Development of Protein-Catalyzed Capture (PCC) Agents with Application to the Specific Targeting of the E17K Point Mutation of Akt1

Thesis By:

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"There is no passion to be found playing small - in settling for a life that is less than the one you are capable of living." – Nelson Mandela

"To give anything less than your best is to sacrifice the gift." – Steve Prefontaine

Abstract

This thesis describes the expansion and improvement of the iterative *in situ* click chemistry OBOC peptide library screening technology. Previous work provided a proof-of-concept demonstration that this technique was advantageous for the production of protein-catalyzed capture (PCC) agents that could be used as drop-in replacements for antibodies in a variety of applications. Chapter 2 describes the technology development that was undertaken to optimize this screening process and make it readily available for a wide variety of targets. This optimization is what has allowed for the explosive growth of the PCC agent project over the past few years.

These technology improvements were applied to the discovery of PCC agents specific for single amino acid point mutations in proteins, which have many applications in cancer detection and treatment. Chapter 3 describes the use of a general all-chemical epitope-targeting strategy that can focus PCC agent development directly to a site of interest on a protein surface. This technique utilizes a chemically-synthesized chunk of the protein, called an epitope, substituted with a click handle in combination with the OBOC *in situ* click chemistry libraries in order to focus ligand development at a site of interest. Specifically, Chapter 3 discusses the use of this technique in developing a PCC agent specific for the E17K mutation of Akt1. Chapter 4 details the expansion of this ligand into a mutation-specific inhibitor, with applications in therapeutics.

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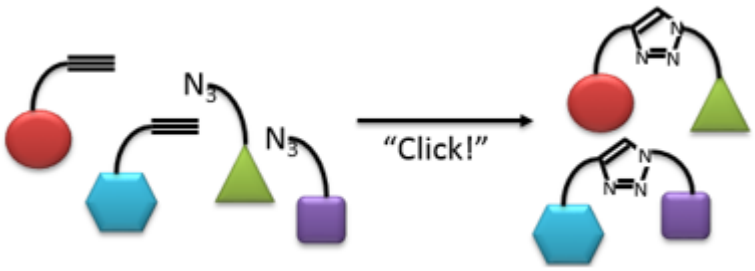
Chapter 1

Introduction



1.1 Protein-Catalyzed Click (PCC) Peptide Capture Agents for Biomarker Detection and Therapeutics

Detecting cancer-associated biomarkers is a necessary step on the road to personalized medicine, as emerging therapeutics require the identification of specific patient populations that will respond to targeted therapies[1]. Methods for protein biomarker detection are highly desirable for rapidly screening changes in protein mutation status, monitoring patient treatment[2], and simple point-of-care diagnostics[3]. Techniques that rely on detecting or monitoring protein levels mainly use antibodies for the capture and measurement of these proteins[4]. Antibodies, however, are biological reagents that are inherently unstable, vary from batch to batch, can exhibit high levels of cross-reactivity with other antibodies, and are expensive to produce[5]. Diagnostic assays are frequently prohibitively limited in both cost and stability due to the restrictions of the gold-standard antibody detection agents.

Peptides can be the missing link for both inexpensive biomarker detection and targeting traditionally undruggable proteins. Peptide - protein interactions cover a large surface area, producing antibody-like affinities with unsurpassed specificities[6]. To date, most peptide discovery techniques use genetically-encoded libraries, which allow for ease of library generation and rapid and simple sequencing. These techniques permit screening of enormous numbers of compounds against a target of interest without any complicated syntheses or detailed knowledge of the target[7]. These libraries, however, are limited by the biological system from which they are derived, both in terms of screening elements and library size. Most of these systems, such as phage display, bacterial display and yeast display, are confined to the natural amino acids because they use the cell machinery to make and express their libraries. These systems limit the suitability of the resulting peptide capture agents due to the instability of biological peptides, which are comprised of naturally-occurring L -amino acid monomers that can be degraded in biological systems and fluids.

The Heath group has sought to alleviate the issue of peptide capture agent instability by relying exclusively on the use of unnatural amino acids. Because biological libraries are not conducive to this type

of work, we have instead adopted a peptide screening method utilizing One-Bead, One-Compound (OBOC)[8] chemically synthesized libraries on 90µm polystyrene beads. This technique trivializes the inclusion of any unnatural amino acid or structure that can be chemically synthesized, allowing for the use of biologically stable D - amino acids and azide-alkyne click chemistry handles in the library[9].

