Effects of high magnetic fields on *in vitro* transcription

Marianna Worczak[1§*], Kim Wadelton[1±*], James Ch. Davis[1], Anna-Lisa Paul[2], and Mark W. Meisel[1]

[1]*Department of Physics, University of Florida, Gainesville, FL 32611-3440*
[2]*Department of Horticulture and the Biotechnology Program, University of Florida, Gainesville, FL 32611-0690*

Abstract:

Transcripts from *in vitro* reactions employing T7 and SP6 RNA polymerases were used to identify potential effects of high magnetic fields on biological molecules. Plant experiments performed exhibited genetic stress responses to high magnetic fields. Our investigations were designed to study the effects on *in vitro* transcription, the process where a RNA transcript is produced from the DNA template of a gene. Commercially available T7 and SP6 *in vitro* transcription kits were used to conduct transcription reactions in high magnetic fields. The length and quantity of the transcripts were subsequently analyzed. Preliminary results with T7 in fields up to 9 Tesla showed no shortening of transcripts but may indicate a delayed transcription rate.

Introduction:

The study of biological molecules in high magnetic fields began in the early 1970's, when major advances in NMR machinery allowed for the observation of subtle effects of high magnetic fields on such molecules. Researchers observed the ability of nucleic acids to align with the magnetic field, as well as certain phospholipids. Magnetic effects on protein orientation were originally considered in theoretical calculations of the magnetic anisotropy of peptide bonds (Pauling 1979). Later, alignment of protein crystals in magnetic fields was observed (Astier 1998). The tendency of phospholipids to align in magnet fields has also lead to their use as solvents for protein NMR. The alignment of these lipid bicelles force partial alignment of globular proteins allowing for increased resolution of NMR images (Zweckstetter 2000). The potential alignment of biological molecules in magnetic fields has raised the question of how biological processes might be affected by the presence of strong magnetic fields. Work involving growth of *Arabidopsis thaliana* in high magnetic fields noted an alcohol dehydrogenase stress response (Paul 2005). This observation indicated that the magnetic field resulted in some alteration of a normal biological function, which affected gene expression. Since many reactions and environmental interactions influence gene expression in a living system, understanding the mechanism of magnetic field effects was difficult. Hence, to

[§] Department of Chemistry, Clarkson University, Potsdam, NY 13699-2389
Contact Information: worczakm@clarkson.edu
[*] Summer 2005 NHMFL REU Program Participant
[±] Department of Physics and Engineering, Sweet Briar College, Sweet Briar, VA 24595
Contact Information: wadelton07@sbc.edu

investigate the affects of high magnetic fields on an isolated biological process, our work choose to investigate *in vitro* transcription.

Transcription is the first of many steps in protein synthesis (Garrett 2005). Upon activation of the promoter region of DNA, RNA polymerases bind to a specified region and initiate the formation of an RNA template, called a transcript, with base pairs complementarily matching those of the template DNA strand. This newly synthesized RNA is either processed further or directly used as a template for the process of translation. Each set of three nucleotides in the RNA transcripts designates one of 20 amino acids to be placed in sequence to form a peptide. Through a variety of interactions, this polypeptide sequence then forms a three dimensional protein potentially used for structural purposes or as an enzyme to facilitate a biochemical reaction. Essentially, the process of transcription is constantly occurring within a living organism and is a critical step in gene regulation.

In vitro transcription reactions were conducted under high magnetic fields in order to provide a simplified model of the effects magnetic fields might induce *in vivo* such as in *Arabidopsis*. Commercially available *in vitro* transcription kits contain RNA polymerases isolated from bacteriophages (Adams 2002). These polymerases, such as T7 and SP6 as used in our work, are single unit globular proteins, which maintain active site structure and function similar to multisubunit polymerases typically found in higher organisms (Place 1999). Since phage polymerases have faster kinetics than their multisubunit homologs, large amounts of RNA can be quickly produced for study (Tahirov 1997).

The working hypothesis fueling our experiments was that the magnetic field acting on anisotropies already present in the RNA polymerases would lead to an alteration in the three dimensional shape of the protein. This alteration, whether it be a partial alignment or substantial distortion, was hypothesized to have some effect on enzymatic function. Companion work by coauthor Wadelton investigates theoretical effects of forces on T7 RNA polymerase in an applied magnetic field (Wadelton 2005). Hence, research proceeded to investigate whether transcription reactions within the field produced shorter, mismatched, or decreased-quantity transcripts compared to control reactions.

