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
Stanford University, Stanford, CA	Postdocoral	06/1987 – 09/1993	Structural Biology

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 (1, 2), and used electron microscopy methods to determine the first low-resolution structures of RNA polymerases (RNAPs). 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. 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 now 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 (3).

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 we 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 (4). 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 (the subject of this grant proposal).

Since establishing my laboratory at The Rockefeller University 28 years ago, I have mentored 16 Postdocs (currently in the following positions: 1 M.D., 10 Profs., 13 in academics, 2 in industry) and 7 Graduate Students (currently: 1 M.D., 2 Profs., 5 in academics, 1 in industry, 1 practicing lawyer, 2 school teachers). 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; http://cbm.msoe.edu/smartTeams/), 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**, Ribi HO, Pierce DW, Kornberg RD (1988) Two-dimensional Crystals of *Escherichia coli* RNA Polymerase Holoenzyme on Positively Charged Lipid Layers, *J. Mol. Biol.* **203**, 269-273.
2. Edwards AM, **Darst SA**, Feaver WJ, Thompson NE, Burgess RR, Kornberg RD (1990) Purification and Lipid Layer Crystallization of Yeast RNA Polymerase II, *Proc. Natl. Acad. Sci. USA* **87**, 2122-2126.
3. 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].
4. 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*. DOI:https://doi.org/10.1016/j.cell.2020.07.033 [PMCID: in process].

B. Positions and Honors

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

Honors

Undergraduate

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

Postdoctoral

1987-1990	American Cancer Society Postdoctoral Fellow

Postdoctoral & PI

1990-1996 Lucille P. Markey Award in Biomedical Science

PI

1994-1999 Career Scientist of the Irma T. Hirschl Charitable Trust
1995-1999 Pew Scholar in the Biomedical Sciences
2008 American Academy of Microbiology Fellow
National Academy of Sciences Fellow

Service (selected)

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

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

C. Contribution to Science

1. As a postdoc (a, b) and then as an independent investigator (c), 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 determined the first X-ray structure in 1999, but we continued to use electron microscopy to examine RNAP complexes with accessory factors to gain mechanistic understanding (d).

a. **Darst SA**, Kubalek EW, Kornberg RD (1989) Three-dimensional Structure of *Escherichia coli* RNA Polymerase Holoenzyme Determined by Electron Crystallography, *Nature* **340**, 730-732.
b. **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.
c. Polyakov A, Severinova E, **Darst SA** (1995) Three-dimensional structure of *Escherichia coli* core RNA polymerase: Promoter recognition and elongation conformations of the enzyme, *Cell*, **83**, 365-373.
d. Opalka N, Chlenov M, Chacon P, Rice WJ, Wriggers W, **Darst SA** (2003) Structure and function of the transcription elongation factor GreB bound to bacterial RNA polymerase, *Cell* **114**, 335-345.

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. We revolutionized the transcription field 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].

4. 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. 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].

5. 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].

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

D. Research Support

Active

R35 GM118130 Seth A. Darst (PI) 5/1/21 - 4/30/26 NIH/NIGMS

Structure, function, and regulation of the bacterial transcription cycle

We will use cryo-electron microscopy, in combination with X-ray crystallography and other approaches, to provide a complete characterization of the bacterial transcription cycle.

Role: PI

Completed

None

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: CAMPBELL, ELIZABETH

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

POSITION TITLE: Research Associate Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
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	Post-doctoral	07/2003	Structural Biology

A. Personal Statement

PhD – Natural transformation in *Streptococcus pneumoniae*: I became interested in studying bacterial pathogens during undergraduate research at Stanford University with Dr. Bruce Stocker, whose work with Salmonella led to the development of vaccines to prevent typhoid. The following year, I worked on purifying macromolecular complexes as a summer research student in Dr. Roger Kornberg's lab, also at Stanford. There, I discovered the power of structural biology as a method for elucidating biochemical mechanisms. As a PhD student at The Rockefeller University, I was trained in the laboratory of Molecular Genetics of Pathogenesis, headed by Drs. Elaine Tuomonen and Robert Masure. I learned how to genetically manipulate the pathogen *Streptococcus pneumonia*e by modifying the then current protocols of molecular biology *for Escherichia coli* and developing genetic screens to identify genes in *S. pneumoniae* required for competence (DNA transformation), a mechanism this pathogen uses to acquire drug resistance.