The Sharpless group showed that the typical azide - alkyne click catalyst, Cu(I)[10], speeds up the reaction but is only barely necessary for it to occur, and demonstrated the ability to replace this catalyst with the surface of a protein. They took advantage of this to assemble small molecule inhibitors for proteins by breaking up known inhibitors into two components and assembling two libraries – each one comprised of pieces similar to its original half of the inhibitor. One of these libraries of molecules was appended with a click handle, the other library with the opposite click handle. When two click reactants bound tightly to the protein surface and in close enough proximity to each other, the long dwell time of these reagents allowed for the click to occur without the use of Cu(I)[11]. In this way, they were able to bring the two libraries, which consisted of variations on the original inhibitor, together and use the surface of the protein to assemble the best possible small molecule inhibitor.

We have adapted this technology to assemble 5-mer peptide sequences displayed on OBOC libraries using the surface of the target protein itself to catalyze a click reaction between peptides that bind tightly to this surface. Hence, we have termed these capture agents “protein catalyzed capture” (PCC) agents. This strategy requires that the two compounds are high-affinity, selective binders for the target that is acting as a catalyst because the click reaction does not occur without a long dwell time between the two agents. PCC agents have been developed against a number of protein targets, and have been shown to exhibit a selectivity and affinity similar to those of monoclonal antibodies. They also can be readily integrated into all standard protein assay formats.

Chapter 2 of this thesis describes the technology development process that was undertaken to optimize the screening stages for the production of high-affinity ligands to targets of interest. Optimizing

the in-depth screening procedure has allowed for the rapid expansion of this project in the past few years. This detailed *in situ* azide-alkyne click screening technology is now regularly used to develop peptide affinity agents that mimic the performance of antibodies[9-15]. These affinity agents that maintain the stability of small molecules can be made to replace biological reagents[9,12,15], lowering the cost and increasing the robustness of detection assays[13,14].

1.2 Epitope Targeting Strategies

The detection of single amino acid point mutations in proteins is critical in the identification of specific patient populations that will respond to targeted therapies in the new era of personalized medicine[1]. The current techniques for mutation detection rely on either capture and measurement of these proteins through antibodies,[4] or on DNA sequencing. DNA sequencing is currently an expensive and time-consuming route to take for mutation screening, especially as most patients need to be screened for mutations before the proper course of their treatment is even decided[16]. Antibodies can provide a faster route for mutation detection and treatment monitoring, as there are methods currently in place for their use as rapid point-of-care diagnostics[3]. These diagnostic tests also provide information about the levels of protein expression in a body, something that cannot be tested through sequencing, which can be used to monitor the response level of a patient to a certain treatment, potentially detecting ineffective medications immediately after they are given. In a diagnostic setting, such binders can be used to assay for the mutant protein within diseased tissues, and thus potentially provide clinical guidance for treatment decisions[3].

A more ambitious application is the development of drugs that can selectively inhibit mutant proteins, and thus avoid those toxic side-effects that stem from the inhibition of the wild-type (WT) variants[17] that reside in non-diseased tissues. Patients on therapies targeted very specifically to the

mutations characteristic of their disease could show significant improvements without the toxic side-effects that stem from of the inhibition of the healthy, wild-type versions of these proteins[17]. A relevant example is compound CO-1686, which is an a epidermal growth factor receptor (EGFR) inhibitor specific for the T790M point mutation associated with certain non-small cell lung carcinomas. That drug, which is currently in clinical trials, is designed to minimize the toxicities (such as skin rash) that can appear when WT EGFR is targeted, since WT EGFR is expressed throughout the healthy tissues in the body[18].

A challenge of drug targeting a single point mutation is that the mutation may not be directly associated with a binding pocket. The presence of a binding pocket is traditionally required for small molecule inhibitor development as is serves as a thermodynamic sink that can attract binders. This requirement does not hold for antibodies and, in fact, several examples of monoclonal antibodies directed against epitopes containing single amino acid mutations do exist[19,2,20]. However, antibodies do not readily enter the living cells that can harbor the mutated proteins[21,22], and so, mutation-selective antibodies are typically only used as diagnostic reagents for staining fixed cells or tissues.