Materials and Methods:

T7 Transcription Reactions:

Reactions were performed evaluating transcripts produced by transcription of the control template provided in the Ribomax T7 Express *In Vitro* Transcription Kit purchased from Promega Corporation. Control reactions were completed to determine the simplest experimental conditions feasible for magnet runs. Reactions included controls at ambient temperatures and at 37°C. An additional frozen-start reaction was conducted in which all reagents except for the enzyme itself were mixed and frozen at -20°C. Preparation for the frozen-start method involved mixing Express T7 2x Buffer, Nuclease-Free Water, and pGem Express Positive Control Template. The frozen-start reaction was then initiated by addition of Ribomax T7 Express Enzyme mix atop the frozen layer. Comparison of control reactions, and the frozen-start reaction

indicated that the T7 reactions could be run at room temperature, and that the frozen-start method did not noticeably affect transcript production.

Support and positioning for reactions conducted within the magnet involved the suspension of a small bucket down the magnet bore. The bucket was positioned such that the sample holding-compartment rested directly in the center of the homogenous region of the magnet. While reactions were not heated during the experiment, the temperature within the bore of the magnet was monitored by a thermocouple and recorded for the sake of completion. The reactions were contained in 1ml eppendorf tubes attached to a rigid lowering apparatus and carefully placed into the sample-holding compartment in the bucket. To remove aliquots of reactions, the lowering apparatus was quickly removed and then replaced. Samples were then quickly plunged into liquid nitrogen to terminate reactions.

For reactions performed in magnetic fields up to 9 Tesla, a mastermix was prepared. This mixture included the Express T7 2x Buffer, nuclease-free water, and pGem Positive Control Template. This solution was then split into three aliquots; one each for control reactions, 4.5 Tesla reactions, and 9 Tesla reactions. Once divided, the individual eppendorf tubes were frozen and stored until reactions were completed. Control reactions were performed in the bore of the magnet prior to generation of a magnetic field. For each magnetic field strength, carefully placing the enzyme atop the frozen mixture and lowering the sample into the sample vesicle initiated reactions. A 10µl aliquot of the reaction was removed at each time point (1, 5, 10 and 20 minutes). Each aliquot was quickly placed into a separate eppendorf and then deposited into a vat of liquid nitrogen to terminate the reaction. The reaction remained in the liquid nitrogen until all time point samples were obtained and frozen. RQ1-RNase-free DNase was then added in a concentration of 1µl/µg of template to remove the DNA template and ensure that the reaction was indeed terminated. Reactions were then stored at -20ºC until analyzed.

SP6 Reactions:

To study magnetic effects on transcription reactions involving SP6 RNA polymerase, SP6 Ribomax *In Vitro* Transcription Kit was purchased from Promega Corporation. While the T7 kit was designed to require a maximum incubation time of thirty minutes, the SP6 reactions required an incubation of 2-4 hours. Control tests at ambient temperature and shorter incubations failed to produce transcripts, requiring all reactions to be run at 37°C. As with the T7, reactions were preceded by preparing a mastermix of all reagents excluding the SP6 polymerase. A frozen start method was also employed. Initially, SP6 Reaction Buffer, Nuclease-Free Water, 100mM rNTPs (ATP, CTP, GTP and UTP), and Control Template (1800base pair luciferase-encoding template) were thoroughly mixed and frozen until needed for transcription reactions.

Control reactions were performed in the magnet bore prior to generation of a magnetic field. Other tests included reactions at 4.5 and 9 Tesla. Immediately prior to the initiation of each test reaction, Enzyme Mix (SP6) was added directly above the frozen mixture of other reagents. Reaction initiation was achieved by lowering the reaction into the magnet. Aliquots were removed from each reaction at time points of 2, 60, 90 and 120 minutes. Removed aliquots were placed in separate eppendorf tubes and immediately terminated by freezing in liquid nitrogen.

Following termination in liquid nitrogen, RQ1-RNase-Free DNase was added to remove the template. Samples were placed in a -20°C freezer for storage until electrophoresis.

The experimental configuration for SP6 reactions resembled that for T7 reactions with the addition of a hot water bath to maintain a temperature of 37°C. Temperature maintenance was achieved by employing a hot water circulator. The circulator bath was set to a temperature of 41.6°C, and pumped through a system of PVC piping and tubing into a copper coil. The coil was suspended in the bucket where it could heat the water within the bucket. A thermocouple was placed alongside the sample to monitor the temperature. The same rigid sample placement device was used to lower the sample into the sample holder. Aliquots were removed at designated time points as with T7 and terminated likewise in liquid nitrogen.

