

ABSTRACT

- 1) NMR Studies of the Spliced Leader RNA from *Crithidia fasciculata* and *Leptomonas collosoma*.
- 2) Hydrodynamic Properties of Nucleic Acids by NMR.

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The first part of this thesis examines the Spliced Leader RNA (SL RNA) from two species of trypanosome, *Crithidia fasciculata* and *Leptomonas collosoma*. Unlike other eukaryotes, trypanosomal genes lack internal introns, rather, they are excised by *trans*-splicing to the SL RNA during pre-mRNA processing. Previous studies have shown that the SL RNA can adopt two alternate secondary structures, form 1 and form 2, and it has been suggested that the RNA may be involved in a conformational switch that could regulate the *trans*-splicing event. Thus, we set out to investigate both the form 1 and form2 secondary structures of the SL RNA. The *in vitro* secondary structure of the *C. fasciculata* SL RNA was found to be in the form 2 and the *L. collosoma* was found to be in the form 1. The form 1 conformation was examined in detail and was found to contain an interesting tri-uridine hairpin loop with the first and third uridine base paired.

The second part of this thesis examines the hydrodynamical properties (translational and rotational diffusion) of nucleic acids using NMR techniques.

The translational diffusion constants for nucleic acids of different sizes and shapes were measured using the pulsed field-gradient NMR technique. The diffusion constants measured in this way were found to be in good agreement with the predicted values using hydrodynamic theory and to the previously published results from other experimental techniques. This technique is shown to be an effective method for solving one of the more common problems in RNA NMR spectroscopy, knowing whether a particular sample is monomeric or not.

The rotational diffusion constants for nucleic acids of different sizes and shapes were examined theoretically and experimentally by NMR via the nuclear Overhauser effect (NOE) and the relaxation matrix. The theory of the hydrodynamics and relaxation matrix calculations are presented in the context of examining molecules that may undergo anisotropic rotation. The results demonstrate that there is a predictable effect on the measured NOEs because of rotational anisotropy of extended shape molecules, such as long DNA fragments.

- 1) NMR Studies of the Spliced Leader RNA from *Crithidia fasciculata* and *Leptomonas collosoma*.
- 2) Hydrodynamic Properties of Nucleic Acids by NMR.

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Jon Lapham

Dissertation Director: Dr. Donald M. Crothers

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For Renata.

And my family,

Mom, Dad, Brian and Laura.

Acknowledgements

As I look over this thesis, I realize that I am unusually indebted to a large number of people for helping me with my research project. Without the help these people have shown me through the years the research presented in this thesis would not have been possible.

Let me begin by thanking my advisor and mentor Prof. Donald M. Crothers. Don is one of those people that you get the feeling knows some truism of life that the rest of us just don't understand. He is one of the only people I have ever met who portrays that most rare of qualities, confidence in others. I really think that is his secret, he *honestly* believes in his students. What a standard for us to emulate!

The other members of my committee are Profs. Peter Moore, Jim Prestegard and Kurt Zilm. Thank you all for reading this manuscript and for your comments and suggestions along the way. I have had the pleasure of writing a paper with Prof. Moore, and I would just like to say that it was a truly rewarding experience. He believes in brevity and clarity above all else in writing, a lesson I hope I have learned. It is difficult, but sometimes (as I like to say) less is more!

I inherited the Spliced Leader RNA project from Karen LeCuyer and Ken Harris and I would like to thank them both for setting the biophysical groundwork for the project. If it were not for Karen, we would not be talking about the form 1 and form 2 secondary structures of these RNAs! Ken is one of the nicest people I have ever had the pleasure to work with, and I hope that one day soon he is a practicing physician in New York City (a dream come true for him).

I would like to thank Jing Xu for collaborating with me on the RNase H cleavage project (Chap. 2). This was the closest thing I ever came to doing "molecular biology", and she helped me to survive unscathed. I also had the pleasure of interacting with Yi Tao Yu in Joan Steitz's lab when we were trying to figure out where RNase H *really* cuts RNA. (PS: It turns out that we were both correct!)

Renata Kover had the original idea and explained the theoretical background for using the isotope selection experiments to distinguish parts of isotope labeled samples (Chap. 3). She has an unusual ability to understand concepts outside her field and to be

able to convey them to others to help them accomplish tasks. All the data from the isotope-selection experiments came from samples prepared by Gauri Dhavan. Getting the pulse sequences to work perfectly was an extraordinarily tedious task, and I couldn't have asked for a better partner than Gauri during the process. Oh yeah, I shouldn't forget to mention that Kevin (spelled with a z!) MacKenzie and John Marino were very helpful with ideas concerning the pulse sequence programming of these experiments.