Thus, there is a need for an approach that can identify small molecules that can be generally targeted against epitopes containing single amino acid point mutations to allow for the rapid detection and assessment of tumor status, and can also potentially be developed into cell-penetrant inhibitors[5]. Our approach is inspired by the technique for developing an epitope-targeted monoclonal antibody (mAb). Such mAbs are made by injecting a small portion of the protein of interest containing the mutation (the epitope) into an animal and screening for an immune response that has the desired selectivity[2,20,19]. This approach can yield an antibody that exhibits focused binding to the specific designated area of the protein surface.

An all-chemical strategy for targeting PCC agent development against epitopes near phosphorylates sites was developed recently[15]. For that approach, an approximately 30-amino fragment representing the phosphorylated epitope of interest was synthesized, and a metalloorganic Zn-chelator

was utilized to bind to the phosphate group and present an azide near that site. That epitope was then screened against a large (1 million element) one-bead-one-compound (OBOC) library of 5-mer alkyne-presenting peptides. Hits were defined as those compounds that bound to the synthesized epitope, and that were coupled to that epitope through a triazole linkage. PCC Agents with high selectivity for the epitope and the full protein, and with affinities as low as 19nM, were developed.

The bulk of my thesis work focuses on the generalization of the epitope targeting strategy by directly substituting an alkyne click handle into the chemically synthesized peptide epitope (around the E17K residue of Akt1) of interest. Chapter 3 describes how this technique was used to develop a 5-mer PCC agent selective for the E17K mutant Akt1 protein. This PCC agent was able to be used as a drop in antibody replacement for the detection of this single amino acid mutation in various assays. It was also possible to render this agent cell-membrane permeable, and this allowed it to be used as a focused imaging agent in live cell experiments. Chapter 4 describes the expansion of this PCC agent into a biligand and then a triligand through the use of iterative *in situ* click chemistry in order to make a bulkier PCC agent. The final triligand PCC agent is capable of blocking the binding of the mutant protein to its substrate at the cell membrane, rendering it inactive and demonstrating the ability of these PCC agents to serve as targeted therapeutics.

1.3 *In Situ* Click Screening Using Azide-Containing Phage Display Libraries

Peptide screening technology has expanded incredibly in the past ten years since the inception of the PCC agent project. Using the protein-catalyzed click screens described above, PCC agents have been developed against only small chunks, or "epitopes" of proteins[15], and various PCC agents that have shown to be unique inhibitors and activators of Akt kinase[23,15], molecular imaging agents[24], detection agents for anthrax[14], suitable as third world detection agents for HIV[13], as well as the single amino acid point mutation specific E17K agents.

The OBOC libraries have their drawbacks, however. The physical size of the library limits the number of total sequences that can be screened. A full library usually contains up to 10^6 members – only a portion of which are screened. The library screening and hit picking methods are exceptionally time-consuming and labor-intensive, hindering rapid peptide discovery. The sequencing of OBOC libraries is also done by either Edman degradation or MALDI TOF/TOF, rendering the sequencing process expensive, time-consuming, and reliant on expert knowledge. Many of these drawbacks are also a huge barrier to entry in this field, limiting the labs that would be able to assist in the advancement of the science. PCC agents could be produced significantly faster and cheaper with library display technology that would combine the advantages of the OBOC product screening techniques and library design with the rapid screening and sequencing of genetically displayed libraries.

Recent advances in biology have made it possible to incorporate unnatural amino acids into the genetic code[25]. Schultz has shown that through the use of amber suppression, azide-containing amino acids can be incorporated in specific locations into the pIII coat protein on an M13 phage[26]. The *Methanococcus jannaschii* amber suppressor tRNATyr (MjtRNA) and the mutant *M.jannaschii* tyrosyl-tRNA synthetase (MjTyrRS) DNA can be contained in one plasmid that can be used to express these amber suppression tools in *E.coli*. In this system, the mutant synthetase is used to attach the unnatural amino acid azidophenylalanine to the tRNA in vivo, allowing for its incorporation into proteins. This tRNA recognizes the amber stop codon and should insert the amino acid in only that location, creating a new amino acid/tRNA combination that can be encoded into proteins.