Electrophoresis:

Determination of transcript length, as well as comparisons of transcript quantity were performed by agarose gel electrophoresis. Samples were run in denaturing agarose gels containing formaldehyde and ethidium bromide. A volume of 4µl of each reaction mixture was combined with 8µl of RNA loading buffer. The samples were then incubated at 55°C for 10 minutes to allow for denaturation. A total volume of 9µl of each sample was then loaded into the gel. Gels were run at a constant voltage of 100V for 25 minutes. Gel images were obtained by the Kodak Gel Logic 440 Imaging System and Kodak 1D 3.6 software. Resulting electrophoresis photographs were then analyzed by BioRad Quantity One gel imaging software determine volume ratios of band intensities to provide quantitative data.

Results and Discussion:

T7 Reactions:

Reactions up to 9 Tesla were initially compared via gel electrophoresis. The pGem Positive Control Template serving as the DNA source for the T7 experiment produces two transcripts of 1.1kb and 2.3kb in length. Figure 1 depicts results from bore control, 4.5 Tesla, and 9 Tesla reactions at time points of 1, 5, 10, and 20 minutes. Direct visual examination of electrophoresis data reveals increased transcript production with time in the control reactions, and those run in 4.5 and 9 Telsa fields. The presence of only the two expected bands (1.1kb and 2.3kb) indicates that the presence of the fields at such strengths does not directly lead to termination of transcription. For all end points, in each of the three reactions, full length transcripts were observed. In addition high concentrations, at the 20 minute endpoints were noted. These results indicate that the catalytic function of T7 RNA polymerase is not significantly disrupted by fields up to 9 Tesla. In addition, these results indicate that peak enzyme activity occurs between 10 and 20 minutes of incubation.

T7 Electrophoresis Results

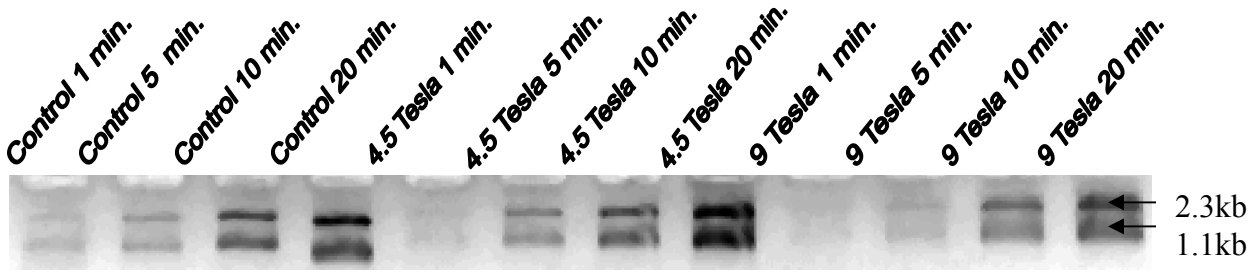

Figure 1: Agarose gel results for bore Control, 4.5 Tesla, and 9 Tesla reactions at time points of 1, 5, 10 and 20 minutes. Control template pGem (Promega) used for the reactions produces bands at 1.1kb and 2.3kb.

While visual inspection verifies the presence of full-length transcripts and a progressive increase in transcript quantity with time, such an evaluation does not provide quantitative evidence. To provide a more precise and quantitative comparison of transcripts produced, band intensities were determined using BioRad Quantity One imaging software. Figure 2 depicts a graphical representation of the relative intensity of transcript bands as a function of time for control, 4.5 and 9 Tesla T7 reactions. Since each reaction set was initiated by the addition of enzyme to one aliquot of the original mastermix, it was possible to directly compare time point intensities within each set. Such a comparison provided the conclusion that in all cases, transcript quantity increased with the reaction duration. However, it was not possible to directly compare rates the intensity measurements at any given time point between conditions. To compensate for the impossibility of direct comparisons, normalization of data was preformed. In doing so, the basal intensity was decreased for all data by a 2300 units. Next, the intensities in each reaction set were divided by the intensity at 1 minute (effectively the zero point of the reaction).

