bmeCURRICULUM VITAE

Fenfei Leng, PhD
Department of Chemistry and Biochemistry,
Florida Internatiopnal University
AHC4 Rm 222, 11200 SW 8th Street, Miami, FL 33199
Phone: 305-348-3277
Email: lengf@fiu.edu
Web: https://case.fiu.edu/about/directory/profiles/leng-fenfei.html

Education

1982-1986:	B.S., Biochemistry, Nanjing University Nanjing, China
1993-1997:	Ph.D., Biochemistry, University of Mississippi Medical Center Jackson, MS
1997-2001:	Postdoctoral training, Biochemistry, Johns Hopkis University, Baltimore, MD

Positions and Employment

2022-present: Founder and President, Top Biosciences, LLC
2018-present: Tenured Professor, Florida International University, Miami, FL
2007-2018: Tenured Associate Professor, Florida International University, Miami, FL
2001-2007: Assistant Professor, Florida International University, Miami, FL
1997-2001: Postdoctoral fellow, Johns Hopkins University, Baltimore, MD
1993-1997: Graduate Assistant, Univ. of Mississippi Medical Center, Jackson, MS
1991-1993: Lecturer, JiangXi Medical College, Nanchang, P. R. China
1989-1991: Assistant Lecturer, Jiangxi Medical College, Nanchang, P. R. China

Other Experience and Professional Memberships

NSF review Panels, 2020, 2021, 2022, 2023, and 2024
Ad Hoc member, NIH ZRG1 MCST-J(10) and 2024 and NIH ZRG1 DCAI-S (02)
Reviewer for the Deutsche Forschungsgemeinschaft (German Research Foundation), 2021
Ad Hoc member, NIH MBRS SCORE review panel (2006, 2008)
Member, Biophysical Society, American Chemical Society
Member, Editorial Board, Scientific Reports

Honors

Robert A. Mahaffey, Jr. Memorial Award (1997)
Member of the Sigma Xi Research Society (1997)
Semifinalist of Student Research Achievement Award of the 41st National Annual Meeting of Biophysical Society (1997)
Member of the Honor Society of Phi Kappa Phi (1996)

Publications (*corresponding author)

1. Rezaei S, Moncada-Restrepo M, Leng S, Chambers JW, **Leng F.*** Synthesizing supercoiled circular DNA molecules in vitro. Nucleic Acids Res. 2025 Sep 5;53(17):gkaf889. doi: 10.1093/nar/gkaf889. PMID: 40930535; PMCID: PMC12421384.
2. Su L, Deng Z, Santos-Fernandez M, Jeanne Dit Fouque K, Chapagain PP, Chambers JW, Fernandez-Lima F, **Leng F.*** Inhibition of HMGA2 binding to AT-rich DNA by its negatively charged C-terminus. Nucleic Acids Res. 2025 Jan 24;53(3):gkaf035. doi: 10.1093/nar/gkaf035. Erratum in: Nucleic Acids Res. 2025 Feb 27;53(5):gkaf193. doi: 10.1093/nar/gkaf193. PMID: 39873271; PMCID: PMC11773362.
3. Jeanne Dit Fouque K, Molano-Arevalo JC, **Leng F**, Fernandez-Lima F. Conformational and Structural Characterization of Knotted Proteins. Biochemistry. 2024 Sep 17;63(18):2293-2299. doi: 10.1021/acs.biochem.4c00218. Epub 2024 Aug 27. PMID: 39189377; PMCID: PMC11790308.
4. Su L, Rezaei S, Mejia G, Pandey P, Dit Fouque KJ, Fernandez-Lima F, McGoron A, He J, **Leng F.*** Conjugating Daunorubicin and Doxorubicin to GTP by Formaldehyde to Overcome Drug Resistance. ChemMedChem. 2024 Dec 2;19(23):e202300481. doi: 10.1002/cmdc.202300481. Epub 2024 Oct 8. PMID: 39136598; PMCID: PMC11620955.
5. Deng Z, Chapagain P, **Leng F.*** Macromolecular crowding potently stimulates DNA supercoiling activity of Mycobacterium tuberculosis DNA gyrase. J Biol Chem. 2023 Dec;299(12):105439. doi: 10.1016/j.jbc.2023.105439. Epub 2023 Nov 7. PMID: 37944619; PMCID: PMC10731242.
6. Khatri S, Pandey P, Mejia G, Ghimire G, **Leng F**, He J. Nanoconfinement and Crowding Enhanced Single-Molecule Detection of Small Molecules with Nanopipettes. J Am Chem Soc. 2023 Dec 27;145(51):28075-28084. doi: 10.1021/jacs.3c09311. Epub 2023 Nov 23. PMID: 37996390; PMCID: PMC11036617.
7. Tantak M, Rayala R, Deng Z, Bunnell A, Wang T, Chaudhari P, **Leng F**, Nefzi A. Polyheterocyclic peptidomimetics: Parallel solid phase synthesis of oligo cyclic guanidines and their inhibition activity against Mycobacterium tuberculosis DNA gyrase. Bioorg Med Chem Lett. 2023 Sep 1;93:129439. doi: 10.1016/j.bmcl.2023.129439. Epub 2023 Aug 8. PMID: 37557925; PMCID: PMC10993493.
8. Hernández-Martínez G, Ares MA, Rosales-Reyes R, Soria-Bustos J, Yañez-Santos JA, Cedillo ML, Girón JA, Martínez-Laguna Y, **Leng F**, Ibarra JA, De la Cruz MA. The nucleoid protein HU positively regulates the expression of type VI secretion systems in Enterobacter cloacae. mSphere. 2024 Apr 22:e0006024. doi: 10.1128/msphere.00060-24. Epub ahead of print. PMID: 38647313.
9. Atomwise AIMS Program. AI is a viable alternative to high throughput screening: a 318-target study. Sci Rep. 2024 Apr 2;14(1):7526. doi: 10.1038/s41598-024-54655-z. PMID: 38565852; PMCID: PMC10987645.
10. Alfonso, E.E., Deng, Z., Boaretto, D., Hood, B.L., Vasile, S., Smith, L.H., Chambers, J.W., Chapagain, P., and **Leng, F.*** Novel and Structurally Diversified Bacterial DNA Gyrase Inhibitors Discovered through a Fluorescence-Based High-Throughput Screening Assay. *ACS Pharmacology & Translational Science*. 2022, 5, 932-944.
11. Alfonso, E.E., Troche, R., Deng, Z., Annamalai, T., Chapagain, P., Tse-Dinh, Y. and **Leng, F.*** Potent inhibition of bacterial DNA gyrase by digallic acid and other gallate derivatives. *ChemMedChem*. (2022) in press. https://doi.org/10.1002/cmdc.202200301

