

**ADVANCED READINGS**  
**THEME 1: EVOLUTION OF CHEMOTAXIS**  
**2017 CPLC SUMMER SCHOOL**

**MODULE 1: EXPERIMENTAL EVOLUTION OF BACTERIAL MIGRATION**

**Part 1: High-throughput single-cell tracking**

Laboratory: Seppe Kuehn (UIUC Physics)

- Fraebel, D. T. *et al.* Environment determines evolutionary trajectory in a constrained phenotypic space. *Elife* **6**, e24669 (2017).

**Part 2: Manipulating genes: precision genetic engineering in *E. coli***

Laboratory: Tom Kuhlman (UIUC Physics)

- Tas, H., Nguyen, C. T., Patel, R., Kim, N. H. & Kuhlman, T. E. An Integrated System for Precise Genome Modification in *Escherichia coli*. *Plos One* **10**, e0136963 (2015).
- Kuhlman TE and Cox EC (2010). Site Specific Chromosomal Integration of Large Synthetic Constructs. *Nucleic Acids Research Methods Online*, PMID: 20047970.

**MODULE 2: MEASURING CHEMOTACTIC RESPONSES IN SINGLE CELLS**

Laboratory: Yann Chemla (UIUC Physics)

- Min, T.I., Mears, P.J., Chubiz, LM, Rao, CV, Golding, I., Chemla, Y.R. (2009), High-resolution, long-term characterization of bacterial motility using optical tweezers. *Nature methods* **6**, 831–835.
- Min, T.I., Mears, P.J., Golding, I., Chemla, Y.R. (2012), Chemotactic adaptation kinetics of individual *Escherichia coli* cells. *PNAS* **109**(25): 9869-9874, doi 10.1073/pnas.1120218109.

**MODULE 3: EVOLVING IN SILICO: STOCHASTIC SIMULATIONS OF EXPERIMENTAL EVOLUTION**

Theory: Nigel Goldenfeld (UIUC Physics)

- Gillespie, Daniel T. (1977). "Exact Stochastic Simulation of Coupled Chemical Reactions". *The Journal of Physical Chemistry*. **81**(25): 2340–2361.
- Netlogo tutorial (please download and review ahead of Summer School)  
<https://ccl.northwestern.edu/netlogo/docs/tutorial1.html>

2017 CPLC SUMMER SCHOOL

THEME 2: LIVE CELL IMAGING OF TRANSCRIPTION

Faculty: Ido Golding and Anna Sokac

TAs: Natalie Biel, Mengyu Wang and Jing Zhang

RECOMMENDED READING

- Lenstra et al., "Transcription dynamics in living cells", Annual Reviews of Biophysics (2016).  
*A recent review of live-cell studies of transcription kinetics across different organisms.*
- Golding et al., "Real-time kinetics of gene activity in individual bacteria", Cell (2005).  
*Describes the MS2-GFP method for labeling RNA in live cells, and how the method is used to measure the stochastic kinetics of transcription in E. coli.*
- Golding, "Single-cell studies of phage lambda: Hidden treasures under Occam's rug", Annual Review of Virology (2016).  
*A recent review of single-cell methods used for studying bacteriophage lambda.*
- Lau et al., "Spatial and temporal organization of replicating Escherichia coli chromosomes", Molecular Microbiology (2003).  
*Describes a method for labeling an individual gene locus in E. coli, and how the method is used to follow chromosome dynamics throughout the cell cycle.*
- Garcia et al., "Quantitative imaging of transcription in living Drosophila embryos links polymerase activity to patterning", Current Biology (2013).  
*Describes use of the MS2 method for following transcription in live Drosophila embryos.*
- Figard and Sokac, "Imaging cell shape change in living Drosophila embryos", Journal of Visualized Experiments (2011).  
*Describes a general method for imaging live Drosophila embryos.*
- Bothma et al., "Enhancer additivity and non-additivity are determined by enhancer strength in Drosophila embryos", eLife (2015).  
*Describes use of the MS2 method for following enhancer strength in live Drosophila embryos.*
- Xu et al., "Combining protein and mRNA quantification to decipher transcriptional regulation", Nature Methods (2015).  
*Describes a method to measure and relate transcription factor binding and mRNA production in Drosophila embryos.*

SOFTWARE TOOLS (Optional, for students comfortable with Matlab)

- <http://easerver.caltech.edu/wordpress/schnitzcells/>

“Schnitzcells”, Matlab code for cell recognition (Elowitz lab).

