

A COCKTAIL OF THERMALLY STABLE, CHEMICALLY SYNTHESIZED CAPTURE AGENTS FOR
THE EFFICIENT DETECTION OF ANTI-GP41 ANTIBODIES FROM HUMAN SERA AND
TECHNIQUES

Thesis by

Jessica A. Pfeilsticker

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



California Institute of Technology

Pasadena, California

2014

(Defended December 2, 2013)

© 2014

Jessica A. Pfeilsticker

All Rights Reserved

ACKNOWLEDGEMENTS

I begin by thanking my advisor Professor James Heath for his incredible guidance and talent. His enthusiasm for science is unrivaled, and his uncanny ability to absorb ideas and tie them together in creative and interesting ways has been a wonderful model for how to conduct research. I have enjoyed my time in his lab immensely, and am grateful to have learned from him for the last three and a half years.

I also want to thank Professor Daniel Weitekamp, from whom I learned many fundamental scientific principles. He helped me transition as a young graduate student from someone who takes classes and obtains grades to someone who thinks about and conducts research. For that, and for sharing with me some of his incredible ideas and vast intellect during my first three years at Caltech, I am thankful.

I want thank my committee members Professor Robert Grubbs, Professor Mitchio Okumura, and Professor William Clemons for their guidance and insight. More than once I sought their advice on how to navigate graduate school, and always received encouragement and enthusiasm in return.

Next, I thank my family. They originally inspired an interest in the natural world and the wish to explore it when I was a child, and then supported me as I pursued my education in science. To Dad and Penny, Mom and Ed, thank you for being my parents and offering love and advice. John and Jason, thanks for being the coolest brothers anyone could ask for. You both have incredible futures in front of you, and I know that

your deep thoughtfulness and curiosity will ensure a never ending supply of fun and interest as you pursue your own paths.

I want to thank my lab mates for their instrumental role in my graduate experience. Valerie Norton, Jason Ollerenshaw, Eduard Chekmenev, Jan Höevener, and Daniel Rowlands in the Weitekamp group; Aiko Umeda, Kaycie Deyle, Blake Farrow, Ryan Henning, Alex Sutherland, Joey Varghese, JingXin Liang, Arundhati Nag, Samir Das, Marilena Dimotsantou, Ann Chung, Steve Millward, Errika Romero, and Connie Hsueh in the Heath group; and Heather Agnew, Bert Lai, Rosemary Rohde, and Suresh Pitram at InDi Molecular. You all kept me sane, and offered much needed scientific and personal feedback and assistance during our time as friends and colleagues. Thank you for being my biggest allies; this experience was made so much richer because you all were in it.

I also need to thank the many members of Caltech staff who make research possible. Agnes Tong, thank you for all that you do to keep chemistry running smoothly. David VanderVelde, thank you for your enthusiasm and interest in sharing your knowledge of NMR. Mona Shahgholi, Jie Zhou, [Felicia Rusnak](#), Darryl Willick, Joe Drew, Cora Carriedo, Steve Gould, Paul Carroad, [Art Seiden](#), Mike Roy, Richard Gerhart, Jose Mendez, Engracia Alvarez, and countless others, thank you all for making the department exist and function. All of you have personally touched my time here in some way, and I wouldn't have been able to do this without you.

Finally, it is imperative that I thank the many friends who make up my support network. Lisa Sherer, thank you for being my pseudo-sister for the last 13 years. Caitlin

Murphy, Lisa Mauger, Tucker Jones, Nick Stadie, Luke Boosey, and Patrick Sanan, thank you for being an awesome group of people who bonded so quickly and tightly when we all first arrived at Caltech as wide-eyed first years. Keenan Crane, thank you for being so thoughtful and kind, and for helping me through all manners of crisis, both personal and professional. Cole DeForest, Robin DeForest, Anya Demianenko, and Michael McCoy, thank you for kindness, compassion, wine and foie gras. Young In Oh and Jean Li, thank you for mutual support in the face of adversities. Sang Tae Park, thank you for unconditionally having my back and being someone I could always rely on for personal and technical advice. Maggie Buckles, Tony Roy, and Kellie Roy, thank you for being smart, kind, wonderful people who gave me balance and kept me sane. Jon-Michael Sanchez, thank you for keeping life exciting, and for becoming someone with whom I could explore new ways to express myself. I love all of you, and thank you so much for being in my life.