12. Sipe SN, Jeanne Dit Fouque K, Garabedian A, **Leng F**, Fernandez-Lima F,* Brodbelt JS.* Exploring the Conformations and Binding Location of HMGA2·DNA Complexes Using Ion Mobility Spectrometry and 193 nm Ultraviolet Photodissociation Mass Spectrometry. *J Am Soc Mass Spectrom.* 2022 Jul 6;33(7):1092-1102. doi: 10.1021/jasms.2c00083.
13. Jeanne Dit Fouque K, Sipe SN, Garabedian A, Mejia G, Su L, Hossen ML, Chapagain PP, **Leng F**, Brodbelt JS,* Fernandez-Lima F.* Exploring the Conformational and Binding Dynamics of HMGA2·DNA Complexes Using Trapped Ion Mobility Spectrometry-Mass Spectrometry. *J Am Soc Mass Spectrom.* 2022 Jul 6;33(7):1103-1112. doi: 10.1021/jasms.2c00101.
14. Garabedian A, Jeanne Dit Fouque K, Chapagain PP, **Leng F**, Fernandez-Lima F.* AT-hook peptides bind the major and minor groove of AT-rich DNA duplexes. Nucleic Acids Res. 2022 Mar 21;50(5):2431-2439. doi: 10.1093/nar/gkac115. PMID: 35212375.
15. Pandey P, Bhattarai N, Su L, Wang X, **Leng F**, Gerstman B, Chapagain PP, He J.* Detecting Individual Proteins and Their Surface Charge Variations in Solution by the Potentiometric Nanoimpact Method. *ACS Sens.* 2022, 25: 555-563.
16. Yan Y, Xu W, Kumar S, Zhang A, **Leng F**, Dunlap D, and Finzi L.* Negative DNA supercoiling makes protein-mediated looping deterministic and ergodic within the bacterial doubling time. *Nucleic Acids Research* 2021**,** 49: 11550-11559.
17. Deng Z and **Leng F***. A T5 Exonuclease-Based Assay for DNA Topoisomerases and DNA Intercalators. *ACS Omega*, 2021, 6: 12205-12212.
18. Jeanne Dit Fouque K, Garabedian A, **Leng F**, Tse-Dinh YC, Ridgeway ME, Park MA, and Fernandez-Lima F.* Trapped Ion Mobility Spectrometry of Native Macromolecular Assemblies. *Anal Chem,* 2021, 93: 2933-2941.
19. Laverde EE, Lai Y, **Leng F**, Balakrishnan L, Freudenreich CH, Liu Y.* R-loops promote trinucleotide repeat deletion through DNA base excision repair enzymatic activities. *J Biol Chem.* 2020, 295: 13902-13913.
20. Su L, Bryan N, Battista S, Freitas J, Garabedian A, D'Alessio F, Romano M, Falanga F, Fusco A, Kos L, Chambers J, Fernandez-Lima F, Chapagain PP, Vasile S, Smith L, **Leng F.** Identification of HMGA2 inhibitors by AlphaScreen-based ultra-high-throughput screening assays. Sci Rep. 2020 Nov 2;10(1):18850. doi: 10.1038/s41598-020-75890-0. PubMed PMID: 33139812; PubMed Central PMCID: PMC7606612.
21. Su L, Deng Z, **Leng F**.* The Mammalian High Mobility Group Protein AT-Hook 2 (HMGA2): Biochemical and Biophysical Properties, and Its Association with Adipogenesis. *Int J Mol Sci.* 2020, 21(10): 3710.
22. Dages S, Zhi X, **Leng F.*** Fis protein forms DNA topological barriers to confine transcription-coupled DNA supercoiling in Escherichia coli. *FEBS Lett.* 2020, 594:791-798.
23. Wang Y, Rakela S, Chambers JW, Hua ZC, Muller MT, Nitiss JL, Tse-Dinh YC, **Leng F**.* Kinetic Study of DNA Topoisomerases by Supercoiling-Dependent Fluorescence Quenching. *ACS Omega.* 2019 4:18413-18422.
24. Jeanne Dit Fouque, K., Garabedian, A., **Leng, F.**, Tse-Dinh, Y.C., and Fernandez-Lima, F.* Microheterogeneity of Topoisomerase IA/IB and their DNA-bound states. *ACS Omega*, 2019, 3619-3629.
25. Garcia, P.K., Annamalai T, Wang W, Bell RS, Le D, Martin Pancorbo P, Sikandar S, Seddek A, Yu X, Sun D, Uhlemann AC, Tiwari PB, **Leng F**, Tse-Dinh YC.* Mechanism

