In Vitro Selection of RNA Binding Peptides

Thesis by

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In Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy

California Institute of Technology
Pasadena, California
2005
(Defended May 11, 2005)
Acknowledgements

It has been said that you learn more from your failures than your successes – if that is true, I must have learned a lot. Scientific research is a lot like gambling: intermittent reward is a powerful motivator. Although the seven years I’ve spent here have been filled with much frustration (and some successes too), I do feel it has been worth it for I have learned not only about science, but about who I am. There have been many people who have helped me be who I am today, and they deserve my thanks.

First and foremost, I have to thank my wife-to-be, Katie Percell, for her love, patience, and support over the last seven years. She is one of the kindest and optimistic people I know. I know too how much she wants to go back to Hawai‘i, yet she has stayed up here for me, and I very much appreciate her sacrifice. I’m just sorry it will be a little longer… hopefully not too much longer, though. She has also read most of my thesis, even though much of it is foreign to her, and has tried to make my thesis writing as bearable as possible.

I have to thank my family – my mom and dad especially – for their love and support. It amazes me how much I can recognize parts of me that can be attributed to them. I appreciate their sacrifices in sending me to ‘Iolani and to Claremont McKenna, both of which aren’t exactly cheap. My mom used to drive me to ‘Iolani, go to work, pick me up, then drive me back home, a >100 mile daily commute, which especially on a small island, is ridiculous. Without you guys, I would not be here today. Tammy and Mike Keller, my sister and brother-in-law, got me through the culture, weather, and all
other types of shock when I first came up to California. I’m sure it would have been
more of an adjustment if they weren’t here to help.

Thanks go out to my housemates Hugh Kimura, Jon Yee, and Shawna Ling, from
the “Real World House,” who made life outside of lab fun and always interesting. Hugh
would be up for pretty much anything, including Donutman runs in the middle of the
night for some strawberry donuts. Stacy Miyake, Michelle Sugihara, and Wendi
Nishikawa were always around for laughs and some good Japanese or Hawaiian food.
Hugh, Corey Chung and Mike Torres were my golf buddies and sources of endless
laughter and entertainment (especially at Scholl Canyon). Alisa Shiraki listened to my
grumbling about graduate school, and always had an optimistic outlook about things.

Special thanks goes to my previous teachers. Pamela Fujinaka, my high school
chemistry teacher, convinced me to take her AP Chemistry class as well as encouraged
me to take the Chemistry Olympiad Exam. Her class was harder and made you think
more creatively than some of my college classes, and gave me a solid foundation in
chemistry. The labs in her class were not follow-the-direction experiments and made me
think outside of the box, a skill extremely useful in research. Todd Richmond was my
undergraduate advisor and all-around cool guy. He taught me much of what I know
about molecular biology and pushed me hard to get started in research. His biochemistry
course got me excited about biological chemistry – otherwise I would have been an
organic chemist.

My thanks go to my advisor at Caltech, Rich Roberts, whose research suggestions
and insights have always been helpful. I joined his lab partly because I was impressed by
his command of the literature and his broad scientific knowledge, partly because I would
have the opportunity to work on a variety of projects, and partly because he’s a nice guy. Rather than telling me what experiment to do next, he let me figure things out for myself, with a nudge here or there.

My thesis committee, Peter Dervan, Steve Mayo, and Doug Rees have always been helpful and provided sound advice. I am grateful for their guidance and support. John Love, John Marino, and Scott Ross taught me many gory details about NMR spectroscopy. Scott has also been extremely helpful in helping me run my NMR samples and I wish him a speedy recovery.

