

**James Chen**

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**EDUCATION**

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**Doctor of Philosophy in Molecular Biophysics**

The Rockefeller University, New York, NY

*Start Date:* September 3, 2013

*Thesis Defense Date:* March 9, 2020

*Thesis Title:* Cryo-EM Studies of Bacterial RNA Polymerase

*Advisors:* Seth A. Darst, Ph.D. and Elizabeth A. Campbell, Ph.D.

*Degree Conferral Date:* June 11, 2020

**Bachelor of Arts with Distinction in Biophysics**

University of Pennsylvania, Pennsylvania, PA

*Start Date:* September 3, 2009

*Graduation Date:* May 13, 2013

**RESEARCH EXPERIENCE**

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**Postdoctoral Fellow****January 2021 – Present**

Department of Cell Biology, New York University School of Medicine

*Advisors:* Damian Ekiert, Ph.D. and Gira Bhabha, Ph.D.

- Characterized the Mammalian Cell Entry (MCE) systems in mycobacterial species
- Utilized genetic editing tools to modify the genomes of *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*
- Purified an endogenous MCE intermembrane transporter complex and determined structure using single-particle cryo-electron microscopy (cryo-EM)
- Optimized cell-based assays to access phenotypes of MCE knockouts in mycobacteria
- Received Biosafety Level 3 (BSL3) training to work with *Mycobacterium tuberculosis*
- Performed cryo-electron tomography (cryo-ET) to examine the cell wall of *Mycobacterium smegmatis*
- Assisted with reviewing articles from journals such as *Science Advances*
- Techniques: Cloning, mutagenesis, recombineering, immunoblotting, cell-based assays, light microscopy, protein purifications, negative stain EM, cryo-EM, cryo-ET

**Postdoctoral Associate****July 2020 – December 2020**

Laboratory of Molecular Biophysics, The Rockefeller University

*Advisors:* Seth A. Darst, Ph.D. and Elizabeth A. Campbell, Ph.D.

- Purified and biochemically characterized nonstructural proteins involved in SARS-CoV-2 replication and transcription
- Solved structures of SARS-CoV-2 replication and transcription complexes coupled to the nsp13 helicase by cryo-EM

**Graduate Research Fellow****September 2013 – June 2020**

Laboratory of Molecular Biophysics, The Rockefeller University

*Advisors:* Seth A. Darst, Ph.D. and Elizabeth A. Campbell, Ph.D.

- Examined the role of the mycobacterial transcription factor, CarD, on bacterial transcription using crosslinking and mutational approaches
- Optimized single-particle cryo-EM sample preparation conditions and data processing pipelines for bacterial RNA polymerase (RNAP) complexes
- Performed cryo-EM experiments on *Escherichia coli* RNAP complex with the alternative sigma factor  $\sigma^{54}$  and promoter DNA
- Elucidated the structure of *Escherichia coli* RNAP in complex with a non-coding regulatory transcript, 6SRNA, using cryo-EM and biochemical assays
- Solved structures of *Mycobacterium tuberculosis* RNAP bound to various inhibitors
- Utilized cryo-ET to examine RNAP particle orientations and distributions in cryo-EM samples prepared with various salts and detergents
- Biochemically and structurally characterized the allosteric regulation of the F plasmid-encoded TraR protein on transcription initiation
- Structurally captured stages of DNA promoter melting by bacterial RNAP with cryo-EM using TraR as a molecular tool to modulate DNA unwinding for ribosomal promoters
- Purified and characterized SARS-CoV-2 nonstructural proteins involved in viral replication and transcription for subsequent single particle cryo-EM studies
- Involved in collaborations with the Gourse Lab at University of Wisconsin–Madison and the Muir Lab at Princeton University
- Assisted with reviewing articles from journals such as *Nature Communications*, *Nucleic Acids Research*, and *Molecular Microbiology*
- Techniques: Cloning, mutagenesis, protein purifications, nucleic acid purification, electrophoretic mobility shift assay (EMSA), *in vitro* transcription, cross-linking, fluorescence-based kinetic assays, X-ray crystallography, negative stain EM, single-particle cryo-EM, cryo-ET, computer science

**Undergraduate Research Fellow****September 2012 – August 2013**

Department of Cell and Developmental Biology, University of Pennsylvania

*Advisor:* Shelley L. Berger, Ph.D.

