Developing peptide based capture agents for diagnostics and therapeutics

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DEDICATIONS

I dedicate this dissertation to my father, friend, philosopher and guide, Mr. Dilip Kumar Nag

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ABSTRACT

Iterative in situ click chemistry (IISCC) is a robust general technology for development of high throughput, inexpensive protein detection agents. In IISCC, the target protein acts as a template and catalyst, and assembles its own ligand from modular blocks of peptides. This process of ligand discovery is iterated to add peptide arms to develop a multivalent ligand with increased affinity and selectivity. The peptide based protein capture agents (PCC) should ideally have the same degree of selectivity and specificity as a monoclonal antibody, along with improved chemical stability. We had previously reported developing a PCC agent against bovine carbonic anhydrase II (bCAII) that could replace a polyclonal antibody. To further enhance the affinity or specificity of the PCC agent, I explore branching the peptide arms to develop branched PCC agents against bCAII. The developed branched capture agents have two to three fold higher affinities for the target protein.

In the second part of my thesis, I describe the epitope targeting strategy, a strategy for directing the development of a peptide ligand against specific region or fragment of the protein. The strategy is successfully demonstrated by developing PCC agents with low nanomolar binding affinities that target the C-terminal hydrophobic motif of Akt2 kinase. One of the developed triligands inhibits the kinase activity of Akt. This suggests that, if targeted against the right epitope, the PCC agents can also influence the functional properties of the protein. The exquisite control of the epitope targeting strategy is further demonstrated by developing a cyclic ligand against Akt2. The cyclic ligand acts as an inhibitor by itself, without any iteration of the ligand discovery process. The epitope targeting strategy is a cornerstone of the IISCC technology and opens up new opportunities, leading to the development of protein detection agents and of modulators of protein functions.

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