Postdoctoral studies – Structural and biophysical training in bacterial transcription: My principal research as a post-doctoral fellow and research associate used a structure-based approach to understand the diverse features of regulators that mediate the basic and pathogenic mechanisms of transcription in bacteria. To understand the molecular mechanisms of regulators, I determined three-dimensional structures of transcription factors and inhibitors, and their complexes with RNA polymerase (RNAP). My structural training was supplemented with biochemical, genetic and *in vivo* techniques, often in highly productive collaborations with leaders in the bacterial transcription field, to gain comprehensive understanding of the molecular mechanisms that modulate transcription. My work led to the first structures of bacterial σ factors and the proteins that regulate them (anti-σ factors) from several different bacteria, including pathogens such as *Bacillus* sp., *Mycobacterium* sp. and *Enterobacter* sp. In collaborative studies, I defined the structural basis for rifampicin inhibition of bacterial RNAP, and for other RNAP inhibitors (e.g., sorangicin) relevant to drug design against tuberculosis and other infectious diseases (see contributions). It was during these studies that I realized the potential of applying the structural and biophysical techniques I learned in Dr. Darst's lab towards understanding transcription in pathogens. I applied to be a Senior Research Associate with the understanding that I will employ my training towards directing projects aimed at understanding transcription in mycobacteria.

Current - Structure/function studies of transcription in *Mycobacterium tuberculosis* and *Clostridioides difficile* with the goal of providing new therapeutics against tuberculosis: Upon promotion to Senior Research Associate and later to Research Associate Professor, I shifted my focus to the structure and function of RNAPs from mycobacteria, and the essential transcription factors that regulate this enzyme. My group has found that unlike *E. coli*, where the overwhelming majority of biochemical characterizations have focused, *Mycobacterium sp*. are substantially different at various kinetic steps of initiation and several essential transcription factors (which are absent in *E. coli*) are critical to boost the activity of the mycobacteria enzyme. Our structural and biochemical studies have revealed the mechanisms by which two essential transcription

factors, CarD and RbpA, activate mycobacteria RNAP. It is our recent success in achieving the first structures of mycobacteria RNAP at high resolution has facilitated our focus on characterizing the mechanisms of inhibitors with the goal to provide therapeutics to treat the disease tuberculosis. One of the significant outcomes of our research has led to the first X-ray crystal and cryo-EM structures of mycobacteria RNAP, positioning us for the proposed research (see contributions). In summary, our structural and biochemical advances encourage future structure/function studies with the goal to dissect the general mechanism of transcription in pathogenic bacteria such including mycobacteria and *Clostridiodes difficile*. We have embarked on these studies in collaboration with Xinyun Cao in the Landick lab. This work should contribute to new mechanistic insights that could lead to new therapeutics that target the RNAP.

In March 2020, during the COVID-19 pandemic, I realized that much of the same approaches we used for mechanistic studies of the bacteria system could be applied to study viral replication and transcription. Upon reviewing the literature of what was known about the regulation of the SARS-CoV-2 RNA dependent RNAP (RdRp), I realized that it was relatively underexplored compared to what we know about bacterial transcription. Because the university shut down, except for SARS-CoV-2 related research, I began to work on the replication/transcription complex of the SARS-CoV-2, with the goal to structurally and biochemically elucidating higher order complexes involved in different steps of viral transcription and replication. My training in structurally and functionally characterizing antimicrobials also positioned me to work with collaborators that are discovering new potential antivirals. This work proved fruitful as listed in contribution and resulted in a new direction of studies for my group.