- <https://code.google.com/archive/p/spatzcells/>

“Spätzcells”, Matlab code for spot recognition (Golding lab).

- <https://github.com/PrincetonUniversity/FlyRNAQuant>

Matlab code for nuclei and RNA spot recognition in fly embryos (Bothma et al., 2015).

- <http://mtshasta.phys.washington.edu/website/SuperSegger.php>

“SupperSegger”, Matlab code for cell and spot recognition.

- <http://oufti.org/>

“Oufti”, software for cell and spot recognition.

**ADVANCED READINGS**  
**THEME 3: NEUROBIOLOGY THEME**  
**2017 CPLC SUMMER SCHOOL**

**MODULE 1: SUPER-RESOLUTION IMAGING OF NEURONAL SYNAPSE PROTEINS**

Laboratory: [Paul Selvin](#) (UIUC Physics)

- En Cai, Pinghua Ge, Sang Hak Lee, Okunola Jeyifous, Yong Wang, Yanxin Liu, Katie M. Wilson, Sung Jun Lim, Michelle A. Baird, John E. Stone, Kwan Young Lee, Michael W. Davidson, Hee Jung Chung, Klaus Schulten, Andrew M. Smith, William N. Green, Paul R. Selvin, "[Stable small quantum dots for synaptic receptor tracking on live neurons.](#)" *Angewandte Chemie International Edition*, Vol. 53 12484-12488 (2014).
- Ahmet Yildiz, Michio Tomishige, Ronald D. Vale, Paul R. Selvin. [Kinesin Walks Hand-Over-Hand.](#) *Science*, 303, 676-678 (2004)
- Yildiz, P. R. Selvin. Fluorescence Imaging with One Nanometer Accuracy (FIONA): Application to Molecular Motors. *Accounts of Chemical Research*, 38(7), 574-82 (2005)
- R. E. Thompson, D. R. Larson and W. W. Webb. Precise Nanometer Localization Analysis for Individual Fluorescent Probes. *Biophysical Journal*, Volume 82, Issue 5, 2775-2783, 1 May 2002
- Betzig, Eric, George H. Patterson, Rachid Sougrat, O. Wolf Lindwasser, Scott Olenych, Juan S. Bonifacio, Michael W. Davidson, Jennifer Lippincott-Schwartz, and Harald F. Hess. "Imaging intracellular fluorescent proteins at nanometer resolution." *Science* 313, no. 5793 (2006): 1642-1645.

**MODULE 2: OPTOGENETIC ACTIVATION OF NEURONAL CELL DIFFERENTIATION**

Laboratory: [Kai Zhang](#) (UIUC Biochemistry)

- Kennedy, M.J., et al., *Rapid blue-light-mediated induction of protein interactions in living cells.* *Nat Methods*, 2010. **7**(12): p. 973-5.
- Wu, Y.I., et al., *A genetically encoded photoactivatable Rac controls the motility of living cells.* *Nature*, 2009. **461**(7260): p. 104-8.
- Strickland, D., et al., *TULIPs: tunable, light-controlled interacting protein tags for cell biology.* *Nat Methods*, 2012. **9**(4): p. 379-84.
- Levskaya, A., et al., *Spatiotemporal control of cell signalling using a light-switchable protein interaction.* *Nature*, 2009. **461**(7266): p. 997-1001.
- Zhang, K. and B. Cui, *Optogenetic control of intracellular signaling pathways.* *Trends Biotechnol*, 2015. **33**(2): p. 92-100.
- Zhang, K., et al., *Light-Mediated Kinetic Control Reveals the Temporal Effect of the Raf/MEK/ERK Pathway in PC12 Cell Neurite Outgrowth.* *PLoS ONE*, 2014. **9**(3): p. e92917.