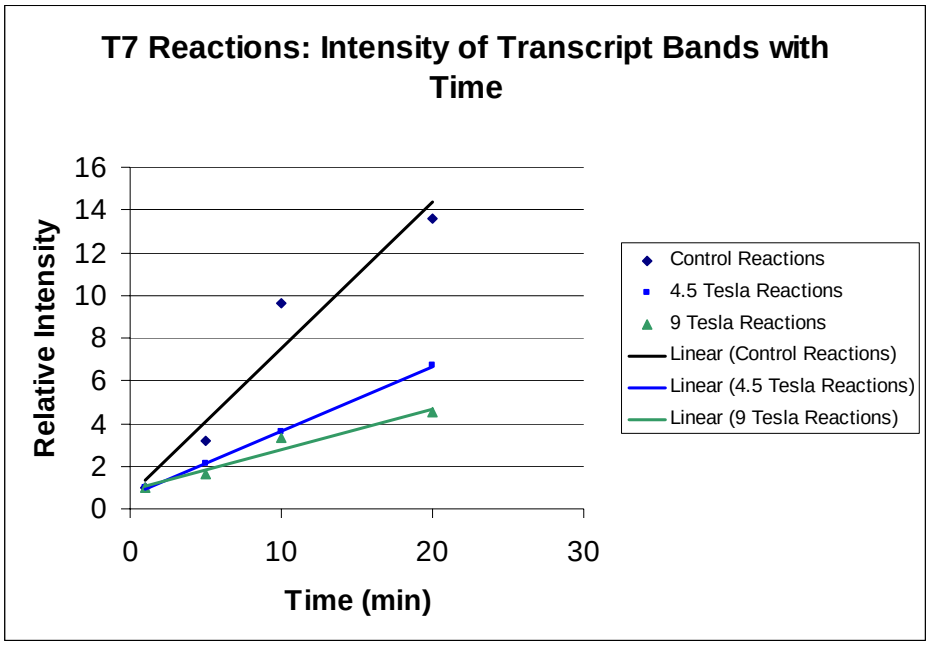

Figure 2: A graphical representation of transcript production with time for control, 4.5 and 9 Tesla T7 reactions. Transcript quantity is depicted as the relative intensity of electrophoresis bands as determined by BioRad Quantity One software. The graph indicates a decrease in rate with reactions occurring in the magnetic field when compared to the bore control.

From the plotted, normalized, data it appears that the rate of T7 production of transcript is slightly delayed with increasing magnetic field. This delay is indicated by an overall decrease in slope with increasing field strength. This subtle change in transcript production could be a result of altered three dimensional morphology of the enzyme, therefore decreasing its effectiveness as a catalysis. However, since large quantities of transcripts are indeed produced even at fields up to 9 Tesla, the enzyme retained the bulk of its activity.

Promega's Ribomax T7 Express *In Vitro* Transcription Kit used in the experiment is designed to produce rapid quantities of RNA in a very short duration of time. Normally T7 polymerase activity is fast-acting, at a rate of approximately 200 base pairs a second (Tahirov 2002). However the kit contains the additional enzyme yeast pyrophosphatase, as well as a concentration of ribonucleotide triphosphates in a ratio that actually increases the speed of transcription (Adams 2002). Hence, in the experiment shortening of transcripts would have been expected if the magnetic field had a rapid and substantial effect on the enzymes structure. Since full length transcripts were produced in control reactions even after one minute, it was unlikely to see such results. Other T7 *in vitro* transcription kits which require two to four hour incubation periods exists but are unlikely to produce shortened transcripts in the field because the normal activity of T7 may still be too rapid to observe such a dramatic effect.

SP6 Reactions:

For SP6 reactions samples for control, 4.5 and 9 Tesla reactions at time points of 2, 60, 90 and 120 minutes were analyzed by gel electrophoresis. Figure 3 depicts gel electrophoresis results for SP6 reactions. Since the DNA template used for SP6 reactions codes for the luciferase gene, normal transcription is indicated by the production of a single 1.8kb transcript. For each reaction set (control, 4.5 and 9 Tesla) transcripts were produced at each time point. Control reactions and 4.5 Tesla reactions appeared to produce approximately the same quantity of transcript. However, reactions run in the 9 Tesla field show significantly less transcript. Decreased transcript suggests that at a field strength of 9 Tesla SP6 RNA polymerase exhibits decreased function. As with T7 full length transcripts were seen in each reaction providing no indication of shortened transcripts. It is also worth noting that the intensity between time point so 60, 90 and 120 minutes were in all cases approximately equal. This indicates that transcripts are produced in large quantities before 60 minutes of incubation. Further test at lower time points may be necessary to visualize any change in the rate of the reaction as observed in T7