Chapter 4 is chock full of collaborative efforts. I must first say that the idea of performing the diffusion measurements on DNA came to me whilst listening to a talk Mike Andrec gave on his proton exchange experiment, so, thanks Mike! Jason Rife has the dubious honor of being the first person I found that had an RNA sample that existed as both a monomer and dimer. I wanted data on that system! It turns out that I was very lucky to work with him, because I have very much benefited from the many scientific discussions we have had over the years. Thanks Jason. (and yes, I know that I am "preaching to the choir", but I just like to argue...). The D14 sample presented in chapter 4 was kindly provided by Dan Zimmer, thanks!

If I understand anything of the theoretical aspects of NMR, I owe it to a number of people. It should be a requirement of everyone at Yale to take Kurt Zilm's spectroscopy course, I give the class a ten on the Seminole-Head (SH) rating system. I will always remember the rotating frame demonstration... It should NOT be a requirement that everyone at Yale take Pat Vaccaro's Quantum Chemistry course, but DAMN did I learn a lot about quantum mechanics (and indirectly, about NMR), I also give his class a ten SHs. Pat, you will no longer have to fear me starting conversations with you by saying, "I have this equation..." (at this point he would look around like a hunted animal and try to escape). Finally, I would like to thank Don Crothers (once again) who has such an intuitive understanding of all things physical that his non-equations based explanations of NMR theory usually had the effect of me actually understanding things.

The inertia tensor calculations are compliments of Charlie Schmuttenmaer, thanks for the help!

Much of the data analysis presented in this thesis required writing a number of computer programs. When I came to Yale, I had never even used email, let alone

programmed a computer. Since then I have, hopefully, picked up some of the rudimentary aspects of programming from a number of people. To the Perl people:

```
%perl_people = (  
    "Dr. Jason Kahn" => "scalars are good",  
    "Dr. Mike Andrec" => "lists are better",  
    "Dr. Dave Schweizguth" => "associative arrays are best",  
);  
  
@people = keys %perl_people;  
  
foreach $person ( @people ) {  
    print "I learned that, in Perl, ";  
    print $perl_people{$person};  
    print " from $person\n";  
}
```

To the C (C++) people:

```
#include <iostream.h>  
#include "myalloc.h"  
  
main () {  
    char *Person1 = NEW1D_C( 16 );  
    strcpy(Person1, "Dr Klaus Fiebig");  
    char *Person2 = NEW1D_C( 16 );  
    strcpy(Person2, "Dan Rosewater");  
  
    char *VARptr;  
    char VAR[9] = "pointers";  
    VARptr = VAR;  
  
    cout << "Thank you " << Person1 << " and " << Person2 << endl;  
    cout << "for showing me how to do dynamic memory allocation\n";  
    cout << "and working with " << VARptr << endl;  
}
```

Finally, but certainly not the least, I would like to thank my friends and family. I was going to rate everyone the SH rating system described before. It turns out, however, that everyone seems to have fallen into two categories, those rated with 10 SHs (Mom, Dad, Brian, Laura, Renata and Chris) and those with 9 SHs (all others). Hmmm, I guess the SH rating system won't be useful after all.

Mom, Dad, thank you for your support over the eons that I have been in graduate school, it meant more than you will know. To Laura "I can't get enough of chemistry" Lapham and Brian "one day I am going to be bigger than you so I can beat you up" Lapham, it seems that under my expert older-brother supervision you have both turned out to be okay. I hope one day we will live nearer each other, because I actually like to hang out with you guys!

To adequately express my gratitude to the friends I have made here at Yale would require writing another volume to this thesis. I cannot mention everyone, but I must mention the following people. When I rotated in the Crothers lab I worked for one John Marino. For my “project” we synthesized some RNA and collected a 1D imino proton spectrum for it on the 490 MHz NMR. This was back in the days when the 490 was accessed using what amounts to a typewriter (no computer screen). Anyway, John, thanks for what ended up being the beginning of a great friendship. Matthew P. Augustine, this is a transcript of the first conversation I had with you, “Hi, my name is Jon”, said I, “Did I ask?!”, replyeth Matt. You haven’t stopped being a bastard ever since, and I thank you for it. You are an oasis of “state school-ness” in an ocean of “private school”. I am looking forward to the first FSU – PSU Rose Bowl game! Who do those Big-Eleven, PAC-10 teams think they are? Of course, I hope it ends in a tie. Rich Roberts, I enjoyed those late nights dog fighting on the SGIs while you collected stopped-flow data. I will (I swear) get out to California to visit you and Maja one day. Dan Zimmer, you have the unfortunate “honor” of being the guy I usually bounce my “great ideas” off of, thanks for listening to them without laughing too much! (I *will* do acrylamide-based NMR, I swear it!). Jason Kahn, thanks for being smart as hell and a nice guy (hi Effie and Evan!). To Anil and the rest of you damn Canadians, I am happy to say that you NEVER “checked me into the boards” (or whatever it is you say when you know how to speak hockey-ese).

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Renata Kover may be the only person who has (or will ever) read every single word of this thesis. She, more than anyone else, helped me scientifically and personally.

Renata, I love you, thanks.

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