- Assayed interactions between the histone acetyltransferase CBP with the long non-coding RNA HOTAIR
- Work acknowledged in: Bose, D. A., Donahue, G., Reinberg, D., Shiekhattar, R., Bonasio, R., & Berger, S. L. (2017). RNA Binding to CBP Stimulates Histone Acetylation and Transcription. *Cell*, 168(1-2), 135-149.e22.
- Techniques: Protein purification and pull-downs, RNA binding assays, RNA *in vitro* transcription/purification, tissue culture, ChIP-seq, Western Blotting

**Summer Undergraduate Research Fellow****June 2012 – August 2012**

Laboratory of Molecular Biophysics, The Rockefeller University

*Advisor:* Seth A. Darst, Ph.D.

- Performed protein purifications and X-ray crystallographic experiments on the transcription factor, CarD
- Determined a high-resolution structure of *Thermus thermophilus* CarD in complex with RpoB- $\beta$ 1 by X-ray crystallography.
- Techniques: Cloning, protein purifications, EMSA, crystallography

**High School/Undergraduate Research Fellow** **September 2006 – May 2013**

Department of Biochemistry and Biophysics, University of Pennsylvania

Advisor: Gregory D. Van Duyne, Ph.D.

- Performed protein purifications and biophysical measurements on the Gemin2-SmD1/D2/F/E/G complex
- Designed structural-driven mutations in Cre recombinase and assessed catalytic consequences of these mutations *in vivo* and *in vitro*
- Characterized induction of Holliday Junction complexes during Cre recombination by gel shift assays
- Mentored high school and undergraduate students
- Techniques: Cloning, mutagenesis, protein purifications, *in vivo*  $\beta$ -galactosidase assay, EMSA, small-angle X-ray scattering (SAXS), analytical ultracentrifugation, crystallography

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**TEACHING AND MENTORING EXPERIENCE**

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**Lab Mentor****January 2021 – Present**

Department of Cell Biology, New York University School of Medicine

- Mentored rotation student (1) and undergraduate students (2) in the Bhabha/Ekiert Lab on techniques in molecular biology, microbiology, and protein purification
- Conducted 1-on-1 meetings with mentees from NYU Summer Immersion Program (2) and Schmidt Science Fellows (2) to discuss career development

**Lab Mentor****September 2013 – December 2020**

Laboratory of Molecular Biophysics, The Rockefeller University

- Taught cryo-EM to research assistants (4), students (1), post-docs (1), research associates (2), visiting investigators (3) in the Darst Lab
- Trained students (1), post-doctoral fellows (1), and research associates (1) in the Campbell group in cryo-EM sample preparation and data collection and processing

**Undergraduate Research Mentor****Summer 2017 and Summer 2018**

Summer Undergraduate Research Fellowship Program, The Rockefeller University

- Mentored undergraduate students from Vassar College (1) and Rutgers University (1)
- Lead research project to examine transcriptional properties of 6S RNA for subsequent structural studies

**Undergraduate Tutor****September 2010 – May 2013**

The Tutoring Center, University of Pennsylvania

- Tutored undergraduates in the subject of Math 114: Multivariable Calculus (Calculus II)
- Mentored less experienced tutors
- Attended various career-building workshops

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**TRAINING**

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**EMBO Virtual Workshop****November-December 2021**

European Molecular Biology Laboratory

- Online workshop about the recent advances in structural biology of membrane proteins

**Schrödinger's Introduction to Molecular Modeling in Drug Discovery** June 2019

Schrödinger, Inc

- Online course on how to use industry-leading computational molecular modeling tools for drug discovery and design

