Name: Emily Hayden
Research Advisor: Noel Clark
Research Field: Chromonic Liquid Crystals and RNA NTPs
Expected Semester of Graduation: Spring 2018

Liquid Crystal Phases of RNA NTPs

Prospectus:

Earlier work with Deoxyribonucleic Acid (DNA) has shown that it can form columnar liquid crystal phases in both short base-pair oligomer solutions and solutions of single DNA Nucleoside Tri-Phosphates (dNTPs) [1]. This increase in complexity may be useful when discussing the effect of liquid crystals and their phase behavior in prebiotic environments and in relation to molecular evolution. Our group's research into this phenomenon and its relevance to the study of pre-biotic systems is currently under review for publication [6].

Ribonucleic acid (RNA) and its nucleoside triphosphates (rNTPs) are the primary focus of this work, and they are closely related to DNA and the dNTPs discussed above. RNA differs from DNA in that, rather than containing a Deoxyribose sugar, it contains a Ribose sugar, which has two hydroxyl groups. These hydroxyl groups make the RNA less stable in solution because of its propensity for hydrolysis [3]. This lack of stability makes RNA difficult to work with, but because it is considered an early precursor of DNA, it is interesting to study--specifically with regards to the RNA world hypothesis. Another difference from DNA is that RNA does not contain Thymine—this is replaced by Uracil (U), which differs from Thymine only in that it lacks a methyl group.

My work is focused on the liquid crystals formed by the complementary rNTPs Cytidine Triphosphate (rCTP) and Guanosine Triphosphate (rGTP). When placed into a solution, these molecules are able to bond with another to form base-pairs. The base-pairs may then stack on top of one another to shield their hydrophobic center from the surrounding solution. These stacks can then orient into a columnar LC phase, where this self-assembly further promotes the stabilization of the aggregates. Additionally, there is the potential for G-quadraplexes to form from four rGTP molecules bonding to one another. A columnar LC phase can be formed by these G-quadraplexes when they stack on top of one another. The LC structure of the mixture can be probed using both polarized light microscopy (PLM), which will provide information regarding the general structure and morphology of the LC phases, and X-ray Diffraction (XRD), which will provide data regarding the type and spacing of the LC structures. The XRD data will also show whether liquid crystals formed by rCTP+rGTP base-pairs co-exist with those formed by G-quadraplexes, and what the temperature and concentration parameters are that determine the type of coexistence.

Bibliography:

[1] Nakata, M., Zanchetta, et. al. *End-to-End Stacking and Liquid Crystal Condensation of 6– to 20–Base Pair DNA Duplexes*. Science. Vol. 318, Issue 5854:1276-1279. (2007). DOI: 10.1126/science.1143826.

[2] Alberts, B., Johnson, A., Lewis, .J, et al. *The Structure and Function of DNA*. Molecular Biology of the Cell. 4th edition. New York: Garland Science; (2002).

[3] Zanchetta, G. *Liquid Crystalline Phases in Oligonucleotide Solutions.* Doctoral Thesis. Universita degli Studi di Milano. Web. 07 July 2017.(2007).

[4] Strey, H., Parsegian, V. and Podgornik, R. *Equation of State for DNA Liquid Crystals: Fluctuation Enhanced Electrostatic Double Layer Repulsion*. Phys. Rev. Lett. Issue:78, 895–898 (1997).

[5] Maniatis, T., Jeffrey, A. and van deSandet, H. *Chain Length Determination of Small Double-and Single-Stranded DNA Molecules by Polyacrylamide Gel Electrophoresis*, Biochem., Issue:14, 3787-3794 (1975).

[6] Smith, G., Fraccia, T, et. al. *Watson-Crick Base-Pairing and Duplex Stacking of Nucleic Acid Monomers in Aqueous Solution.* Article submitted for publication, (2017).

[7] Davis, J.T. & Spada, G.P. *Supramolecular architectures generated by self-assembly of guanosine derivatives*. Chem. Soc. Rev. 36, 296–313 (2007). DOI: 10.1039/B600282J.

[8] Joyce, G.F. *The antiquity of RNA-based evolution.* Nature. Vol. 41, 214-221. (2002). DOI: 10.1038/418214a.

[9] Senyuk, B. “Liquid Crystals: A Simple View on a Complex Matter.” SPIE Student Chapter at Kent State University. Kent State University. Web. 7 July 2017.

[10] Yamaguchi, Akihiro, "Self-Assembly of Lyotropic Chromonic Liquid Crystal Mixtures". *Undergraduate Honors Theses*. 973. (2015).

[11] Kleman, M and Lavrentovich, O.D., *Soft Matter Physics*, Springer (2003).

[12] Dierking, I., *Textures of Liquid Crystals,* Wiley-VCH (2003).

[13] Seddon, J.M., *Structural Studies of Liquid Crystals by X-Ray Diffraction*. In Demus, D., Goodby, J., Gray, G. W., Spiess, H.-W. and Vill, V., Handbook of Liquid Crystals Set, Wiley-VCH. (1998). DOI: 10.1002/9783527619276.ch8ca.

Timeline

Fall 2016

- Replicate Sunset Yellow phase diagram using PLM data
- Make Allura Red+Sunset Yellow phase diagram
- dNTP's phase diagram (various mixtures)
- DNA Oligomers Phase diagrams

Spring 2017

- RNA NTP's analysis
    - Mixtures of rATP+rUTP—PLM and XRD
        - pH adjustments, ion replacement, etc.
        - rATP+dTTP, dATP+rUTP
        - Begin work with rCTP+rGTP—A+U mixtures do not form LC's

Summer 2017

- Analyze X-ray data--Synchrotron (rATP+rUTP)
- dATP+dUTP images for SMRC paper
- Begin work with rC, rG, and combinations
    - Analyze domain development and adjustment, morphology, etc. (PLM)
        - G-quartets
    - X-ray Diffraction Capillaries and Data
    - Prepare initial and adjusted phase diagram of mixtures
- Begin writing Thesis Chapters (can use for REU paper)
- Poster Presentation at ISSOL conference in San Diego
- 08/10: REU final presentation and paper submitted

Fall 2017:

- Experimentation (complete by 12/12)
    - X-ray data of C+G samples (varying temperature and concentration)
    - Calculate sign of birefringence
    - Flat capillaries to see ordering of domains dependent on evaporation
    - 4 NTP's combined in evap. Cell—LC morphology
- Thesis Application
    - Determine/Contact Committee Members
    - Choose date
    - Complete Prospectus, Bib and Timeline
    - Submit Application (10/03)
- Thesis Writing goals/deadlines
    - Sections to write:
        - Concentration Adjustment Methods
        - Methods and Materials—XRD section
        - Experiment and Analysis—XRD Data/Correlation with PLM
        - Conclusion and Results
            - Phase Diagram
            - rA+rU analysis section

Spring 2018

- 01/2018: RD of thesis complete
  - Further experimentation work finalized
- 02/2018: First edit of thesis to Dr. Clark and Greg
  - Finalize graphs, figures, and tables.
  - Complete XRD data analysis and PLM photography
- 03/2018: Defend