and resistance for antimycobacterial activity of a fluoroquinophenoxazine compound. *PLOS One*, 2019, 14, e0207733.
26. Dages S, Dages K, Zhi X, **Leng F.*** Inhibition of the gyrA promoter by transcription-coupled DNA supercoiling in Escherichia coli. Sci Rep. 2018 Oct 3;8(1):14759. doi: 10.1038/s41598-018-33089-4. PMID: 30282997; PMCID: PMC6170449.
27. Garabedian, A., **Leng, F.**, Ridgeway, ME, Park, MA, and Fernandez-Lima, F.* Tailoring peptide conformational space with organic gas modifiers in TIMS-MS. *Int. J. Ion Mobil. Spec.* 2018, 21: 43-48.
28. Garabedian, A., Bolufer, A., **Leng, F.**, and Fernandez-Lima, F.* Peptide sequence influence on the conformational dynamics and DNA binding of the intrinsically disordered AT-hook 3 peptide. *Scientific Reports*, 2018, 8: 10783.
29. Butcher, D., Chapagain, P., **Leng, F.**, Fernandez-Lima, F.* Differentiating Parallel and Antiparallel DNA Duplexes in the Gas Phase Using Trapped Ion Mobility Spectrometry. *Journal of Physical Chemistry*, 2018, 122: 6855-6861.
30. Zhi, X., Dages, S., Dages, K., Makemson, J., and **Leng, F.*** Transient and dynamic DNA supercoiling potently stimulates the *leu-500* promoter in *Escherichia coli. Journal of Biological Chemistry,* **2017***,* 292: 14566-14575.
31. Liu, Y., Hua, Z.-C., and **Leng, F.*** DNA supercoiling measurement in bacteria, *Methods Mol Biol.* **2018,** 1703: 63-73.
32. Liu, Y., Berrido, A., He, J., Hua, Z., Tse-Dinh, Y., and **Leng, F.*** Biochemical and biophysical properties of positively supercoiled DNA. *Biophysical Chemistry* **2017,** 230: 68-73.
33. Yan, Y., **Leng, F.**, Finzi, L., and Dunlap, D.* Supercoiling drives protein-mediated looping of DNA under slight tension. *Nucleic Acids Research* **2018, 46: 2370-2379**.
34. Yan Y, Ding Y, **Leng F**, Dunlap D, Finzi L. Protein-mediated loops in supercoiled DNA create large topological domains. Nucleic Acids Res. 2018 May 18;46(9):4417-4424. doi: 10.1093/nar/gky153. PMID: 29538766; PMCID: PMC5961096.
35. Mathivathanan, L., Yong, G., **Leng, F.**, and Raptis, R. Crystal structure and conformational analysis of doxorubicin nitrate. *Acta Cryst*, **2018**, E74: 400-405.
36. Arya, D.*, Wang, W., **Leng, F.,** Tse-Dinh, Y., Ahmad, M., Fulcrand, G., Story, S., and Ranjan, N. Selective inhibition of E. coli RNA and DNA topoisomerase I by Hoechst 33258 derived mono and bisbenzimidazoles. *Journal of Medcinal Chemistry,* **2017**, 60: 4904-4922.
37. Gu, M., Berrido, A., Gonzalez, W.G., Miksovska, J., Chambers, J., and **Leng, F.*** Fluorescently labeled circular DNA molecules for DNA topology and topoisomerases. *Sci Rep.* **2016**, 6:36006.
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39. **Leng, F.*** Protein-induced DNA linking number change by sequence-specific DNA binding proteins and its biological effects. **2016**, *Biophysical reviews*, 8: 197-207.

40. Fulcrand, G., Dages, S., Zhi, X., Chapagain, P., Gerstman, B. S., Dunlap, D., and **Leng, F.*** DNA supercoiling, a critical signal regulating the basal expression of the lac operon in Escherichia coli. *Sci Rep.* **2016**, 6:19243.
41. Fulcrand, G., Chapagain, P., Dunlap, D., and **Leng, F.*** Direct observation of a 91 bp LacI-mediated, negatively supercoiled DNA loop by atomic force microscope. *FEBS Letters*, **2016**, 590, 613-618.
42. Sun, P., Leeson, C., Zhi, X., Leng, F., Pierce, R.H., Henry, M.S., Rein K.S.* Characterization of an epoxide hydrolase from the Florida red tide dinoflagellate, Karenia brevis. *Phytochemistry*, **2016**, 122: 11-21.
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44. Frost, L., Baez, M.A.M., Harrilal, C., Garabedian, A., Fernandez-Lima F., and **Leng, F.*** The dimerization state of the mammalian high mobility group protein AT-hook-2 (HMGA2). *PLOS ONE,* **2015**, 10(6): e0130478.
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48. **Leng, F.*** DNA Bending by Proteins: Utilizing Plasmid pBednAT as a Tool. *Methods Mol Biol.* **2013**, 1054: 267-282.
49. Deng T., Zhu Z. L., Zhang, S. **Leng, F.,** Cherukuri, S., Hansen, L., Marino-Ramirez, L., Meshorer, E., Landsman, D., and Bustin, M.* HMGN1 Modulates Nucleosome Occupancy and DNase I Hypersensitivity at the CpG Island Promoters of Embryonic Stem Cells. **2013**, *Mol Cell Biol*, 33: 3377-3389.
50. Fulcrand, G., Zhi, X., and **Leng, F.*** Transcription-coupled DNA supercoiling in defined protein systems and in *E. coli topA* mutant strains. **2013**, *IUBMB Life*, 65: 615-622 **(cover story).**
51. Zhi, X. and **Leng, F.*** Dependence of transcription-coupled DNA supercoiling on promoter strength in Escherichia coli topoisomerase I deficient strains. **2013**, *Gene*, 514: 82-90.
52. Xu, X., Zhi, X., and **Leng, F.*** Determining DNA Supercoiling Enthalpy by Isothermal Titration Calorimetry. **2012**, *Biochimie,* 94, 2665-2672.
53. **Leng, F.***, Chen, B. and Dunlap, D. Dividing a supercoiled DNA molecule into two independent topological domains, *Proceedings of the National Academy of Sciences U S A*, **2011,** 108, 19973-19978.
54. Xu, X. and **Leng, F.*** A rapid procedure to purify E. coli DNA topoisomerase I, **2011**, *Protein Expression and Purification*, 77, 214-219.
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56. Chen, B., Young, J., and **Leng, F.*** DNA bending by the Mammalian High Mobility Group Protein AT-hook 2. *Biochemistry,* **2010,** 49(8):1590-5.