Expertise

My expertise in the field of bacterial transcription and pathogenesis is evidenced by the chairing of multiple sessions at FASEB meetings on Mechanisms of Prokaryotic Transcription from 2009 to 2017. I served as co-organizer for the 2017 meeting. I also served as convener and speaker at the ASM microbe 2017 and 2019 meetings in the symposium 'Structural Basis of Antibiotic Mechanism of Action and Resistance' and 'Second messengers, antibiotics, and small molecule regulators of gene expression. I am a repeat invited speaker at the IAS Focused Program on Mechanisms of Transcription and Its Regulation, an invited seminar speaker at The University of Wisconsin-Madison (a hub of bacterial transcription studies), and served on the scientific advisory board on of the European ERASynBio initiative. I have also served as an *ad hoc* reviewer for many peer-reviewed journals and the NIH PCMB panel. More recently, as chair I have initiated a Gordon Research Conference on Microbial Transcription, which was approved and took take place in the summer of 2019 and was highly successful. Finally, my recent (2018) election to the American Academy of Microbiology, my invitation to write two peer-reviewed reviews on transcription initiation in mycobacteria (Transcription) and more recently in bacteria (Nature Reviews in Microbiology), affirms my expertise in the field of bacterial transcription. Lastly, I was invited to be a panelist in the November NIH antiviral Summit on Summit (panel: Targeting Viral Replication Machinery). In addition to my research expertise, I have enthusiastically and successfully mentored over 30 high school and undergraduate students, nine graduate students, four post-doctoral fellows, and eight research scientists.

1. 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.
2. Hubin EA, Lilic M, Darst SA, **Campbell EA**. Structural insights into the mycobacteria transcription initiation complex from analysis of X-ray crystal structures. *Nature Comm.* 2017 Jul13;8:16072. PMCID:PMC5511352.
3. Peek J, Lilic M, Montiel D, Mishteyn A, Woodworth I, Biggins JB, Ternei MA, Calle P, Danziger M, Warrier T, Saito K, Braffman N, Fay A, Glickman MS, Darst SA, **Campbell EA***, Brady SF*. (*Co-corresponding authors). Rifamycin congeners kanglemycins are active against rifampicin-resistant bacteria via a distinct mechanism. *Nature Comm.* 2018 Oct8; 9:4147. PMCID:PMC6175910.
4. Chen J, Boyaci H, **Campbell EA**. Diverse and unified mechanisms of transcription initiation in bacteria. *Nat Rev Microbiol*. 2020 Oct 29; doi: 10.1038/s41579-020-00450-2. [Epub ahead of print] Review. PubMed PMID: 33122819.

B. Positions and Honors

Positions and Employment

2018 - current 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 Experience and Professional Memberships

2020	Panelist, NIH Antiviral Summit (Targeting Viral Replication Machinery)
2017-2019	Chair, Gordon Research Conference on the Mechanism and Regulation of Microbial Transcription
Ongoing-	Contributing member, American Society of Microbiology
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.
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 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 Conference on the Mechanism and Regulation of Prokaryotic Transcription
2016-2020	Invited speaker at the IAS Focused Program on Mechanisms of Transcription in HKST
2017, 2019	Convener and speaker at Symposium entitled Structural Basis of Antibiotic Mechanism of Action and Resistance, American Society of Microbiology, Microbe 2017 Meeting
2017	NIH PCMB panel reviewer, ad hoc

Honors

1991 - 1992	Hunter Grubb Foundation Scholar, Swarthmore College
1991 - 1992	Pew Minority Undergraduate Fellow, Swarthmore College
1992 - 1992	Distinction in Biology in Graduation, Swarthmore College
1992	Sigma Xi Fellow, Swarthmore College
1992 - 1993	John Kluge Graduate Fellow, The Rockefeller University
1993 - 1996	NSF Minority Graduate Fellow, National Science Foundation
2000 – 2003	Individual National Research Service award, National Institutes of Health
2017	Structural Genomics Consortium research award
2018	Elected to Fellowship in the American Academy of Microbiology
2019	Keystone Symposia Early Career Investigator Travel Award

