biology. I started as an undergraduate in the group of Prof. Anna Akhmanova (Utrecht University), learning various fluorescent microscopy and biochemistry techniques to study the interaction between a microtubule-associated protein and clathrin. Subsequently, as a graduate student, I joined the structural biology group of Prof. Friedrich Föster (Utrecht University), to employ cryo-electron tomography to two different types of isolated, cellular organelles. I learned the basics of cellular organelle isolation and was exposed to the essential techniques and principles involved in cryo-electron tomography. Equipped with experience in both cell biology and structural biology, I started in the structural biology group of Prof. Peijun Zhang (Oxford University). There, I utilized advanced fluorescent microscopy, various organelle and virus capsid isolation techniques, cryo-focused ion beam milling and cryo-electron tomography to visualize HIV-1 nuclear import with high throughput. This work made me excited to continue studying virus infections on the ultrastructural level, in particular coronaviruses. Therefore, I started my PhD track in the group of Dr. Montserrat Barcena, aiming to deepen our understanding of coronavirus and arterivirus replication organelles using a combination of structural and functional assays.

B. Positions, Scientific Appointments, and Honors

2024 – present PhD candidate, NWO-funded research project on coronavirus replication organelles
2023 – 2024 Research assistant, STRUBI, University of Oxford
2022 – 2023 Master internship, STRUBI, University of Oxford
2020 – 2021 Voluntary research assistant, SBC, Utrecht University
2019 – 2020 Master internship, SBC, Utrecht University
2018 – 2019 Bachelor internship, Dep. Cell biology, Neurobiology and Biophysics, Utrecht University

C. Contributions to Science

During my Bachelor studies, I investigated the interaction between clathrin heavy chain (CHC) and the microtubule-associated protein MAP7D2, a neuronal protein that specifically localizes in the axon initial segment. I managed to validate the minimal binding regions of both CHC and MAP7D2 using truncations in

pulldown experiments followed by Western blots. Subsequently, I determined the exact binding motif of MAP7D2 using bioinformatics screening and mutagenesis experiments, validated by pulldown experiments. Finally, I characterized the interaction and the effect of mutagenesis using immunofluorescence microscopy assays.

As a master student, I worked on several projects, with one involving endoplasmic reticulum (ER) stress. I found a treatment that induced high levels of UFMylation, an indicator of translational stalling. We investigated this at the ultrastructural level using cryo-electron tomography on isolated, rough ER microsomes. In addition, we applied advanced fluorescence microscopy techniques to visualize ERphagy, the cellular response of ribosome stalling. The initial data proved crucial for another study in the same group about translational reorganization in ribosome collision stress, which was published in Mach 2024.

Secondly, as a master student, I worked on establishing a system for high-throughput, ultrastructural imaging of human immunodeficiency virus type 1 (HIV-1) nuclear import using cryo-ET. HIV-1 nuclear import is a rare event, and thus difficult to capture using cryo-ET. I established a method to improve the number of HIV-1 capsids localizing at the nuclear pore complex (NPC) of individual nuclei, which included the isolation of HIV-1 capsids from virions and the isolation of CD4+ T-cell nuclei. Combined with a correlative fluorescence microscopy approach for cryo-focused ion beam milling, we reached an unprecedented throughput of about 50% for detecting HIV-1 capsids in the process of nuclear import, in contrast to the 2-3% described in literature. This enabled us to do accurate, statistical analyses of this rare event, investigating the dynamic nature of the interaction between the HIV-1 capsid and the NPC. We found that both capsid elasticity and NPC adaptability are essential for nuclear import of HIV-1. A paper describing this work is currently under revision.

Currently, during my PhD track, I am working on several projects, including establishing an affinity-based purification method of coronavirus replication organelles from infected cells. In addition, I am involved in a project to visualize and characterize the replication organelles of the arteriviruses equine arteritis virus (EAV) and porcine reproductive and respiratory syndrome virus (PRRSV) on the ultrastructural level using cryo-ET. In this project, we discovered the presence of RO pore complexes in arterivirus replication organelles. Moreover, for EAV, we determined the minimal components required to form pore complexes to be nsp2 and nsp3. This work highlights the possible conservation and functional relevance of the RO pore complex across the nidovirus order. A paper describing this work is currently in preparation.

1. Fedry, J., Silva, J., Vanevic, M., **Fronik, S**., Mechulam, Y., Schmitt, E., ... & Förster, F. (2024). Visualization of translation reorganization upon persistent ribosome collision stress in mammalian cells. *Molecular cell*, *84*(6), 1078-1089.
2. Hou, Z., Shen, Y., **Fronik, S**., Shen, J., Shi, J., Xu, J., ... & Zhang, P. (2025). Correlative in situ cryo-ET reveals cellular and viral remodeling associated with selective HIV-1 core nuclear import. *bioRxiv*.