**SEMC Appion: Tomography Workshop** May 2019

New York Structural Biology Center

- Introductory course and hands-on tutorial for cryo-electron tomography analysis using Leginon, Appion-Protomo, and Dynamo

**SBGrid EM Data Processing Workshop** March 2017

Harvard Medical School

- Introductory course and hands-on tutorial for single particle analysis using RELION

**SBGrid/NE-CAT PHENIX Workshop** November 2016

Harvard Medical School

- Course on experimental phasing, automated model building, and real-space refinement

**GRANTS AND FELLOWSHIPS**

Jane Coffin Childs Postdoctoral Fellowship (New York University School of Medicine)	2021
Schmidt Science Fellows (The Rockefeller University)	2020
Summer Undergraduate Research Fellowship (The Rockefeller University)	2012

**HONORS AND AWARDS**

Phi Sigma Biological Science Honor Society (University of Pennsylvania)	2012-2013
Philadelphia's Mayor's Scholarship (University of Pennsylvania)	2009-2013
Lindsay G. Dunham Scholarship (University of Pennsylvania)	2009-2013

**PUBLICATIONS**

1. Malone, B. F., Perry, J. K., Olinares, P. D. B, **Chen, J.**, Appelby, T. K., Feng, J. Y., Bilello, J. P., Ng, H., Sotiris, J., Ebrahim, M., Chua, E. Y. D., Mendez, J. H., Eng, E. T., Landick, R., Chait, B. T., Campbell, E. A., Darst, S. A. (2022). Structural basis for substrate selection by the SARS-CoV-2 replicase. *BioRxiv* 2022.05.20.492815; DOI: <https://doi.org/10.1101/2022.05.20.492815>
2. Cao, X., Boyaci, H., **Chen, J.**, Bao, Y., Landick, R., Campbell, E. A. (2022). Basis of narrow-spectrum activity of fidaxomicin on *Clostridioides difficile*. *Nature* 604, 541–545; DOI: 10.1038/s41586-022-04545-z
3. **Chen J.\***, Wang Q.\*, Malone B.\*, Llewellyn E., Pechersky Y., Maruthi K., Eng E. T., Perry J. K., Campbell E. A., Shaw D. E., Darst S. A. (2022). Ensemble cryo-EM reveals conformational states of the nsp13 helicase in the SARS-CoV-2 helicase replication–transcription complex. *Nat Struct Mol Biol* 29, 250–260. DOI: 10.1038/s41594-022-00734-6