57. Joynt, S., Morillo, V., and **Leng, F.*** Binding the Mammalian High Mobility Group Protein AT-hook 2 to AT-Rich Deoxyoligonucleotides: Enthalpy-Entropy Compensation. *Biophysical Journal*, **2009**, 96(10):4144-52.
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60. Samul, R. and **Leng, F**.* Transcription-coupled Hypernegative Supercoiling of Plasmid DNA by T7 RNA Polymerase in Escherichia coli Topoisomerase I-Deficient Strains. *Journal of Molecular Biology*, **2007**, 374, 925-935.
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62. Cui, T., Wei, S., Brew, K., and **Leng, F.*** "Energetics of Binding the Mammalian High Mobility Group Protein HMGA2 to poly(dA-dT)$_2$ and poly(dA)poly(dT)." *Journal of Molecular Biology*, **2005**, 325, 629-645.
63. **Leng, F.***, Amado, L., and McMacken, R. "Coupling DNA supercoiling to transcription in defined protein systems." *Journal of Biological Chemistry*, **2004**, *279*, 47564-47571.
64. **Leng, F.**, Chaires, J.B., and Waring, M.J.* "Energetics of echinomycin binding to DNA" *Nucleic Acids Research*, **2003**, *31*, 6191-6197.
65. **Leng, F.** and McMacken, R.* "Potent Stimulation of Transcription-coupled DNA Supercoiling by Sequence-Specific DNA-Binding Proteins" *Proceedings of the National Academy of Sciences U S A*, **2002**, *99*, 9139-9144.
66. **Leng**, **F.,** Graves, D., and Chaires, J.B.* "Chemical Cross-linking of Ethidium Bromide to DNA" *Biochemica Biophysica Acta,* **1998**, *1442*, 71-81.
67. **Leng**, **F.,** Priebe, W., and Chaires, J.B.* "Ultratight DNA Binding of a New Bisintercalating Anthracycline Antibiotic" *Biochemistry*, **1998**, *37*, 1743-1753.
68. **Leng, F.** and Leno, G.H.* "Daunomycin Disrupts Nuclear Assembly and the Coordinate Initiation of DNA Replication in Xenopus Egg Extracts" *Journal of Cellular Biochemistry*, **1997**, *64*, 476-491.
69. Chaires, J.B.*, **Leng, F.**, Przewloka, T., Fokt, I., Ling, Y.-H, Perez-Soler, R., and Priebe, W. "Structure Based Designed of a New Bisintercalating Anthracycline Antibiotic" *Journal of Medicinal Chemistry*, **1997**, *40*, 261-266.
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71. **Leng, F.**, Savkur, R., Fokt, I., Przewloka, T., Priebe, W., and Chaires, J.B.* "Base Specific and Regioselective Chemical Cross-linking of Daunorubicin to DNA". *Journal of the American Chemical Society*, **1996**, *118*, 4731-4738.

Selected Abstracts

1. Fenfei Leng. Synthesizing supercoiled circular DNA molecules in a novel in vitro enzymatic system. The 2025 Biophysical Society Annual Meeting, Los Angles, CA, February 15-19.
2. German Mejia and Fenfei Leng. Serum Starvation and the Stringent Response in *H. pylori.* 2024 DNA Topoisomerases in Biology and Medicine Miami, Fl July 14-18 2024.
3. German Mejia and Fenfei Leng. RNA-seq Analysis of Serum Starvation in *H. pylori.* American Society for Biochemistry and Molecular Biology (ASBMB) Chicago, Il April 12-15 2025.
4. Sepideh Rezaei, Monica Moncada-Restrepo, Sophia Leng, Jeremy W. Chambers and Fenfei Leng. A novel cell-free enzymatic system to synthesize supercoiled circular DNA molecules. 2024 Topoisomerase in Biology and Medicine, Miami, FL, July 14-18, 2024.
5. Matthew Dias, Sophia Leng, Thirunavukkarasu Annamalai, and Fenfei Leng. A Novel High Throughput Screening Method to Discover Bacterial DNA Gyrase Poisons. American Society for Biochemistry and Molecular Biology (ASBMB) Chicago, IL, April 12-15, 2025.
6. Fenfei Leng. Novel bacterial DNA gyrase inhibitors discovered through high throughput screening assays. FIU TSC seminar, Port St. Lucie, FL, October 20, 2023.
7. Fenfei Leng, Observing DNA Topological Barriers in Action. The online TORC seminar, December 6, 2023.
8. Sepideh Rezaei and Fenfei Leng. A novel in vitro enzymatic system to synthesize closed circular DNA molecules. The 2024 Biophysical Society Annual Meeting, Philadelphia, PA, February 10-14.
9. Matthew Dias and Fenfei Leng- Biophysical Society Annual Meeting (Poster Presentation) - "A Novel High Throughput Screening (HTS) Assay to Discover Bacterial DNA Gyrase Poisons"- February 10-14, 2024
10. Eddy Alfonso and Fenfei Leng. Novel bacterial DNA gyrase inhibitors discovered through a fluorescence-based ultra-high throughput screening assay (poster and oral presentation). Gordon Research Conference: DNA Topoisomerase in Biology and Medicine. Bryant University, RI, August 7- 12, 2022.
11. Zifang Deng and Fenfei Leng, Novel catalytic inhibitors of human DNA topoisomerase I identified from screening the Torrey Pines Institute Library. Gordon Research Conference: DNA Topoisomerase in Biology and Medicine. Bryant University, RI, August 7- 12, 2022.
12. German Mejia and Feifeng Leng. Interactions between doxorubicin, a human DNA topoisomerase 2 poison and nucleotides. Gordon Research Conference: DNA Topoisomerase in Biology and Medicine. Bryant University, RI, August 7- 12, 2022.
13. Eddy Alfonso, Zifang Deng, Daniel Alonso Boaretto and **Fenfei Leng**. Target bacterial DNA gyrase for new antibiotic discovery. The 2022 Biophysical Society Annual Meeting, San Francisco, CA, February, 2022.
14. Eddy Alfonso, Zifang Deng, and **Fenfei Leng**, Identification of novel bacterial DNA gyrase inhibitors using supercoiling-dependent fluorescence quenching assays. EMBO virtual Workshop: DNA Topology in genomic transactions (poster presentation). September 20th - 23rd, 2021.

