RAPID CONSTRUCTION OF PROTEIN CAPTURE AGENTS WITH CHEMICALLY DESIGNED STABILITY AND ANTIBODY-LIKE RECOGNITION PROPERTIES

Thesis by

Heather Dawn Agnew

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ABSTRACT

This thesis describes technologies for the rapid and scalable production of high-affinity, high-specificity protein capture agents which possess the affinities and specificities of antibodies, but also exhibit improved chemical, biochemical, and physical stability. I will discuss how the chemical flexibility of comprehensive, one-bead-one-compound (OBOC) libraries of oligopeptides may be combined with *iterative* in situ click chemistry to select multi-ligand capture agents. Large OBOC libraries form the basis of individual peptide ligands, and also permit chemically designed stability through the incorporation of artificial (azide or acetylene) and non-natural amino acid building blocks. The in situ click chemistry method then utilizes the target protein as the catalyst, or template, for assembling its own biligand via formation of a 1,2,3-triazole linkage between two individual ligands (azide and acetylene). This process can be repeated to produce triligands, tetraligands, and other higher-order multi-ligands with an accompanying increase in affinity and specificity through cooperative interactions. Once found, multi-ligand capture agents can be produced in gram amounts via conventional synthetic methods such as the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC). This is a general and robust strategy for the inexpensive, high-throughput construction of protein capture agents that can be exploited to detect protein biomarkers in multi-parameter clinical diagnostic assays.

While high-affinity protein capture agents represent a significant technology advance, they are just one component of what is necessary for highly multiplexed measurements of protein biomarkers. It is also important to develop or optimize the actual assay platforms that can enable sensitive multi-parameter protein measurements using these capture agents. Silicon nanowire (SiNW) nanoelectronic sensors can

provide quantitative, label-free multi-parameter measurements of protein biomarkers in real time. However, SiNW sensors can be challenging to deploy because unprotected Si forms a native oxide layer that can significantly reduce the detection sensitivity of the nanowire sensors via dielectric shielding. Another technical challenge is the development of chemistries which allow for the selective encoding of nanowire surfaces with the capture agents. To overcome these challenges, the final part of this thesis presents a general method to functionalize organic and biological molecules on highly passivated Si(111) surfaces with minimal surface oxidation.

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