4. Saecker R. M., **Chen J.**, Chiu C. E., Malone B., Sotiris J., Ebrahim M., Yen L. Y., Eng E. T., Darst S. A. (2021). Structural origins of Escherichia coli RNA polymerase open promoter complex stability. *PNAS* 118(40):e2112877118. DOI: 10.1073/pnas.2112877118
5. Malone, B.\*, **Chen, J.\***, Wang, Q., Llewellyn, E., Choi, Y. J., Olinares, P. D. B., Cao, X., Hernandez, C., Eng, E. T., Chait, B. T., Shaw, D. E., Landick, R., Darst, S. A., Campbell, E. A. (2021). Structural basis for backtracking by the SARS-CoV-2 replication–transcription complex. *PNAS*. 118 (19) e2102516118; DOI: 10.1073/pnas.2102516118
6. Kang, J. Y., Llewellyn, E., **Chen, J.**, Olinares, P. D. B., Brewer, J., Chait, B. T., Campbell, E. A., Darst, S. A. (2021). Structural basis for transcription complex disruption by the Mfd translocase. *eLife*. 10:e62117; DOI: 10.7554/eLife.62117
7. Olinares, P. D. B., Kang, J. Y., Llewellyn, E., Chiu, C., **Chen, J.**, Malone, B., Saecker, R. M., Campbell, E. A., Darst, S. A., Chait, B. T (2020). Native Mass Spectrometry-Based Screening for Optimal Sample Preparation in Single Particle Cryo-EM. *Structure*. 29, 2, 186-195.e6; DOI: 10.1016/j.str.2020.11.001
8. Lilic, M., **Chen, J.**, Boyaci, H., Braffman, N., Hubin, E. A., Herrmann, J., Müller, R., Mooney, R., Landick, R., Darst, S. A., Campbell, E. A. (2020). The antibiotic sorangicin A inhibits promoter DNA unwinding in a Mycobacterium tuberculosis rifampicin-resistant RNA polymerase. *PNAS*. 202013706; DOI: 10.1073/pnas.2013706117
9. **Chen, J.**, Boyaci, H., Campbell, E.A (2020). Diverse and unified mechanisms of transcription initiation in bacteria. *Nat Rev Microbiol*; DOI: 10.1038/s41579-020-00450-2
10. Dandey, V. P., Budell, W. C., Wei, H., Bobe, D., Maruthi, K., Kopylov, M., Eng, E. T., Kahn, P. A., Hinshaw, J. E., Kundu, N., Nimigeon, C. M., Fan, C., Sukomon, N., Darst, S. A., Saecker, R. M., **Chen, J.**, Malone, B., Potter, C. S., Carragher, B. (2020). Time-resolved cryo-EM using Spotiton. *Nat Methods*. 17, 897-900; DOI: 10.1038/s41592-020-0925-6
11. **Chen, J.\***, Malone, B.\*, Llewellyn, E., Grasso, M., Shelton, P. M. M., Olinares, P. D. B., Maruthi, K., Eng, E. T., Vatandaslar, H., Chait, B. T., Kapoor, T. M., Darst, S. A., Campbell, E. A. (2020). Structural Basis for Helicase-Polymerase Coupling in the SARS-CoV-2 Replication-Transcription Complex. *Cell*. 182, 6,1560-1573.e13; DOI: 10.1016/j.cell.2020.07.033
12. **Chen, J.**, Chiu, C., Gopalkrishnan, S., Chen, A. Y., Olinares, P. D. B., Saecker, R. M., Winkelman, J. T., Maloney, M. F., Chait, B. T., Ross, W., Gourse, R. L., Campbell, E. A., Darst, S. A. (2020). Stepwise Promoter Melting by Bacterial RNA Polymerase. *Mol. Cell*. 78, 275-288.e6; DOI: 10.1016/j.molcel.2020.02.017
13. **Chen, J.**, Gopalkrishnan, S., Chiu, C., Chen, A. Y., Campbell, E. A., Gourse, R. L., Ross, W., and Darst, S. A. (2019). E. coli TraR allosterically regulates transcription initiation by altering RNA polymerase conformation. *eLife*. 8, 1–81; DOI: 10.7554/eLife.49375.
14. **Chen, J.\***, Noble, A. J.\*, Kang, J. Y., Darst, S. A. (2019). Eliminating effects of particle adsorption to the air/water interface in single-particle cryo-electron microscopy: Bacterial RNA polymerase and CHAPSO. *Journal of Structural Biology: X*. 1 (January–March 2019):100005; DOI: 10.1016/j.jysbx.2019.100005.
15. Boyaci, H., **Chen, J.**, Jansen, R., Darst, S. A., Campbell, E. A. (2019). Structures of an RNA polymerase promoter melting intermediate elucidate DNA unwinding. *Nature*. 565 (7739): 382-385; DOI: 10.1038/s41586-018-0840-5.
16. Guo, X., Myasnikov, A. G., **Chen, J.**, Crucifix, C., Papai, G., Takacs, M., Schultz, P., Weixlbaumer, A. (2018). Structural Basis for NusA Stabilized Transcriptional Pausing. *Mol Cell*. 69 (5): 816-827; DOI: 10.1016/j.molcel.2018.02.008.
17. **Chen, J.\***, Boyaci, H.\*, Lilic, M., Palka, M., Mooney, R. A., Landick, R., Darst, S. A., Campbell, E. A. (2018). Fidaxomicin jams Mycobacterium tuberculosis RNA polymerase motions needed for initiation via RbpA contacts. *eLife*. 7: e34823; DOI: 10.7554/eLife.34823.
18. **Chen, J.**, Wassarman, K. M., Feng, S., Leon, K., Feklistov, A., Winkelman, J. T., Li, Z., Walz, T., Campbell, E. A., Darst, S. A. (2017). 6S RNA Mimics B-Form DNA to Regulate