C. Contributions to Science

Transcription in bacteria is performed by the enzyme RNAP and is highly regulated at initiation. Factors regulating which genes are transcribed in response to specific signals include s factors, subunits of RNAP critical for promoter recognition and melting, as well as other transcription factors such as activators and repressors. RNAP is a proven target for antibiotic therapy against pathogens, most notably *Mycobacterium tuberculosis*, the causative agent of tuberculosis. My path in science started as a graduate student studying genetic mechanisms of how pathogens acquire antibiotic resistance. I then switched disciplines to learn biochemistry and structural

biology and to apply these approaches to understanding basic mechanisms in transcription, including the basis of antibiotic inhibition of RNAP. The culmination of this training has now allowed me to apply these fundamental disciplines of structural biology, biophysics and biochemistry toward understanding the mechanisms of current and novel antibiotics against mycobacteria RNAP with the goal to contribute to the development of new therapeutics against tuberculosis.

1. When I joined the Darst lab as a postdoctoral fellow, the understanding of the structural mechanisms of σ factor function was limited due to the lack of structures. Using my previous training in microbial genetics, I was able to identify, purify and crystallize the σ factor from the thermophilic bacterium, *Thermus aquaticus*, leading to the first structures of discrete σ domains, including bound to substrate promoter DNA and RNAP. This work led to the understanding of how σ /promoter recognition occurs. I served as experimental investigator, first author and a contributing author on this work.
   a. Murakami KS, Masuda S, **Campbell EA**, Muzzin O, Darst SA. Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex. *Science*. 2002 May 17;296(5571):1285-90. PubMed PMID: 12016307.
   b. **Campbell EA**, Muzzin O, Chlenov M, Sun JL, Olson CA, Weinman O, Trester-Zedlitz ML, Darst SA. Structure of the bacterial RNA polymerase promoter specificity sigma subunit. *Molecular Cell*. 2002 Mar;9(3):527-39. PubMed PMID: 11955433.
2. An additional step of regulation in bacterial transcription is mediated by a class of factors (anti-σ's) which negatively regulate σ factors. As a postdoctoral fellow, I elucidated their mechanism of inhibition by solving the first structures of anti-σ's in complex with their cognate σ factors. I showed that anti-σ's function in a variety of ways, including preventing the σ factor from binding RNAP and binding promoter DNA. This work led to the definition of a conserved anti-σ structural domain in all bacteria, revealing the mechanism of inhibition within a wide spectrum of bacterial σ/anti-σ pairs. I served as the primary experimental investigator/first author and principal investigator on these projects.
   a. **Campbell EA**, Masuda S, Sun JL, Muzzin O, Olson CA, Wang S, Darst SA. Crystal structure of the Bacillus stearothermophilus anti-sigma factor SpoIIAB with the sporulation sigma factor sigmaF. *Cell*. 2002 Mar 22;108(6):795-807. PubMed PMID: 11955433.
   b. **Campbell EA**, Tupy JL, Gruber TM, Wang S, Sharp MM, Gross CA, Darst SA. Crystal structure of Escherichia coli sigmaE with the cytoplasmic domain of its anti-sigma RseA. *Molecular Cell*. 2003 Apr;11(4):1067-78. PubMed PMID: 12718891.
   c. Masuda S, Murakami KS, Wang S, Anders Olson C, Donigian J, Leon F, Darst SA, **Campbell EA**. Crystal structures of the ADP and ATP bound forms of the Bacillus anti-sigma factor SpoIIAB in complex with the anti-anti-sigma SpoIIAA. *Journal of Molecular Biology*. 2004 Jul 23;340(5):941-56. PubMed PMID: 15236958.
   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, *Molecular Cell*. **27**, 793-805. PMCID: PMC2390684.
3. I then decided to apply a structural approach towards understanding inhibitors of transcription. I therefore solved the first structure of RNAP bound to an inhibitor rifampicin (Rif), a current antimicrobial therapeutic for treating tuberculosis, thus elucidating the mechanisms of inhibition as well as understanding the molecular nature of resistance to the medically important antibiotic Rif. An important finding was that Rif kills bacteria by inhibiting transcription via steric clash with the nascent RNA transcript, thus preventing completion of RNA synthesis. I subsequently solved the structure of RNAP bound to another inhibitor, sorangicin, which suggested that sorangicin, despite occupying the Rif pocket, inhibits Rif-resistant RNAP because of its flexible nature, suggesting a desirable feature of future antibiotics. I served as the primary experimental investigator/first author on these projects.
   a. **Campbell EA**, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. *Cell*. 2001 Mar 23;104(6):901-12. PubMed PMID: 11290327.
   b. **Campbell EA**, Pavlova O, Zenkin N, Leon F, Irschik H, Jansen R, Severinov K, Darst SA. Structural, functional, and genetic analysis of sorangicin inhibition of bacterial RNA polymerase. *EMBO* J. 2005 Feb 23;24(4):674-82. PubMed Central PMCID: PMC549610.