ABSTRACT

This thesis reports on a method to improve *in vitro* diagnostic assays that detect immune response, with specific application to HIV-1. The inherent polyclonal diversity of the humoral immune response was addressed by using sequential *in situ* click chemistry to develop a cocktail of peptide-based capture agents, the components of which were raised against different, representative anti-HIV antibodies that bind to a conserved epitope of the HIV-1 envelope protein gp41. The cocktail was used to detect anti-HIV-1 antibodies from a panel of sera collected from HIV-positive patients, with improved signal-to-noise ratio relative to the gold standard commercial recombinant protein antigen. The capture agents were stable when stored as a powder for two months at temperatures close to 60°C.

TABLE OF CONTENTS

LIST OF FIGURES, SCHEMES, AND TABLES.....	xiii
--------------------------------------------------	-------------

CHAPTER 1: Introduction.....	1
-------------------------------------	----------

1.1 PCC Agent Cocktail for HIV-1 Diagnostics.....	2
---------------------------------------------------	---

1.2 References.....	5
---------------------	---

CHAPTER2: Developing a PCC Agent Cocktail for the Detection of Anti-HIV

Antibodies.....	7
------------------------	----------

2.1 Introduction.....	8
-----------------------	---

2.2 <i>In Situ</i> Click.....	9
-------------------------------	---

2.3 Anchor Selection.....	10
---------------------------	----

2.4 Screening.....	11
--------------------	----

2.5 Biligand Characterization.....	16
------------------------------------	----

2.6 Materials and Methods.....	21
--------------------------------	----

2.6.1 Anchor Synthesis.....	21
-----------------------------	----

2.6.2 OBOC Screens.....	22
-------------------------	----

2.6.3 Biligand Synthesis.....	24
2.6.4 Assays.....	27
2.7 Conclusion.....	29
2.8 Acknowledgements.....	29
2.9 References.....	31
 CHAPTER 3: PCC Agent Based Assay for the Detection of Anti-HIV Antibodies from Human Sera.....	 33
3.1 Introduction.....	34
3.2 Patient Sample Assay.....	34
3.3 PATH Sample Assay.....	37
3.4 Stability Assay.....	39
3.5 Materials and Methods.....	41
3.5.1 Patient Serum ELISA.....	41
3.5.2 PATH Samples ELISA.....	41
3.5.3 Stability Assay.....	42
3.6 Conclusion.....	43

3.7 Acknowledgements.....	44
3.8 References.....	45
CHAPTER 4: A Selective ^{15}N-to-^1H Polarization Transfer Sequence for More Sensitive	
Detection of ^{15}N-Choline.....	46
4.1 Abstract.....	47
4.2 Introduction.....	48
4.3 Theory.....	49
4.4 Experimental.....	56
4.5 Results and Discussion.....	61
4.6 Conclusion.....	68
4.7 Acknowledgements.....	68
4.8 References.....	69
APPENDIX A: Understanding PCC Agent Binding to Single Point Mutation E17K of Akt1	
Pleckstrin Homology Domain through Molecular Dynamics.....	71
A.1 Introduction.....	72

A.2 Peptide Capture Agent Against E17K Mutation of Akt1 PHD.....	73
A.3 Construction of E17K and WT Systems.....	73
A.3.1 yleaf-tosyl-biotin.....	73
A.3.2 Ligand/Protein Complexes.....	74
A.4 Molecular Dynamics.....	78
A.5 Binding Energy.....	80
A.6 Conclusion.....	85
A.7 Acknowledgements.....	85
A.8 References.....	86
 APPENDIX B: Peptide-Based General Antibody Detection Agent.....	87
B.1 Introduction.....	88
B.2 Wells Peptide.....	88
B.3 Biligand Screen.....	89
B.4 1,4 Triazole Linked Biligand Characterization.....	92
B.5 1,5 Triazole Linked Biligand Characterization.....	92
B.6 Click Cyclized Wells.....	97

B.7 Materials and Methods.....	100
B.7.1 Anchor Synthesis.....	100
B.7.2 OBOC screen.....	100
B.7.3 Biligand Synthesis.....	102
B.7.4 Click Cyclized Wells Synthesis.....	103
B.7.5 Assays.....	104
B.8 Conclusion.....	106
B.9 Acknowledgements.....	106
B.10 References.....	107
 APPENDIX C: Algorithm for Peptide Clustering	 108
C.1 Introduction.....	109
C.2 Persistence Clustering.....	109
C.3 Algorithm Description.....	111
C.4 Output.....	111
C.5 Function Code.....	117
C.6 Conclusion.....	123