- Escherichia coli RNA Polymerase. *Mol Cell*. 68 (2): 388-397; DOI: 10.1016/j.molcel.2017.09.006.
19. Kang, J. Y., Olinares, P. D., **Chen, J.**, Campbell, E. A., Mustaev, A., Chait, B. T., Gottesman, M. E., Darst, S. A. (2017). Structural basis of transcription arrest by coliphage HK022 N<sub>un</sub> in an Escherichia coli RNA polymerase elongation complex. *eLife*. 6: e25478; DOI: 10.7554/eLife.25478.
  20. Garner, A. L., Rammohan, J., Huynh, J. P., Onder, L. M., **Chen, J.**, Bae, B., Jensen, D., Weiss, L. A., Manzano, A. R., Darst, S. A., Campbell, E. A., Nickels, B. E., Galburt, E. A., Stallings, C. L. (2017). Effects of Increasing the Affinity of CarD for RNA Polymerase on Mycobacterium tuberculosis Growth, rRNA Transcription, and Virulence. *Journal of Bacteriology*. 199 (4): e00698-16; DOI: 10.1128/JB.00698-16.
  21. Bae, B., **Chen, J.**, Davis, E., Leon, K., Darst, S. A., & Campbell, E. A. (2015). CarD uses a minor groove wedge mechanism to stabilize the RNA polymerase open promoter complex. *eLife*. 4: e08505; DOI: 10.7554/eLife.08505.
  22. Davis, E., **Chen, J.**, Leon, K., Darst, S. A., & Campbell, E. A. (2014). Mycobacterial RNA polymerase forms unstable open promoter complexes that are stabilized by CarD. *Nucleic Acids Research*. 43 (1): 433-445; DOI: 10.1093/nar/gku1231.
  23. Gibb, B., Gupta, K., Ghosh, K., Sharp, R., **Chen, J.**, & Van Duyne, G. D. (2010). Requirements for catalysis in the Cre recombinase active site. *Nucleic Acids Research*. 38 (17): 5817-5832; DOI: 10.1093/nar/gkq384.
- \* These authors contributed equally to this work.

## ORAL SCIENTIFIC PRESENTATIONS

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1. **Chen, J.** *Structure of an endogenous mycobacterial transport complex*. Oral talk present at Women Leading CryoEM NYC Retreat hosted at Flatiron institute, New York, NY, US (July 2022).
2. **Chen, J.** *Structure of an endogenous mycobacterial transport complex*. Oral talk present at New York Bacillus Interest Group symposium hosted at Columbia University, New York, NY, US (July 2022).
3. **Chen, J.** *Cryo-EM structure of a mycobacterial inter-membrane transport complex*. Oral talk presented at NYU Biochemistry and Molecular Biophysics Work-In-Progress seminar series in NYU School of Medicine, New York, NY, US (February 2022).
4. **Chen, J.\***, Malone, B.\*, Wang, Q., Llewellyn, E., Olinares, P. D. B., Grasso, M., Shelton, P. M. M., Choi Y. J., Cao, X., Vatandaslar, H., Eng, E. T., Shaw D. E., Chait, B. T., Kapoor, T. M., Landick, R., Darst, S. A., Campbell, E. A. *Structural perspectives of SARS-CoV-2 RNA synthesis*. Poster presented at GRS and GRC: Three Dimensional Electron Microscopy, Waterville Valley, NH, US (October-November 2021).
5. **Chen, J.** *Understanding structural dynamics*. Discussion Leader at the GRS: Three Dimensional Electron Microscopy (October-November 2021).
6. **Chen, J.\***, Malone, B.\* *CoV-er all the bases: Structural perspectives of SARS-CoV-2 RNA synthesis*. Virtual online talk for CryoEM Current Practice Webinar Series hosted by NCCAT (July 2021).
7. **Chen, J.**, Gopalkrishnan, S., Chiu, C., Chen, A. Y., Campbell, E. A., Gourse, R. L., Ross, W., Darst, S. A. *The Escherichia coli F element-encoded TraR protein allosterically regulates transcription initiation by altering RNA polymerase conformation and dynamics*. Poster presented at Tri-Institutional Ph.D. Program in Chemical Biology (TPCB) Recruitment, Memorial Sloan Kettering Cancer Center in New York, NY, US (January 2020).