15. Eddy Alfonso, Zifang Deng, Daniel Boaretto, **Fenfei Leng**. Target bacterial DNA gyrase for new antibiotic discovery. 1st South Florida Translational Research Symposium (Poster presentation). April 13-14, 2022.
16. Linjia Su and **Fenfei Leng**. Synthesis and characterization of daunorubicin-GTP, doxorubicin-GTP, daunorubicin-dGTP, and doxorubicin-dGTP conjugates. EMBO virtual Workshop: DNA Topology in genomic transactions (poster presentation). September 20th - 23rd, 2021.
17. Zifang Deng and **Fenfei Leng**, A T5 exonuclease-based high-throughput screening assay for topoisomerase inhibitors and DNA intercalators. EMBO virtual Workshop: DNA Topology in genomic transactions (poster presentation). September 20th - 23rd, 2021.
18. Miguel Santos-Fernandez, Kevin Jeanne Dit Fouque, **Feifeng Leng**, Francisco Fernandez-Lima. Structural Characterization of Peptide-Peptide complexes using tandem Trapped Ion Mobility, Electron Capture dissociation, and ToF Mass Spectrometry. 2021 ASMS.
19. **Fenfei Leng**, Discover new bacterial DNA gyrase inhibitors using high throughput screening assays, FIU Department of Environmental Health Sciences Seminar, November 12, 2020.
20. Eddy Alfonso, Zifang Deng, and **Fenfei Leng**, Identification of novel inhibitors of bacterial DNA gyrase, FIU BSI Symposium, April 29, 2021.
21. Zifang Deng, Travis LaVoi, Marcello Giulianotti, Clemencia Pinilla, and **Fenfei Leng**, Identification of novel inhibitors targeting Mycobacterium Tuberculosis DNA gyrase by screening the Torrey Pines Institute combinatorial libraries. FIU BSI Symposium, April 29, 2021.
22. Linjia Su and **Fenfei Leng**, Synthesis and characterization of GTP-daunorubicin, GTP-doxorubicin, dGTP-daunorubicin, and dGTP-doxorubicin conjugates. FIU BSI Symposium, April 29, 2021.
23. Linjia Su, Zifang Deng, Jeremy Chambers, and **Fenfei Leng** (2020) "Inhibition of HMGA2 binding to AT-rich DNA by its own negatively charged C-terminus." The 2020 Annual BSI Research Symposium. Florida International University, Miami, FL, US.
24. Eddy Alfonso, Rogelio Troche, Zifang Deng, and **Fenfei Leng**, Gallic acid based bacterial DNA gyrase inhibitors. The 2020 Annual BSI Research Symposium. Florida International University, Miami, FL, US.
25. Zifang Deng and **Fenfei Leng**, A new fluorescence-based, high throughput screening (HTS) method to identify DNA topoisomerase inhibitors. The 2020 Annual BSI Research Symposium. Florida International University, Miami, FL, US.

Patents

1. Patent Number: US 12,371,725 B2
   Date of patent: July 29, 2025
   Title: Synthesis of DNA molecules in in vitro enzymatic systems
   Inventor: Fenfei Leng

2. Patent number: 12234504
   Date of patent: February 25, 2025
   Title: High throughput screening assay to identify DNA topoisomerase inhibitors
   Inventors: Fenfei Leng, Matthew Dias

3. Patent Number: 12121528
   Date of Patent: October 22, 2024
   Title: Bacterial DNA Gyrase Inhibitors and Methods of Use Thereof
   Inventors: Fenfei Leng, Eddy Alfonso, Zifang Deng

4. Patent number: 11732286
   Date of patent: August 22, 2023
   Title: T5 exonuclease-based method to identify DNA topoisomerase inhibitors
   Inventors: Fenfei Leng, Zifang Deng

5. Patent number: US 11,613,780 B2
   Date of patent: March 28, 2023
   Title: Bacterial DNA Gyrase Inhibitors and Methods of Use Thereof
   Inventors: Fenfei Leng, Xiaoduo Zhi, Samantha Dages, and Kelley Dages

6. Patent number: US 11,286,514 B1
   Date of patent: March 29, 2022
   Title: T5 exonuclease-based method to identify DNA topoisomerase inhibitors
   Inventors: Fenfei Leng and Zifang Deng

7. US patent number: 9,890,416
   Date of Patent: February 13, 2018
   Title: Labeled circular DNA molecules for analysis of DNA topology and topoisomerases and for drug screening
   Inventor: Fenfei Leng

8. US patent number: 9920014
   Date of Patent: March 20, 2018
   Title: Selective inhibition of bacterial topoisomerase I
   Inventors: Nihar Ranjan, Dev P. Arya, Fenfei Leng

Research Funds

1. Title: Novel gyrase inhibitors targeting Mycobacterium tuberculosis
   Project Number: 1 R21 AI178134-01
   Name of PD/PI: Leng (Contact) /Nefzi/Rohde
   Source of Support: NIH/NIAID
   Primary Place of Performance: Florida International University
   Project/Proposal Start and End Date: 05/2023 - 04/2026
   Total Requested Amount (including Indirect Costs): $421,640
   Major Goals: This project is designed to determine structure activity relationships (SARs) of a particular chemotype of 4-(1-methylimidazo[1,2-a]pyridine-1-ium (aka CPD229 and analogs), new antimycobacterial gyrase inhibitors discovered in our recent HTS campaign targeting bacterial DNA gyrase.

2. Title: Serum Starvation and the Stringent Response in H. pylori
   Project Number:
   Name of PD/PI: Fenfei Leng
   Source of Support: NSF
   Primary Place of Performance: Florida International University
   Project/Proposal Start and End Date: 08/2023 – 07/2026
   Total Requested Amount (including Indirect Costs): $147,000
   Major Goals: This is an NSF graduate research fellowship to support my graduate student German Mejia's graduate studies in my lab at fIU.

3. Title: High Throughput Screening to Discover Bacterial DNA Gyrase Poisoning Inhibitors
   Project Number: 1R41TR005250-01
   Name of PD/PI: Fenfei Leng; co-PI: Thirunavukkarasu Annamalai (FIU)
   Source of Support: NIH/NCATS
   Primary Place of Performance: Top Biosciences, LLC and Florida International University
   Project/Proposal Start and End Date: 05/2024 - 01/2026
   Total Requested Amount (including Indirect Costs): $298,251.80
   Major Goals: This is an NIH/NCATS STTR grant submitted through Top biosciences, LLC (founder and president, Fenfei Leng). The PI at FIU is Dr. Thirunavukkarasu Annamalai. The work will be mainly performed in Fenfei Leng's lab at FIU. FIU receives a subaward of $103,848 from this grant.