### MODULE 3: NEURAL NETWORK MODELING OF ZEBRAFISH LOCOMOTION

Laboratories: [Martin Gruebele](#) (UIUC Chemistry & Physics) & [Yann Chemla](#) (UIUC Physics)

- Kiran Girdhar, Martin Gruebele, Yann R. Chemla. (2015) The behavioral space of zebrafish locomotion and its neural network analog. *PLoS ONE* 10(7): e0128668.
- Kandice Fero, Tohei Yokogawa, Harold A. Burgess. (2011) The behavioral repertoire of larval zebrafish. Chapter in *Neuromethods* 52: 249-291.
- Greg J. Stephens, Bethany Johnson-Kerner, William Bialek, William S. Ryu. (2008) Dimensionality and Dynamics in the Behavior of *C. elegans*. *PLoS Comput Biol* 4(4): e1000028.
- Harold A. Burgess, Michael Granato. (2007) Sensorimotor gating in larval zebrafish. *J. Neurosci.* 27(18): 4984-4994.
- Scott A. Hill, Xiao-Ping Liu, Melissa A. Borla, Jorge V. Jose, Donald M. O'Malley (2005) Neurokinematic modeling of complex swimming patterns of the larval zebrafish. *Neurocomputing* 65: 61-68.

**ADVANCED READINGS**  
**THEME 4: PHYSICS OF DNA**  
**2017 CPLC SUMMER SCHOOL**

**MODULE 1: EXAMINING DNA FLEXIBILITY BY SINGLE-MOLECULE FRET AND COMPUTATIONAL ANALYSIS.**

**Part 1: smFRET measurement of DNA flexibility on sequences with varying modifications and genomic contexts**

Laboratory: [Taekjip Ha](#) (Johns Hopkins University)

**smFRET Technique**

- Single-Molecule Techniques: A Laboratory Manual. Cold Spring Harbor Laboratory Press ISBN 978-087969775-4, 507 pp (2008), Chapter 2, Joo, C. and T. Ha, "Single-Molecule FRET with Total Internal Reflection Microscopy."
- Roy, R., S. Hohng and T. Ha., "A practical guide to single-molecule FRET.", *Nat. Methods* 5, 507-516 (2008)

**DNA Flexibility**

- R. Vafabakhsh and T. Ha, "Extreme bendability of DNA less than 100 base pairs long revealed by single molecule cyclization", *Science* 337, 1097-1101 (2012).

**Hidden Markov Modeling-**

- Rabiner, LR. 1989. A Tutorial on Hidden Markov-Models and Selected Applications In Speech Recognition. *Proc. IEEE* 77 (2): 257-286.
- Liu, Y; Park, J; Dahmen, KA; Chemla, YR; Ha, T. 2010. A Comparative Study of Multivariate and Univariate Hidden Markov Modelings in Time-Binned Single-Molecule FRET Data Analysis. *J. Phys. Chem. B* 114 (16): 5386-5403.

**Part 2: Microscopic Mechanics of DNA**

Laboratory: [Alek Aksimentiev](#) (UIUC Physics)

- The VMD tutorial: <http://www.ks.uiuc.edu/Training/Tutorials/vmd/tutorial-html/index.html>
- The NAMD tutorial: <http://www.ks.uiuc.edu/Training/Tutorials/namd/namd-tutorial-unix-html/index.html>
- Thuy Ngo, Jejoong Yoo, Qing Dai, Qiuqen Zhang, Chuan He, Aleksei Aksimentiev and Taekjip Ha. Effect of Cytosine Modifications on DNA Flexibility and Nucleosome Mechanical Stability. *Nature Communications* 7:10813 (2016).
- Lu, X. and Olson, W. K. 3DNA: a versatile, integrated software system for the analysis, rebuilding and visualization of three-dimensional nucleic-acid structures. *Nature Protocols* 3(7): 1213-1227 (2008). *Describes the method of analyzing DNA conformations that we will be using.* (pdf attached)
- Maffeo, C., Yoo, J., Comer, J., Wells, D.B., Luan, B., and Aksimentiev, A. Close Encounters with DNA. *J Phys Condens Matter* 26:413101(2014). *A review covering DNA mechanics.* (pdf attached)

