

**Title: A pipeline to develop antibody-based tools to visualize and manipulate molecules in living systems**

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**Abstract:**

The process of how a fertilized egg becomes a functional organism has fascinated us for thousands of years (Aristotle, *Generation of Animals*). Our ability to peer down the eyepieces of microscopes to observe fluorescently labeled proteins in the embryos of genetically modified model organisms has taught us important lessons from how stem cells maintain self-renewal potential to how cancer cells metastasize. As our technological capabilities in live imaging and genetic manipulation of embryos by CRISPR-mediated genome editing techniques have grown, fundamental limitations exist, impeding further progress. For example, insertion of fluorescent tags into endogenous genes with CRISPR-based tools remain technically challenging for most organisms. Even if tagging of a gene is successful, the tag itself can negatively impact the normal function of the translated protein. Further, given that the functional output of a protein often changes upon interaction with partner proteins, developing methods to differentially visualize and manipulate a protein when bound to specific partner proteins is needed, the methods for which currently do not exist.

In our proposed study, we seek to broadly solve these technological impediments by developing a non-covalent labeling strategy to detect and manipulate endogenous proteins in their native conformations inside the cells of live animals. Taking advantage of a synthetic Nanobody (Nb)-based technology, we will prove this concept by developing biosensors to  $\beta$ -catenin ( $\beta$ cat), a signaling transducer involved in a myriad of developmentally regulated processes and human diseases. are the antigen recognition domains of the heavy-chain-only antibodies found in camelids. But instead of immunizing the animals, we will utilize a synthetic library of Nbs presented on the surface of yeast cells to identify candidate Nbs that bind to purified  $\beta$ cat protein in vitro. Biosensors consisting of  $\beta$ cat-specific Nbs and moieties for live tracking or targeted inhibition will be introduced in zebrafish embryos to interrogate the spatiotemporal function of  $\beta$ cat during development. Given that Nbs could distinguish conformational variance of proteins in complex with different binding partners, these Nb-based biosensors could be applied to deconvolute the context-dependent function of  $\beta$ cat. The impact of this project will extend far beyond basic science research, as Nb-based reagents hold tremendous promise as therapeutics against viruses and traditionally undruggable targets in human diseases.