4. Title: Self-crowding and ligand-binding assisted single-molecule detection of small molecules by nanopipettes
   NSF grant 2434524
   Role: co-PI. PI (Jin He)
   Amount: $ 442,106
   Project/Proposal Start and End Date: 09/2025 - 08/2028
   Major goal: The overall objective of this proposal is to develop a nanopipette based ultrasensitive label-free electrical detection method for small biomolecules (typically < 2 kDa in molecular weight) and bioactive small molecules (<1 kDa in molecular weight) at the single-molecule level under the physiological condition based on their size, charge, mobility and affinity.

Services

Professional

- Editor, **Scientific Reports**
- Grant reviewer for **National Institutes of Health**, **National Science Foundation, and National Science Center of Poland**.
- Reviewer for Nature Chemical Biology, Nucleic Acids Research, Reviewer for Biopolymers, Biochem. Biophy. Acta, Journal of Inorganic Biochemistry, Gene, Journal of American Chemical Society, Biochemistry, Journal of Physical Chemistry, Planta Medica, Biochimie, Scientific Report, Photochemistry and Photobiology Journal, Biophysical Journal, PLOS ONE, Biotechnology Progress, Chemical Reviews,

Analytical Biochemistry, Molecular BioSystems, International Journal of Molecular Sciences, Clinical Microbiology and Infection, Biophysical Chemistry, Chemical Communications, European Biophysics Journal, Molecular Pharmaceutics, ACS books, Science China Life Sciences, and Taylor & Francis Group LLC Book "Physical Principles in Nucleic Acid Chemistry," by David Draper.

Service to the University

To the University

- Faculty senator, 2013-2014
- Faculty senator alternate, 2014-2015
- Faculty senator, 2015-2019
- Radiation Control Committee, 2008-2011
- FIU NIGMS RISE Graduate Student Selection Committee, 2017
- Chair Biochemistry section of ARCH 2014, FIU
- Reviewer and Chair Chemistry & Biochemistry section, FIU-URC Conference, 2015

To the College of Arts and Sciences

- Biomedical and Behavioral Sciences Committee (Integrated Lif Sciences Committee) to establish the School of Integrated Science and Humanity, 2008-2011
- Biomolecular Science Committee to establish Biomolecular Sciences Institute, 2011-2012

To the Department of Chemistry and Biochemistry

- Instrumental facilities committee
- Graduate student recruitment committee
- Radiation safety officer
- Budget committee
- Tech representative
- FAR revision committee
- Biochemistry PhD program executive committee
- Public Relation Committee
- Graduate committee for Forensic Sciences
- Faculty search committee
- Human resource committee
- Biochemistry graduate committee
- Director, the Biochemistry PhD Program

Courses taught at FIU

CHM4304 (Biological Chmistry 1), CHM4304L (Biological Chmistry 1 Lab), CHM4307 (Biological Chmistry 2), CHM5503 (Physical Chemistry of Nucleic Acids), CHM6382 (Advance Biochemistry), CHM6930 (Chemistry seminar), CHM6936 (Chemistry Colloquium), CHM5506 (Physical Biochemistry), BCH6108 (Biochemical Techniques), CHM5306 (Special Topics in Biochemistry), CHM 6306 (Advanced Biochemistry I, CHM6307 (Advanced Biochemistry II), Biochemistry Graduate Seminar (BCH 7930)

Graduate Student Supervision

Thesis/Dissertation Advisor

1. Linda M. Erdei 2001-2002 M.S. "Use of whole genome amplification to improve performance of the AmpliType PM and QDA1 amplification and typing kit for forensic samples with low copy number of DNA templates." October 2002. Current position: Laboratory director, Lake County Crime Lab, Ohio

2. Angelica Mendoza 2002-2004 M.S. "Conformational Analysis of the Mammalian High Mobility Group Protein HMGA2", November 2004. Current position: Forensic Scientist, Ventura County Sheriff's Office, Ventura, CA

3. Lorraine Edwards 2003-2006 M.S. "Biochemical and biophysical characterization of high mobility group protein A2," March, 2006. Current position: manager of reference laboratory for exotic animal viruses, London, UK

4. Rebecca Samul, 2004-2007 M.S. "Transcription-Induced Hypernegative Supercoiling of Plasmid DNA By T7 RNA Polymerase in *E. coli* Topoisomerase Deficient Strains," April, 2007. Current position: Director, Quest Diagnostics Nichols Institute, Chantilly, VA

5. Tengjiao Cui, 2001-2007 Ph.D. "Specific Binding of the Mammalian High Mobility Group Protein AT-hook 2 to the Minor Groove of AT-rich DNAs: Thermodynamic and Spcificity Studies," May, 2007. Current position: Assistant Professor of Pathology, University of Miami Medical School, Miami, FL

6. Xiaozhou Xu, 2007-2010 M.S., Kinetics of *E. coli* Topoisomerase I and Energetic Studies of DNA Supercoiling of Isothermal Titration Calorimetry, October, 2010. Current position: Chemist II at Sigma-Aldrich, Louisville, Kentucky

7. Xiaoduo Zhi, 2007-2013 Ph.D., Transcription-coupled DNA supercoiling in Escherichia coli: mechanisms and biological functions, May, 2013. Current position: Scientist, Advanced Fertility Center of Chicago, Chicago, IL

8. Samantha Dages 2015-2016 M.S. Transcription-coupled DNA supercoiling, December, 2016. Current position:

9. Linjia Su 2016-2021 Ph.D., Identifying anticancer drugs targeting HMGA2-DNA interaction using High throughput Screening (HTS) assays.