## MODULE 2: EXAMINING GENOME SCALE DNA FLEXIBILITY

### **Part 1: Loop-Seq: Genome scale DNA flexibility measurement using sequencing**

Laboratory: [Taekjip Ha](#) (Johns Hopkins University)

- R. Vafabakhsh and T. Ha, “Extreme bendability of DNA less than 100 base pairs long revealed by single molecule cyclization”, *Science* 337, 1097-1101 (2012).

### **Part 2: Loop-Seq: Computational analysis of nucleosome positioning sequences**

Laboratory: [Jun Song](#) (UIUC Physics and Bioengineering)

- H. Jin, H.T. Rube, J.S. Song. Categorical spectral analysis of periodicity in nucleosomal DNA. [Categorical spectral analysis of periodicity in nucleosomal DNA](#). *Nucleic Acids Research*, 44(5), 2047-2057, (2016).
- Widom, J. Role of DNA sequence in nucleosome stability and dynamics *Quarterly Reviews of Biophysics* 34, 3 (2001), pp. 269–324. (pdf attached)
- Heng Li and Richard Durbin. Fast and accurate short read alignment with Burrows–Wheeler transform. *BIOINFORMATICS Vol. 25 no. 14 2009, pages 1754–1760*, doi:10.1093/bioinformatics/btp324. (pdf attached)
- Ben Langmead, Cole Trapnell, Mihai Pop and Steven L Salzberg. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* 2009, 10:R25 (doi:10.1186/gb-2009-10-3-r25). (pdf attached)
- Ch. 3 Linear Methods for Regression from <http://statweb.stanford.edu/~tibs/ElemStatLearn/>. (pdf attached)

**ADVANCED READINGS**  
**THEME 5: QUANTITATIVE IMAGING AND CELL SIMULATION**  
**OF SMALL REGULATORY RNA (FEI/LUTHEY-SCHULTEN LABS)**  
**2017 CPLC SUMMER SCHOOL**

**MODULE 1: FISH/STORM IMAGING OF sRNA**

**Laboratory:** [Jingyi Fei](#) (University of Chicago Biochemistry and Molecular Biology)

- Fei J, Singh D, Zhang Q, Park S, Balasubramanian D, et al. (2015) RNA biochemistry. Determination of in vivo target search kinetics of regulatory noncoding RNA. *Science* 347: 1371-1374.
- Huang B, Wang W, Bates M, Zhuang X (2008) Three-dimensional super-resolution imaging by stochastic optical reconstruction microscopy. *Science* 319: 810-813
- Bates M, Blosser TR, Zhuang X (2005) Short-range spectroscopic ruler based on a single-molecule optical switch. *Phys Rev Lett* 94: 108101.
- Rust MJ, Bates M, Zhuang X (2006) Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat Methods* 3: 793-795.

**MODULE 2: LATTICE MICROBES CELL SIMULATIONS OF RNA METABOLISM**

**Laboratory:** [Zaida Luthey-Schulten](#) (UIUC Chemistry & Physics)

- Peterson JR, Cole JA, Fei J, Ha T, Luthey-Schulten ZA (2015) Effects of DNA replication on mRNA noise. *Proc Natl Acad Sci USA* 112: 15886-15891.
- E. Roberts, J. E. Stone, and Z. Luthey-Schulten (2013) Lattice microbes: high-performance stochastic simulation method for the reaction-diffusion master equation. *J. Comput. Chem.*, 34(3), 245-55.
- T.M. Earnest, R. Watanabe, J.E. Stone, J. Mahamid, W. Baumeister, E. Villa, Z. Luthey-Schulten (2017) Challenges of Integrating Stochastic Dynamics and Cryo-Electron Tomograms in Whole-Cell Simulations, *J. Phys. Chem. B*, 121(15):3871-3881
- T.M. Earnest, J.A. Cole, J.R. Peterson, M.J. Hillock, T.E. Kullman, Z. Luthey-Schulten (2016), Ribosome biogenesis in replicating cells: Integration of experiment and theory, *Biopolymers*. 105(10):735-51. doi: 10.1002/bip.2289.
- PDFs can be found here:  
<https://drive.google.com/drive/folders/0B3CnojVyw9uQVlhJUlowc0hDWE0?usp=sharing>