Chapter 5 discusses the ongoing development of a screening technology that combines the *in situ* click screen advantages of the OBOC process with the rapid screening of large libraries characteristic of biological display systems. For this project, a phage display library containing azidophenylalanine for use in *in situ* click chemistry screening has been made and is being used to develop a PCC agent. These phage

libraries can be screened in place of the OBOC peptide libraries described in previous chapters for the more rapid development of PCC agents.

1.4 References

1. Hanash, S. M.; Baik, C. S.; Kallioniemi, O., Emerging molecular biomarkers--blood-based strategies to detect and monitor cancer. *Nature reviews. Clinical oncology* **2011,** *8* (3), 142-50.
2. Yu, J.; Kane, S.; Wu, J.; Benedettini, E.; Li, D.; Reeves, C.; Innocenti, G.; Wetzel, R.; Crosby, K.; Becker, A.; Ferrante, M.; Cheung, W. C.; Hong, X.; Chirieac, L. R.; Sholl, L. M.; Haack, H.; Smith, B. L.; Polakiewicz, R. D.; Tan, Y.; Gu, T.-L.; Loda, M.; Zhou, X.; Comb, M. J., Mutation-Specific Antibodies for the Detection of EGFR Mutations in Non–Small-Cell Lung Cancer. *Clinical Cancer Research* **2009,** *15* (9), 3023-3028.
3. Rusling, J. F.; Kumar, C. V.; Gutkind, J. S.; Patel, V., Measurement of biomarker proteins for point-of-care early detection and monitoring of cancer. *Analyst* **2010,** *135* (10), 2496-2511.
4. Borrebaeck, C. A., Antibodies in diagnostics - from immunoassays to protein chips. *Immunology today* **2000,** *21* (8), 379-82.
5. Kodadek, T.; Reddy, M. M.; Olivos, H. J.; Bachhawat-Sikder, K.; Alluri, P. G., Synthetic molecules as antibody replacements. *Accounts of chemical research* **2004,** *37* (9), 711-8.
6. Edwards, P. J.; LaPlante, S. R., Peptides as Leads for Drug Discovery. In *Peptide Drug Discovery and Development*, Wiley-VCH Verlag GmbH & Co. KGaA: 2011; pp 1-55.
7. Sun, N.; Funke, S. A.; Willbold, D., Mirror image phage display – Generating stable therapeutically and diagnostically active peptides with biotechnological means. *Journal of Biotechnology* **2012,** *161* (2), 121-125.
8. Lam, K. S.; Lebl, M.; Krchňák, V., The “One-Bead-One-Compound” Combinatorial Library Method. *Chemical Reviews* **1997,** *97* (2), 411-448.
9. Agnew, H. D.; Rohde, R. D.; Millward, S. W.; Nag, A.; Yeo, W.-S.; Hein, J. E.; Pitram, S. M.; Tariq, A. A.; Burns, V. M.; Krom, R. J.; Fokin, V. V.; Sharpless, K. B.; Heath, J. R., Iterative In Situ Click Chemistry Creates Antibody-like Protein-Capture Agents. *Angewandte Chemie International Edition* **2009,** *48* (27), 4944-4948.
10. Kolb, H. C.; Finn, M. G.; Sharpless, K. B., Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angewandte Chemie International Edition* **2001,** *40* (11), 2004-2021.
11. Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radić, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B., Click Chemistry In Situ: Acetylcholinesterase as a Reaction Vessel for the Selective Assembly of a Femtomolar Inhibitor from an Array of Building Blocks. *Angewandte Chemie International Edition* **2002,** *41* (6), 1053-1057.
12. Millward, S. W.; Henning, R. K.; Kwong, G. A.; Pitram, S.; Agnew, H. D.; Deyle, K. M.; Nag, A.; Hein, J.; Lee, S. S.; Lim, J.; Pfeilsticker, J. A.; Sharpless, K. B.; Heath, J. R., Iterative in situ click chemistry assembles a branched capture agent and allosteric inhibitor for Akt1. *Journal of the American Chemical Society* **2011,** *133* (45), 18280-8.
13. Pfeilsticker, J. A.; Umeda, A.; Farrow, B.; Hsueh, C. L.; Deyle, K. M.; Kim, J. T.; Lai, B. T.; Heath, J. R., A Cocktail of Thermally Stable, Chemically Synthesized Capture Agents for the Efficient Detection of Anti-Gp41 Antibodies from Human Sera. *PLoS ONE* **2013,** *8* (10), e76224.
14. Farrow, B.; Hong, S. A.; Romero, E. C.; Lai, B.; Coppock, M. B.; Deyle, K. M.; Finch, A. S.; Stratis-Cullum, D. N.; Agnew, H. D.; Yang, S.; Heath, J. R., A Chemically Synthesized Capture Agent Enables the