10. Eddy E. Alfonso 2018-2023, Ph.D. Discovering novel bacterial DNA gyrase inhibitors.

11. Zifang Deng 2018-2023, Ph.D. Mtb DNA gyrase and inhibitors.

12. Monica Restrepo 2019-2025, Ph.D. TARGETING MUSCLE-SPECIFIC PROTEIN KINASE ACTIVITY

13. German Mejia 2021-present, Ph.D., Molecular mechanism of the starvation/stress respons.

14. Matthew Dias 2022-present, Ph.D., Bacterial DNA gyrase and its inhibitors.

15. Sepideh Rezaei 2022-present, Ph.D., In vitro DNA synthesis.

16. Jeffrey Caldwell, 2024-present, Ph.D., DNA binding ligands

Thesis/Dissertation Committee Member

Vanessa Thompson, CHM, PHD
Adriana Galvis, Biology, PhD
Erika Doctor, CHM, PhD
Mark Clifton, Biology, PhD
Alicia Fernandez-Fernandez, Biomedical Engineering, PhD
Juan Jeanniton, CHM, PhD
Hui Tian, CHM, PhD
Yuxiang Mao, CHM, PhD
Li Liu, CHM, PhD
Dragan Simovic, CHM, PhD
Julie Lynn Langdon, CHM, MS
Robert Perez Jr., CHM, PhD
Jamie Winshell, CHM, MS
Richard Snyder, CHM, PhD
Mingping Di, CHM, PhD
Charles L. Parkins, CHM, MS
Minakshi C. Gurbhele, CHM, MS
Danny S. Gonzalez, Biomedical Engineering, MS
Megan Bottegal, CHM, PhD
Zhonghua Wang, CHM, PhD
Iru Paudel, Medicine, PhD
Georgiana Gibson-Daw, CHM, PhD
Shayna Sandhaus, Biochemistry, PhD
Seongshin Gwak, CHM, PhD
Alyssa Garabedian, CHM, PhD
Christopher L. De Jesus, CHM, MS
Meghan Roig, CHM, MS
Pamela Garcia, Biochemistry, PhD
Juan Arevalo, CHM, PhD
Vanesa Mendez, CHM, PhD
Elina Barredo, Biology, PhD
Maria Santiago, Biochemistry, PhD
Katelyn Lambert, CHM, PhD
Santosh Khatri, Physics, PhD
Shomita Ferdous, Biochemistry, PhD
Alexandria Roach, Biochemistry, PhD
Somaia Haque Chadni, Biochemistry, PhD

Undergraduate students

Pablo Penaloza, 2001-2002
Luciana Amado, 2001-2003
Hilda Ramon, 2003-2004
Luisel Rodriguez, 2004-2006
Suzanne Joynt, 2006-2009
Victor Morrilo, 2006-2008
Jasmine Young, 2009-2010
Tiffany Chin-You, 2009-2010
Rebeca Armenteros, 2010-2011
Robert Wright, 2012
Nicole Alonso 2013-2015
Andrea Berrido 2012-2015
Andrew Chen 2013
Kelley Dages 2013-2015
Samantha Dages 2013-2016
Catherine Perez 2013-2015
Ashley Tschiggfrie 2013-2015
Roboan Guillen 2013-2015
Maria de Cabrera 2014-2016
Juan Medina 2015-2017
Gabriela Ortega 2015-2017
Daniel Moy 2015-2018
Alex Capaldo 2016-2017
Natalie Rosa 2016-2018
Loreinn Ruiz 2017-2018
Joanna Herrera 2016-2017
Daniel Alonso Boaretto 2018-2021
Jazmin Espinoza 2021-2023
Dominique Gonzalez 2023-2025
Ashley Bush, 2024-present
Ngan Ellie Linh, 2024-present

High school students

Maxwell Gu (2015)

Post-doctor Associates

Weijuan Zheng (2003-2004), Current position: Associate Professor of Biochemistry, Nanjing University, Nanjing, China

Shengji Mao (2004-2005), Current position: unknown

Yazhong Xiao (2005-2007), Current position: Professor of Biology, Anhui University, Hefei, China

Bo Chen (2007-2012), Current position: Associate Professor of Biochemistry, the Institute of Blood Transfusion (IBT), Chinese Academy of Medical Sciences, China

Geraldine Fulcrand (2009-2013), current position: lecturer, University of Montpellier, France

Visiting or exchange students

YintingLiu (2016-2017), visiting graduate student from Nanjing University, China

Yunke Wang (2016-2017), visiting graduate student from Nanjing University, China

Contributions to Science

1. *The discovery of DNA topological barriers:* Both prokaryotic and eukaryotic chromosomes are organized into many independent topological domains. For instance, the *E. coli* chromosome is comprised of a 4.6 Mb circular, negatively supercoiled DNA molecule. A single-stranded nick or double-stranded break should release all superhelical tension and therefore relax the circular DNA molecule. However, early studies showed that multiple single-stranded nicks are required to fully relax the *E. coli* DNA molecule. Further studies showed that the *E. coli* chromosome consists of 400 to 500 independent topological domains *in vivo.* One critical question is what forms topological barriers in DNA. Using two biochemical assays and atomic force microscopy (AFM), we discovered that several sequence-specific DNA-binding proteins, *i.e., lac* repressor, *gal* repressor and Lambda O protein, are able to divide a supercoiled DNA molecule into two independent topological domains. These results can be explained by a topological barrier model in which nucleoprotein complexes confine DNA supercoils to localized regions. The DNA topological barrier model may be a general mechanism for certain DNA-binding proteins, such as histone or histone-like proteins, to modulate topology of chromosome DNA *in vivo*. Using biochemical assays and magnetic tweezers, we found that DNA supercoiling is a regulatory signal for coliphage lambda to decide the lytic vs. lysogenic switch in which lambda repressor serves as a topological barrier, constrains supercoils to the 2.3 kb regulatory DNA element, efficiently represses transcription of cI. These results suggest that the phage genome may have evolved to facilitate quiescent propagation through lysogeny regulated by efficient, negative supercoiling enhanced looping during favorable growth conditions for the bacteria. Additionally, we demonstrated that DNA supercoiling is an epigenetic signal for *E. coli* to control the basal level gene expression of the *lac* operon where the LacI-mediated topological barrier plays an essential role. Our results are consistent with a hypothesis that LacI functions as a topological barrier to constrain (-) supercoils to the 401-bp DNA loop of the *lac* promoter, enhance the LacI's DNA-binding affinity, and therefore increase the promoter inhibition. In this way, LacI is able to tightly control the expression of *lacZYA* in the *lac operon* for different growth conditions.