**ADVANCED READINGS**  
**THEME 6: RNA REGULATION IN EUKARYOTES**  
CPLC Summer School 2017  
University of Illinois at Urbana-Champaign

**MODULE 1: EXAMINATION OF SPLICEOSOMAL RNA FOLDING BY SINGLE-MOLECULE FRET & MOLECULAR DYNAMICS**

**Part 1: smFRET measurement of RNA folding**

Laboratory: [Aaron Hoskins](#) (U Wisconsin Biochemistry)

**Advanced Reading Materials**

- Roy, R., Hohng, S., and T. Ha. "A practical guide to single-molecule FRET" *Nature Methods* (2008) **5(6)**:507-16
- van der Feltz, C., and A. A. Hoskins. "Methodologies for studying the spliceosome's RNA dynamics with single-molecule FRET" *Methods* (2017) doi: 10.1016/j.ymeth.2017.05.011
- Rodgers, M.L., Didychuk, A.L., Butcher, S.E., Brow, D.A., and A. A. Hoskins. "A multi-step model for facilitated unwinding of the yeast U4/U6 RNA duplex" *Nucleic Acids Res.* (2016) **44(22)**:10912-28

**Further Reading Material**

- Joo, C., Balci, H., Ishitsuka, Y., Buranachai, C., and T. Ha. "Advances in Single-Molecule Fluorescence Methods for Molecular Biology" (2008) *Annu. Rev. Biochem.* **77**:51-76
- Blanco, M., and N. G. Walter. "Analysis of Complex Single-Molecule FRET Time Trajectories" (2010) *Methods in Enzymology* **472**:153-78
- Wahl, Markus C., Cindy L. Will, and Reinhard Luhrmann. "The Spliceosome: Design Principles of a Dynamic RNP Machine" *Cell* (2009) **136**:701-18.

**Part 2: Simulation of Spliceosome**

Laboratory: [Zan Luthey-Schulten](#) (UIUC Chemistry)

**Advanced Reading Materials**

**Background**

- Core structure of the U6 small nuclear ribonucleoprotein at 1.7-Å resolution, E Montemayor, E Curran, H Liao, K Andrews, C Treba S Butcher & D Brow, *Nat Struc Mol Biol*, 2014 Jun;21(6):544-51

**Molecular Dynamics simulations tutorials**

- NAMD tutorial: <http://www.ks.uiuc.edu/Training/Tutorials/namd/namd-tutorial-unix-html/index.html>
- VMD tutorial: <http://www.ks.uiuc.edu/Training/Tutorials/vmd/tutorial-html/index.html>
- QwikMD tutorial: <http://www.ks.uiuc.edu/~trudack/QwikMD/index.html>

**MODULE 2: RNA INTERFERENCE IN MAMMALIAN CELLS MEASURED BY SINGLE-MOLECULE FISH**

Laboratory: [Sua Myong](#) (Johns Hopkins University Biochemistry & Biophysics)

TAs Hye Ran Koh ([hkoh6@jhu.edu](mailto:hkoh6@jhu.edu)); Amirhossein Ghanbari Niaki ([aghanba1@jhu.edu](mailto:aghanba1@jhu.edu))

- Raj, Arjun, et al. "Imaging individual mRNA molecules using multiple singly labeled probes." *Nature methods* 5.10 (2008): 877-879. ([Link](#))
- Padovan-Merhar, Olivia, et al. "Single mammalian cells compensate for differences in cellular volume and DNA copy number through independent global transcriptional mechanisms." *Molecular cell* 58.2 (2015): 339-352. ([Link](#))
- Mueller, Florian, et al. "FISH-quant: automatic counting of transcripts in 3D FISH images." *Nature methods* 10.4 (2013): 277-278. ([Link](#))