BIOGRAPHICAL SKETCH

NAME: de Beijer, Nina Lynn

eRA COMMONS USERNAME (credential, e.g., agency login): N/A (non-US based, not on NIH grant)

POSITION TITLE: PhD candidate

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Leiden University, Leiden, the Netherlands	BSc	06/2019	Biomedical sciences
Leiden University, Leiden, the Netherlands	MSc	12/2021	Biomedical sciences
Leiden University Medical Center, Leiden, the Netherlands	PhD	08/2026 (expected)	Molecular virology

A. Personal Statement

I am a PhD candidate in the final year and my project is focused on the structure and function of the nidovirus replication organelles. This research is embedded in the Molecular Virology group of the Leiden University Center for Infectious Diseases (LUCID) at Leiden University Medical Center (LUMC). The project involves a close collaboration with the electron microscopy group in the same institute.

My scientific journey began with an internship at the Radiology department, where I worked on a project to develop a protocol for the purification of malaria sporozoites from infected mosquitoes without the need for labor-intensive dissection of the mosquito salivary glands. Although a perfect method was not established during that short period, this experience introduced me to diverse laboratory techniques and the art of combining them for a better outcome.

The following year, I had the opportunity to go to Edinburgh for an internship on proteins implicated in Parkinson's disease. We isolated the synaptosome protein fractions from knockout and wild-type mouse brains to identify differences in protein expression. Here I really got the opportunity of experiencing what was possible with a well-established protocol for the purification of a protein fraction of interest and the amount of work that goes into verifying the method.

For my master's thesis, I engineered recombinant murine hepatitis virus, a model virus of the betacoronavirus cluster, by attaching an affinity-tag to nsp3, a non-structural protein essential for the formation of viral replication organelles. To evaluate the effect of these tags on the morphology of the replication organelles, I performed TEM on plastic-embedded samples. This work launched a project aiming to purify the replication organelles from coronavirus-infected cells, in particular the double-membrane vesicles (DMVs) in which viral RNA synthesis occurs. In collaboration with my supervisor, we used cryo-EM to confirm the presence of intact pores spanning the DMV's double membrane in the initial infected cell-derived samples. These pores are assumed to serve as RNA export channels, which may transport newly made viral RNA from the DMV's interior to the cytosol. This early work really inspired me and fueled my enthusiasm to continue my research in the field.

Currently, I am working on several projects focused on elucidating the structure of putative RNA export pore and its influence on the function of the nidovirus replication organelles. While my expertise lies in molecular virology, my research increasingly integrates structural biology, supported by collaborations with the in-house EM group and the labs of Elizabeth Campbell and Seth Darst at Rockefeller university.

This interdisciplinary environment, combining molecular virology with cutting-edge structural biology, motivates me to further our understanding of viral replication organelles.

B. Positions, Scientific Appointments, and Honors

2025	travel grant from the Leiden University fund to travel to the American Society for Virology meeting
2022-present	PhD candidate at the Leiden University Center of Infectious Diseases, LUMC, Leiden, the Netherlands
2021	Master internship at the Department of Cell and Chemical Biology, LUMC, Leiden, the Netherlands
2020	Master internship at the Center for Discovery Brain Sciences, University of Edinburgh, Scotland
2019	undergraduate internship at the Radiology Department, LUMC, Leiden, the Netherlands

C. Contributions to Science

During my PhD track, I have primarily focused on studying the replication organelles of nidoviruses, and had the opportunity to write an invited scientific review highlighting the remarkable similarities among replication organelles formed by various positive-stranded RNA viruses. Despite the morphological diversity and distinct membrane origins of these organelles, cryo-EM studies have revealed crown-shaped multimeric pore complexes on replication organelles of several well-studied viruses. These complexes are believed to regulate the export of newly-synthesized RNA from the interior of the replication organelles into the cytoplasm. In some viruses, there is evidence to suggest that these protein complexes actively participate in viral RNA synthesis. For coronaviruses, a high-resolution structure of pore complexes from DMVs purified from an expression system was only recently published. My ongoing research projects aim to elucidate how the nidovirus pore complex regulates RNA export and whether it plays a direct role during the RNA replication.

In addition, I have gained experience in a wide range of molecular virology techniques and fluorescence microscopy. For example, I contributed provided microscopy images and quantitative image analysis for a fellow PhD candidate's project investigating the host-cell interaction partners of Usutu virus (Orthoflavivirus) non-structural protein 4A. A key finding was the induction of the autophagy pathway by the virus. I provided microscopy images and quantified LC3 puncta in infection and by each of the non-structural proteins individually. This work supported the conclusion that Usutu virus indeed induces autophagy and identified NS4A as an important contributor to this process.

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