# Point of Care Protease Sensing Cut-N-Glow Mapping & Diagnostic Tool

Published date: Feb. 27, 2015

## Technology description

Cut-N-Glow is the first fully, biological in vivo protease mapping tool that emits fluorescence. Our assay is easily tailored via standard cloning techniques to detect different proteases or to map protease specificity. No chemical synthesis is required therefore there is no need for co-factors or co-substrates. Additionally, this assay only requires two reagents and both are proteins that can be easily obtained following over-expression of E. coli. In addition to the advantage of emitting a fluorescent signal in the presence of proteases, Cut-N-Glow, as its name implies, is designed with a conditional distortion to convert self-assembling GFP proteins into a site specific protease switch. Our approach to the distortion is reversible through proteolysis by constraining the N and C termini of GFP 11 with a protease-sensitive tether, eglin C. The feature distinguishing our system from other GFP reporters is that there is a gain of fluorescence, rather than a loss of fluorescence in proteolysis both in vitro and in vivo.

Proteases occur naturally in all organisms and are valuable tools in medical diagnostics serving as initiators of cell signaling, as regulators of immune responses, and as agents of infectious disease. Therefore, mapping proteases in parasitic diseases and bacteria as well as assayable proteases associated with cancer could lead to the identification of shared structural similarities validating potential drug targets. Our strategy utilized split proteins in a conditionally inactive form with the aid of a conformational distortion maintained by a cleavable tether. We applied this method to convert split GFP into a latent fluorophore that can be activated by site-specific proteolysis. The chimeric GFP served as substrate for representative enzymes from the three major protease classes: serine, cysteine, and aspartic acid.

## Application area

Human clinical diagnostic protease detection for diseases & infection: In bacterial infections such as M.tuberculosis, C. botulism and MARTX toxins such as V. Cholera. In assayable proteases associated with cancer such as human kallikrein-3, commonly known as prostate specific antigen (PSA). Targeting the HIV protease, for the AIDS virus. In matrix metalloproteinases's (MMPs) involved in tissue remodeling such as morphogenesis, angiogenesis, cirrhosis and arthritis. In parasitic infections such as Cryptosporidium parvum, Plasmodium falciparum, Schistosomiasis, and Trypanosoma cruzi. Potential

utility as a diagnostic and as a research tool in vitro or in vivo: Identification of peptide sequences cleaved by a particular protease (substrate discovery) Identification of the protease responsible for cleaving a specific peptide sequence (protease discovery) Identification of protease variants, created through mutation, that cleave at a user-defined peptide sequence (protease evolution) Detection of a characterized protease in chromatographic fractions or laboratory buffers (protease detection).

## Advantages

Great potential to be implemented as an in vivo technique, to stand alone as the first fully biological, gain-of-fluorescence protease mapping tool. Features a highly stable output signal. Our results indicate that the fluorescent signal that follows site-specific proteolysis S/N level ΓÇô despite the presence of E. coli lysate. Efficient and affordable. This assay only requires two reagents and both are proteins that can be easily obtained following over-expression of E. coli. No chemical synthesis is required. No need for co-factors or co-substrates. Easily tailored assay via standard cloning techniques to detect different proteases or to map protease specificity. Effectively adapted to portable field testing.

## Institution

Health Research Inc

## Inventors

Brian Callahan

叶先生

电 话：021-65679356

手 机：13414935137

邮 箱：yeyingsheng@zf-ym.com