Selective, Sensitive, and Robust Electrochemical Detection of Anthrax Protective Antigen. *ACS Nano* **2013,** *7* (10), 9452-9460.
15. Nag, A.; Das, S.; Yu, M. B.; Deyle, K. M.; Millward, S. W.; Heath, J. R., A Chemical Epitope-Targeting Strategy for Protein Capture Agents: The Serine 474 Epitope of the Kinase Akt2. *Angewandte Chemie International Edition* **2013,** *52* (52), 13975-13979.
16. Mardis, E. R., A decade's perspective on DNA sequencing technology. *Nature* **2011,** *470* (7333), 198-203.
17. Chong, C. R.; Janne, P. A., The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med* **2013,** *19* (11), 1389-1400.
18. Tjin Tham Sjin, R.; Lee, K.; Walter, A. O.; Dubrovskiy, A.; Sheets, M.; St Martin, T.; Labenski, M. T.; Zhu, Z.; Tester, R.; Karp, R.; Medikonda, A.; Chaturvedi, P.; Ren, Y.; Haringsma, H.; Etter, J.; Raponi, M.; Simmons, A. D.; Harding, T. C.; Niu, D.; Nacht, M.; Westlin, W. F.; Petter, R. C.; Allen, A.; Singh, J., In vitro and In vivo Characterization of Irreversible Mutant-Selective EGFR Inhibitors that are Wild-type Sparing. *Molecular Cancer Therapeutics* **2014**.
19. Capper, D.; Zentgraf, H.; Balss, J.; Hartmann, C.; von Deimling, A., Monoclonal antibody specific for IDH1 R132H mutation. *Acta neuropathologica* **2009,** *118* (5), 599-601.
20. Capper, D.; Preusser, M.; Habel, A.; Sahm, F.; Ackermann, U.; Schindler, G.; Pusch, S.; Mechtersheimer, G.; Zentgraf, H.; von Deimling, A., Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. *Acta neuropathologica* **2011,** *122* (1), 11-9.
21. Marschall, A. L. J.; Frenzel, A.; Schirrmann, T.; Schüngel, M.; Dubel, S., Targeting antibodies to the cytoplasm. *mAbs* **2011,** *3* (1), 3-16.
22. Rondon, I. J.; Marasco; A., W., Intracellular AntibodiesS (Intrabodies) For Gene Therapy Of Infectious Diseases. *Annual Review of Microbiology* **1997,** *51* (1), 257-283.
23. Millward, S. W.; Henning, R. K.; Kwong, G. A.; Pitram, S.; Agnew, H. D.; Deyle, K. M.; Nag, A.; Hein, J.; Lee, S. S.; Lim, J.; Pfeilsticker, J. A.; Sharpless, K. B.; Heath, J. R., Iterative in Situ Click Chemistry Assembles a Branched Capture Agent and Allosteric Inhibitor for Akt1. *Journal of the American Chemical Society* **2011,** *133* (45), 18280-18288.
24. Millward, S. W.; Agnew, H. D.; Pitram, S.; Lai, B. T.; Rohde, R. D.; Hardman, N., Protein catalysed capture agents for molecular imaging. *Internation Hospital Equipment and Solutions* **2013**.
25. Wang, L.; Xie, J.; Schultz, P. G., Expanding the Genetic Code. *Annual Review of Biophysics and Biomolecular Structure* **2006,** *35* (1), 225-249.
26. Tian, F.; Tsao, M. L.; Schultz, P. G., A phage display system with unnatural amino acids. *J Am Chem Soc* **2004,** *126* (49), 15962-3.