4. More recently, as a Senior Research Associate and Research Associate Professor, I have directed an independent research program to characterize the transcriptional properties of RNAP from the pathogen *M. tuberculosis*, the causative agent of tuberculosis. *M. tuberculosis* contains two essential transcription factors not found in *E. coli* and our biochemical and structural studies led to the finding that the paradigm of transcription initiation, previously defined by studies in *E. coli*, does not apply to mycobacteria. In addition, an impediment to drug design and optimization as well as understanding basic transcriptional mechanisms, was the lack of structures of RNAP from "gram-positive" bacteria, including those in the Actinobacteria phylum to which *M. tuberculosis* belongs. Our work has provided the first structure of RNAP from the Actinobacteria clade, *Mycobacterium smegmatis*, in complex with promoter DNA and the initiation factor RbpA. In addition, we compared the kinetics of transcription between *M. tuberculosis* RNAP and *E. coli* and found critical differences in their function. Importantly, recently we applied the unique kinetic properties of *M. tuberculosis* RNAP to capture a promoter melting intermediate revealing the pathway of DNA unwinding. This work is significant for understanding how RNAPs in general work, the specific structural and kinetic properties of RNAP from this pathogen, as well as setting up a platform for studying and devising molecules to target this enzyme. Another of our most recent cryo-EM structures reveals how the Actinobacteria specific transcription factor, RbpA, interacts with the antibiotic Fidaxomicin and confers clade specific sensitivity of this antibiotic to *M. tuberculosis*. This work is especially relevant to the medical community as RNAP is a target for first-line treatment against tuberculosis. Here, I served as the principal investigator and corresponding author for these projects. We have recently renewed our proposal to include similar studies of RNAP from the deadly opportunistic pathogen, *Clostridium difficile*, with a focus on a clinical inhibitor.

   a. 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.

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

   c. 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.

5. This year, in light of the pandemic, I combined efforts with other PIs at the Rockefeller University (Drs. Darst, Chait and Kapoor) to study the replication transcription complex of SARS-CoV-2. In order to identify regulators of this process we used gel shift assays and native mass-spectrometry to show that the helicase nsp13 makes a stable complex with the holo-RdRp. We then proceeded to resolve the structure of this complex, which led to structure-based models of backtracking, template switching and replication. This work was recently published.