8. **Chen, J.** *Deconvolution of bacterial RNAP clamp dynamics using multi-body refinement.* Talk presented at Second New York Area CryoEM Meeting, CUNY Advanced Science Research Center, New York, NY, US (October 2019).
9. **Chen, J.**, Gopalkrishnan, S., Chiu, C., Chen, A. Y., Campbell, E. A., Gourse, R. L., Ross, W., Darst, S. A. *The Escherichia coli F element-encoded TraR protein allosterically regulates transcription initiation by altering RNA polymerase conformation and dynamics.* Poster presented at GRC: Mechanisms of Microbial Transcription, Bates College in Lewiston, ME (July-August 2019).
10. **Chen, J.**, Noble, A. J., Kang, J. Y., Darst, S. A. *Eliminating effects of particle adsorption to the air/water interface in single-particle cryo-electron microscopy: Bacterial RNA polymerase and CHAPSO.* Poster was presented at The Rockefeller University Chemistry & Structural Biology Retreat, Edith Macy Conference Center, Briarcliff Manor, NY (October 2018).
11. **Chen, J.** *6S RNA mimics B-form DNA to regulate Escherichia coli RNA polymerase.* Talk presented at The New York Structural Biology Discussion Group Summer 2017 Meeting, Mount Sinai School of Medicine, New York, NY (August 2017).
12. **Chen, J.**, Wassarman, K. M., Feng, S., Leon, K., Feklistov, A., Winkelman, J. T., Li, Z., Walz, T., Campbell, E. A., Darst, S. A. *6S RNA mimics B-form DNA to regulate Escherichia coli RNA polymerase.* Poster presented at FASEB: Mechanism and Regulation of Prokaryotic Transcription, Saxtons River, VT (June 2017).
13. **Chen, J.**, Wassarman, K. M., Feng, S., Leon, K., Feklistov, A., Winkelman, J. T., Li, Z., Walz, T., Campbell, E. A., Darst, S. A. *6S RNA mimics B-form DNA to regulate Escherichia coli RNA polymerase.* Poster presented at SBGrid EM Data Processing Workshop, Harvard Medical School, Boston, MA (March 2017).
14. **Chen, J.** *Structural Characterization of 6S RNA in Complex with Bacterial RNA Polymerase.* Talk was presented at The Rockefeller University Chemistry & Structural Biology Retreat, Edith Macy Conference Center, Briarcliff Manor, NY (October 2016).
15. **Chen, J.**, Bae, B., Davis, E., Leon, K., Darst, S. A., & Campbell, E. A. *CarD uses a unique mode of interaction to stabilize the RNA polymerase open promoter complex.* Poster presented at FASEB Science Research Conference: Mechanism and Regulation of Prokaryotic Transcription, Saxtons River, VT (June 2015).
16. **Chen, J.**, Bae, B., Campbell, E.A., Darst, S.A. *Structural Study of CarD and RpoB-β1.* Poster presented at The Rockefeller University, New York, NY (August 2012).