I have had the privilege to work in a lab of very nice people. I thank Shuwei Li who helped me a lot when I first started and for his continuing advice and suggestions after I joined the lab. Bill Ja also helped me get up and running, and has always been a critically thinking scientist with good experimental suggestions, and is an expert in finding free stuff. Bill works hard and is always in lab, but plays hard too. Jie Xu and Joan He were not only fun to talk to but also had helpful experimental suggestions. I have to thank Jeff Barrick who was an undergrad in a graduate student’s body and worked with me on the N project and always had great scientific ideas. Chris Balmaseda (Balms) helped me a lot with protein purification and was fun to hang out with. Jinsong Ren taught me a lot about biophysical chemistry and was always around to cheer people up. Tianbing Xia taught me about nucleic acids as well as NMR, has a great scientific mind, and has made me see problems from different perspectives. His wife, Cindy (Xin) Qi, has taught me much about organic chemistry and made me think more about life after Caltech. Shelley (Starck) Green gave me many good experimental suggestions, especially controls, but was also a fiercely loyal and good friend who has always been
supportive of me. Her husband Harry Green provided a biologists’ perspective to things, often during rounds of golf. Over the past year, I’ve worked closely with Christine Ueda and Steve Millward on a variety of projects, some of which have finally come to fruition, and their help has been indispensable. Ryan Austin has not only helped me with science, but has made me think more about non-science things, and along with Adam Frankel, Anders Olson, and Premal Shah, have had many interesting discussions over an Ernie’s lunch. Anders is also my baymate and always has insightful scientific suggestions.

My thanks go to Margot Hoyt, who has kept things running behind the scenes as well as Alison Ross who always helped me out when I had administrative questions and problems. Po-Ssu Huang deserves thanks for his help with science as well as his friendship. I still remember rooming with him during the Biology retreat my first year here and he helped me a lot in Bch170 and Bch176. Possu is also the original Mac evangelist and guru and helped me whenever I’ve had computer problems. I would imagine that he would like me to attribute the problems to the Microsoft programs, rather than OSX. Lastly, others that deserve thanks at Caltech are, Chris Otey, Corey Hu, Justin Bois, Greg Drummond, Robert Dirks, Shantanu Sharma, and Geoff Hom.
Abstract

RNA is recognized to play an increasing number of roles in the cell: transcription regulation, translation, and catalysis. Peptides that bind RNA would therefore be useful as biochemical tools and lead compounds for therapeutics. Existing genetic methods of isolating RNA binding peptides are prone to biases and can only search millions of sequences. In vitro selections using mRNA display provide an avenue to discover specific, high affinity peptides that bind to any RNA target from libraries composed of trillions of molecules.

Here, we describe initial experiments to optimize the mRNA display selection cycle for the isolation of RNA binding peptides. We use this optimized cycle to show that enrichment of specific sequences is possible using mRNA display, and select mutants of the λ N peptide which bind in a different conformation than wild-type. Characterization of these peptides demonstrates that affinity is not enough for in vivo activity; binding in a correct conformation is also important.

Based on these experiments, we designed a strategy to isolate RNA binding peptides to targets for which no natural ligand is known. We test this strategy and isolate peptides that bind to functionally important domains of telomerase RNA with nanomolar affinity and high specificity. Using mutagenic PCR and additional rounds of selection, we increase the specificity of several peptides for telomerase RNA and also isolate other peptides which bind an important domain of the Hepatitis C Virus internal ribosome entry site.
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>RT</td>
<td>Reverse transcription/reverse transcriptase</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris-(hydroxymethyl)-aminomethane</td>
</tr>
<tr>
<td>pmol</td>
<td>Picomole</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetate</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Double distilled water</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer ribonucleic acid</td>
</tr>
<tr>
<td>Ni-NTA</td>
<td>Nickel-nitriloacetic acid</td>
</tr>
<tr>
<td>RNase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>TMV</td>
<td>Tobacco mosaic virus</td>
</tr>
<tr>
<td>Bsa</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>IRES</td>
<td>Internal ribosome entry site</td>
</tr>
<tr>
<td>hTR</td>
<td>Human telomerase RNA</td>
</tr>
<tr>
<td>hTERT</td>
<td>Human telomerase reverse transcriptase</td>
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