   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

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

D. Additional Information: Research Support and/or Scholastic Performance

2R01 GM114450-06 CAMPBELL, ELIZABETH A (PI) 04/01/15-03/31/24NIH/NIGMS
Structural and Functional Characterization of RNA polymerase and its Regulators from *Mycobacterium tuberculosis* and *Clostridioides difficile.*

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: Brandon Malone

eRA COMMONS USER NAME (credential, e.g., agency login): BMALONE31

POSITION TITLE: Graduate student

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 College Cork, Cork, Ireland	BPharm	05/2018	Pharmacy & Pharmacology
The Rockefeller University	Ph.D	Ongoing	Molecular Biophysics

A. Personal Statement

The proposed research project will focus on structurally elucidating key steps of the SARs-Cov-2 RNA dependent RNA polymerase lifecycle. As an early stage graduate student, I am well versed in many of the experimental techniques that I will be employing from protein purification to cryo-EM data processing having conducted similar workflows when studying RNA polymerases from both *E.coli* and *M. tuberculosis* under the supervision of Drs. Darst & Campbell.

B. Positions and Honors

2014-2018 – Undergraduate researcher in UCC Cork Cancer Research Centre (Dr Mark Tangney)
2016-2016 Summer undergraduate researcher at the John Innes Centre, Norwich (Dr Tony Maxwell)
2017-2017 Summer undergraduate researcher – Amgen Scholars program, Institut Pasteur (Dr Ludovic Deriano)
2019- Graduate student, Laboratory of Molecular Biophysics (Seth A. Darst), The Rockefeller University, New York, NY

Honors

2014 "All-Ireland Scholarship"-University scholarship based on High school examination results
2014 Pfizer's National High School Seniors (Leaving Certificate) Chemistry Quiz 1st place
2015-2016 Gold medal winner at IGEM 2015 & iGEM 2016
2016 UCC Blackstone Launchpad Entrepreneur of the Year winner 2016, University College Cork, Ireland
2015-2018 Awarded title of "College Scholar", University College Cork, Ireland

C. Contributions to Science

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 in the Darst-Campbell Laboratory, we felt we could make an impact towards understanding the SARS-CoV-2 replication-transcription complex (RTC). Me, another senior Graduate Student, and a Research Assistant in the laboratory, starting essentially from scratch, in four months we published our manuscript describing the SARS-CoV-2 RTC complexed with the essential nsp13 helicase within four months (1). In this manuscript we made the provocative hypothesis that the nsp13 helicase contributed to RTC backtracking, and a short while later we published another manuscript describing SARS-CoV-2 RTC backtracked complexes (2). My work on the SARS-CoV-2 RTC is continuing with our detailed characterization of the remdesivir mechanism.

1. 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].
2. **Malone B**, Chen J, Llewellyn E, Choi YJ, Olinares PDB, Cao X, Hernandez C, Eng ET, Chait BT, Landick R, Darst SA, Campbell EA (2021) Structural basis for backtracking by the SARS-CoV-2 replication-transcription complex, *Proc Natl Acad Sci USA* **118**, e2102516118 [PMCID: in process].

D. Additional Information: Research Support and/or Scholastic Performance

None.

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	05/2025 (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. While I do not have experience with cryo-EM, I have joined a laboratory with several experts who can guide me through the process and assist in my transition to this structural determination method. My prior work within structural biology has led to a significant amount of exposure to the field, lending me a familiarity with the theory of cryo-EM that will expedite the practical learning process. Additionally, since joining the lab for my graduate studies, I have had the opportunity to learn how to prepare samples and freeze grids, as well as begin to touch on the computational side of screening and assessing the quality of samples. While early in my career, I have demonstrated an ability to succeed in structural biology, and have entered an environment optimized to encourage my growth even further in the pursuit of higher order replicase complexes from SARS-CoV-2.

B. Positions, Scientific Appointments, and Honors

2021 – Present	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 a 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 utilizing 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.

1. 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. PMID: 30550789
2. Batra J., Anantpadma M., **Small G.I**., Shtanko O., Mira M., Liu D., Biedenkopf N., Becker S., Davey R.A., LaCount D.J., Amarasinghe G.K., Krogan N.J., & Basler C.F. Multiple host proteins possessing PPxPxY motifs target Ebola virus VP30 to modulate its phosphorylation and viral gene expression. *BioRXiv*.