SP6 Electrophoresis Results

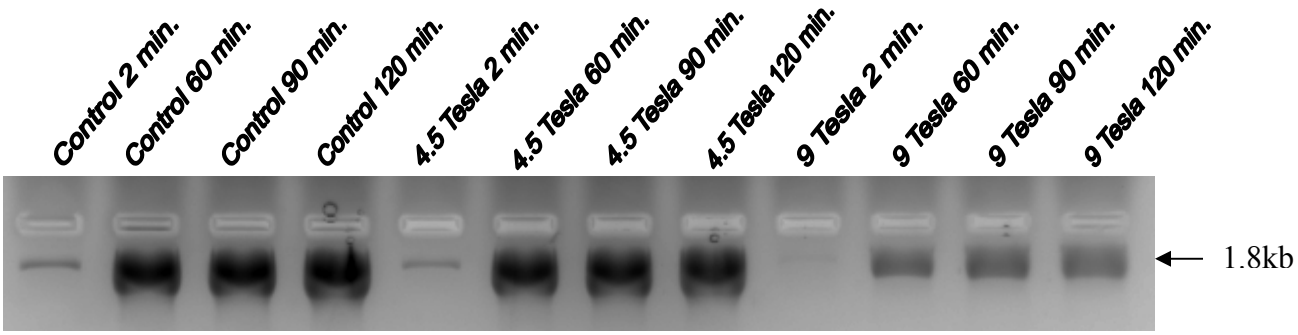

Figure 3: Agarose gel results for bore Control, 4.5 Tesla, and 9 Tesla reactions at time points of 1, 5, 10 and 20 minutes. The control template contains the luciferase gene coding for a single 1.8kb transcript. A decrease total transcript production may be noted for 9 Tesla reactions in comparison to control and 4.5 Tesla reactions.

Conclusions:

The purpose of our work was to determine if high magnetic fields have measurable effects on single transcription processes. This was investigated to help explain stress responses in plants in high magnetic fields, and to better understand the effects of magnetic fields on living organisms. The hypothesis was that the magnetic field would somehow affect RNA polymerases leading to shortened transcripts, mistranscription, or an overall decrease in transcript produced. To test the hypothesis, experiments employing T7 and SP6 polymerase *in vitro* transcription kits were preformed in control environments in the presence of 4.5 Tesla and 9 Tesla fields. Results analyzed by gel electrophoresis indicate that shortening of transcripts did not occur. However, T7 results suggest a subtle delay in transcript production with increasing magnetic field; which indicates the potential of some minor effects of the magnetic field on transcription. Results from SP6 indicate an overall decrease in transcript produced for reactions at 9 Tesla but not at 4.5 Tesla. While SP6 and T7 are both bacteriophage RNA polymerases, they differ slightly in structure. If results observed are indeed occurring due to some alteration of polymerase

conformation, the observed difference in effects between SP6 and T7 reactions may be due to their slight difference in structure. Future tests are necessary at higher field strengths to determine if greater fields augment and verify effects already observed. In addition, experiments in simple organisms such as bacteria may allow for control of some variables confounding original plant studies, while still providing a system more sensitive to subtle magnetic field effects than detectable with the *in vitro* kits.

Acknowledgements:

This work was partially supported by the National High Magnetic Field Laboratory (NHMFL) summer 2005 REU program at the University of Florida in Gainesville. Work was also supported in part by NSF DMR-0305371 and NASA grant NNA04CC61. We acknowledge R.J. Ferl and W.B. Gurley for enlightening conversations.

Additional Notes:

A significant portion of early work on this REU project involved an extensive literature search. Several works not explicitly detailed cited in this manuscript were vital to understanding the function, structure and kinetics of T7 and SP6 RNA polymerases. Works assisting our experimental design are included in Appendix A.

Works Cited:

Adams, Julie, Bill Patterson, and Mandy Wautlet. (2002) *Take the "Express Way" to RNA. Fast and Efficient Production of RNA with the T7 Ribomax Express System.* Promega Notes (www.promega.com) 5-9.

Astier, Jean Pierre, Stephane Veesler, and Roland Boistelle (1998) *Protein crystals orientation in a magnetic field.* Acta Crystallographica Section D (D54) 703-706.