2. *Transcription-coupled DNA supercoiling (TCDS).* Transcription by RNA polymerases can stimulate (-) DNA supercoiling both *in vitro* defined protein systems and in *E. coli topA* strains. This phenomenon has been successfully explained by a "twin-supercoiled-domain" model of transcription in which (+) supercoils are produced in front of the transcribing RNA polymerase and (-) supercoils behind it. Using defined protein systems; we found that a variety of sequence-

specific DNA-binding proteins, such as the bacteriophage lambda O replication initiator (O) or the *E. coli lac* repressor (LacI) or *gal* repressor (GalR), strongly stimulated TCDS. We demonstrated that this stimulation required the presence in the DNA template of a recognition sequence for the relevant DNA-binding protein and depended on the production of long RNA chains by an RNA polymerase. Our data are most consistent with a model in which specific DNA-binding proteins facilitate a twin-domain mechanism to enhance DNA supercoiling during transcription. We also demonstrated that TCDS in the defined protein systems takes place by two separate, and apparently independent, mechanistic pathways *in vitro*. The first supercoiling pathway, which is not likely to be significant *in vivo*, was found to be dependent on R-loop formation but not on the length of the RNA transcripts, and could be suppressed by the presence of RNase HI or HU protein. The second pathway for TCDS was much more potent, but became predominant *in vitro* only when sequence-specific DNA-binding proteins were present during transcription. This major supercoiling route was shown to be resistant to RNase HI and had functional properties consistent with those predicted for the twin-supercoiled-domain model. Under optimal conditions, the twin domain pathway of TCDS rapidly and efficiently generated superhelicity levels more than twice that typically found *in vivo.* We also developed *in vivo* system to study TCDS in *E. coli.* Using these in vivo systems, we found that transcription by T7 RNA polymerase and *E. coli* RNA polymerase strikingly induced the formation of (--) supercoiled plasmid DNA. We also discovered that TCDS was dependent on the length of RNA transcripts, precisely predicted by the twin-supercoiled-domain model. One surprising finding is that (--) supercoiling of plasmid DNA did not require anchoring of DNA to the bacterial cytoplasmic membrane contrarily to what had been described previously, indicating that a transcribing RNA polymerase alone is sufficient to cause change of local DNA superhelicity. Finally, our results showed that TCDS in *topA* strains is dependent on promoter strength.

3. <u>*The mammalian high mobility group protein AT-hook 2 (HMGA2).*</u> The mammalian high mobility group protein AT hook 2 is a nuclear transcription factor associated with many physiological functions including oncogenesis, obesity, stem cell youth, human height, and human intelligence. It contains three “AT hook” DNA binding motifs and specifically recognizes the minor group of AT-rich DNA sequences. After over a decade of studies, my coworkers and I made numerous contributions to understand the structures and functions of this intriguing protein. We developed a simple and rapid purification procedure to purify HMGA2 in milligram range for biophysical studies. Using different biochemical methods, such as electrophoretic mobility shift assay (EMSA), and biophysical methods, such as isothermal titration calorimetry, we characterized how HMGA2 binds to different AT-rich DNA sequences. Using a PCR-based systematic evolution of ligands by exponential enrichment (SELEX) procedure, we have found that HMGA2 specifically binds to two specific DNA sequences 5’-ATATTCGCGAWWATT-3’ and 5’-ATATTGCGCAWWATT-3’ where W represents A or T. Additionally, we demonstrated that HMGA2 is a DNA bending protein. Recently, we invented a rapid and sensitive high throughput screening method to identify compounds targeting HMGA2-DNA interactions. Guided by this screening excise, we showed that netropsin, the specific inhibitor of HMGA2-DNA interactions strongly inhibited the differentiation of the mouse preadipocyte 3T3-L1 cells into adipocytes, most likely through a mechanism by which the inhibition is through preventing the binding of HMGA2 to the target DNA sequences. This method should be broadly applicable to identify compounds or proteins modulating many DNA-binding or RNA-binding proteins.

<u>*4. Fluorescently labeled circular DNA molecules for DNA topology and topoisomerases.*</u> DNA topology plays essential roles in several fundamental biological processes, such as DNA

replication, recombination, and transcription. Typically, agarose gel electrophoresis is employed to study DNA topology. Since gel electrophoresis is time-consuming and labor intensive, it is desirable to develop other methods, such as fluorescence-based methods, for such studies. In this paper we report the synthesis of a type of unique fluorescence-labeled DNA molecules that can be used to study DNA topology and topoisomerases by fluorescence resonance energy transfer (FRET) or supercoiling dependent fluorescence quenching (SDFQ). Specifically, we inserted an 82 nt. synthetic DNA oligomer FL905 carrying a 42 nt. AT sequence with fluorescein and dabcyl labels into a gapped DNA molecule to generate relaxed (rx) and supercoiled (sc) pAB1_FL905. Since the fluorescence intensity of pAB1_FL905 is dependent on its supercoiling status, pAB1_FL905 is a powerful tool to study DNA topology and topoisomerases by FRET. pAB1_FL905 can also be developed into rapid and efficient high-throughput screening assays to identify inhibitors that target various DNA topoisomerases.

5. *Synthesizing circular DNA in vitro.* Recently, I invented two novel biochemical methods for *in vitro* synthesis of Sc circular DNA. Linear DNA with two *loxP* sites in the same orientation is generated by PCR or rolling circle amplification (RCA). Cre recombinase efficiently converts the linear DNA into relaxed circular DNA. T5 exonuclease is then used to digest unwanted linear DNA, and topoisomerases are employed to generate Sc circular DNA. Using this approach, we synthesized EGFP-FL, a 2 kb mini-circular DNA encoding essential EGFP expression elements. EGFP-FL transfected HeLa and C2C12 cells with significantly higher efficiency than its *E. coli*-derived counterpart. These methods enable the efficient production of Sc circular DNA from 196 bp to several kb, and in quantities from micrograms to milligrams, providing a versatile, scalable, and bacteria-free platform for basic research and therapeutic applications. We have been awarded a patent for this invention (Patent Number: US 12,371,725 B2).