C.7 Acknowledgements.....	123
C.8 References.....	124
APPENDIX D: A cocktail of thermally stable, chemically synthesized capture agents for the efficient detection of anti-gp41 antibodies from human sera (<i>PloS one</i> 2013, 8, e76224).....	125
APPENDIX E: A Selective ^{15}N-to-^1H Polarization Transfer Sequence for More Sensitive Detection of ^{15}N-Choline (<i>Journal of Magnetic Resonance</i> 2010, 205, 125-129).....	131

LIST OF FIGURES, SCHEMES, AND TABLES

FIGURES, Chapter 2:

Figure 2.1. Differential detection of 3D6 and 4B3 by anchor ligands.....	12
Figure 2.2. Structures of peptide ligands in PCC Agent cocktail.....	17
Figure 2.3. Apparent affinity of A21 and biligands directed against 3D6 as determined by SPR.....	18
Figure 2.4. Apparent affinity of A22 and biligand directed against 4B3 as determined by SPR.....	19
Figure 2.5. Performance of PCC agent cocktail to detect 3D6 and 4B3 from human serum.....	20

FIGURES, Chapter 3:

Figure 3.1. Patient sample ELISA.....	36
Figure 3.2. PATH sample ELISA.....	38
Figure 3.3. Stability assay.....	40

FIGURES, Chapter 4:

Figure 4.1. A refocused INEPT pulse sequence for polarization transfer from ^{15}N to ^1H for detection.....	51
Figure 4.2. The structure of ^{15}N -choline.....	52
Figure 4.3. Selective refocused INEPT pulse sequence for coherent polarization transfer from ^{15}N to methyl ^1H in ^{15}N -choline.....	57
Figure 4.4. Numerical simulation of the selective ^{15}N -to- ^1H INEPT pulse sequence acting on a simplified spin system.....	60
Figure 4.5. Comparison of ^1H spectrum using selective ^{15}N -to- ^1H INEPT sequence to ^{15}N spectrum.....	62
Figure 4.6. Measurement of the ^{15}N longitudinal relaxation time of ^{15}N -choline in D_2O solution using a modified version of selective ^{15}N -to- ^1H INEPT sequence.....	63
Figure 4.7. Amplitude of the methyl ^1H signal observed in ^{15}N -to- ^1H INEPT spectra of ^{15}N -choline using selective and non selective pulses.....	65

FIGURES, Appendix A:

Figure A.1. Structure of yleaf-tosyl-biotin.....	75
--------------------------------------------------	----

Figure A.2. Top three ZDOCK predicted conformations.....	76
Figure A.3. Selected ligand conformation complexed with the mutant and wild type PH domains.....	77
Figure A.4. Rmsd plots for the MD trajectories and restarted trajectories of the E17K and WT complexes.....	79
Figure A.5. Energy landscapes obtained from Rosetta <i>ab initio</i> structure prediction simulations on Rosetta@home.....	81
Figure A.6. Energy landscape for E17K complex.....	82
Figure A.7. Energy landscape for WT complex.....	83
Figure A.8. Structures of the E17K and WT reference states.....	84

FIGURES, Appendix B:

Figure B.1. Structures of peptide biligands identified in screen against IgG Fc.....	93
Figure B.2. Apparent affinity of Wells and biligands directed against Fc as determined by sandwich ELISA.....	94
Figure B.3. Structure of 1,5 triazole linked biligand.....	95

Figure B.4. Apparent affinity of Wells and 1,5 triazole linked WA directed against Fc as determined by sandwich ELISA.....	96
Figure B.5. Structure of Cu(I) click cyclized Wells peptide.....	98
Figure B.6. Performance of click cyclized Wells peptide variants compared to Wells peptide tested by sandwich ELISA.....	99

FIGURES, Appendix C:

Figure C.1. Discs formed around points with growing radius ϵ	110
Figure C.2. Illustrative example of how lifetimes of clusters are computed.....	112
Figure C.3. Projection of the peptides onto the top two eigenvectors taken from the diagonalization of the covariance matrix.	114
Figure C.4. Plot of eigenvectors vs. their associated eigenvalues.....	115
Figure C.5. Visual representation of the components of the top four eigenvectors.....	116

SCHEMES, Chapter 2:

Scheme 2.1. Screening strategy for selecting capture agents against

anti-HIV antibodies 3D6 and 4B3.....14

SCHEMES, Appendix B:

Scheme B.1. Screening strategy for selecting capture agents against

human IgG Fc.....90

TABLES, Chapter 2:

Table 2.1. Biligand screen results.....15

TABLES, Appendix B:

Table B.1. Biligand screen results.....91

TABLES, Appendix C:

Table C.1. List of clusters and their associated lifetimes calculated for the hits

resulting from the A22/4B3 screen.....113