Paul , A.-L. et al (2005) *Strong Magnetic Field Induced Changes of Gene Expression in Arabidopsis.* Materials Processing in Magnetic Fields: Proceedings of the International Workshop on Materials Analysis and Processes in Magnetic Fields (NHMFL, Tallahassee, 17-19 March 2004). To appear fall 2005.

Pauling, Linus (1979) *Diamagnetic anisotropy of the peptide group.* Biophysics (76) 2293-2294.

Place, Christopher et al. (1999) *Studies of Contacts between T7 RNA Polymerase and Its Promoter Reveal Features in Common with Multisubunit RNA Polymerases.* Biochemistry (38) 4948-4957.

Garret, Reginald H. and Charles M. Grisham.(2005) Biochemistry (third edition) Thompson Brooks/Cole, Belmont, CA. pgs 943-984.

Tahirov, Tahir H et al. (2002) *Structure of a T7 RNA polymerase elongation complex at 2.9 A resolution.* Nature (420) 43-50.

T7 RiboMax Express Large Scale RNA Production System Technical Bulletin. Promega. (www.promega.com).

Wadelton, Kim et al. (2005) *Diamagnetic Anisotropy of T7 RNA polymerase.* Report of research preformed Summer 2005 as part of NHMFL REU Program.

Zweckstetter, Markus and Ad Bax (2000) *Prediction of Sterically Induced Alignment in a Dilute Liquid Crystalline Phase: Aid to Protein Structure Determination by NMR*. Journal of the American Chemical Society (122) 3791-3792.

Appendix A:

Alexander, Kukarin, Minqing Rong and William T. McAllister (2003) *Exposure of T7 RNA Polymerase to the Isolated Binding Region of the Promoter Allows Transcription from a Single-stranded Template.* The Journal of Biological Chemistry. (278) 2419-2424.

Adelman, Karen et al. (2002) *Single molecule analysis of RNA polymerase elongation reveals uniformed kinetic behavior*. Proceedings of the National Academy of Sciences. (99) 13538-13543.

Cheetham, Graham M. T., David Jeruzalmi, and Thomas A Steitz (1999) *Structural basis for initiation of transcription from an RNA polymerase-promoter complex.* Nature (399) 80-83.

Clore, G. Marius, Angela M. Gronenborn, and Nico Tjandra. (1998) *Direct Structure Refinement against Residual Dipolar Couplings in the Presence of Rhombicity of Unknown Magnitude.* Journal of Magnetic Resonance (131) 159-162.

Cramer, Patrick, David A. Busnell, and Roger D. Kornberg (2001) *Structural Basis of Transcription: RNA Polymerase II at 2.8 Angstrom Resolution.* Science (292) 1863-1876.

Cunningham, P.R. and Fengrand, J. (1900) *Use of Inorganic Pyrophosphatase to Improve the Yield of In Vitro Transcription Reactions Catalyzed by T7 RNA Polymerase.* Biotechniques (9) 713-714.

He, Biao et al. (1997) *A Mutant T7 RNA Polymerase that is Defective in RNA Binding and Blocked in the Early Stages of Transcription.* Journal of Molecular Biology. (265) 275-288.

Kung, Hsiang Chuan et al. (1995) *Magnetic Alignment of Duplex and Quadraplex DNAs*. Journal of Magnetic Resonance, Series B. (109)323-325.

Place, Christophe et al. (1999) *Studies of Contacts between T7 RNA Polymerase and Its Promoter Reveal Features in Common with Multisubunit RNA Polymerases.* Biochemistry (38) 4948-4957.

Schenborn, Elaine T. and Robert c. Mierendorf, Jr. (1985) *A novel Transcription Property of SP6 and T7 RNA polymerase: dependence on template structure.* Nucleic Acids Research (13) 6223-6263.

Schenck, John F. (2005) *Physical interactions of static magnetic fields with living tissues.* Progress in Biophysics and Molecular Biology (87) 185-204.

Tohyama, Kohji and Nahonori Miyata (1973) *Diamagnetic Susceptibility and its Anisotropy of Poly-γ-Ethyl-L-Glutamate.* Journal of the Physical Society of Japan (34) 1699.

Williams, Mark C and Ioulia Rouzina. (2002) *Force Spectroscopy of single DNA and RNA molecules.* Current Opinion In Structural Biology (12) 330-336.

Worchester, D.L. (1978) *Structural Origins of diamagnetic anisotropy*. Pro.Natl. Acad. Sci. (75) 5475-5477.