



HEALTH RESEARCH
I N C O R P O R A T E D

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

Background:

Cut-N-Glow is the first fully biological gain-of-fluorescence protease activity sensor. Our assay is easily tailored via standard cloning techniques to detect proteases of varying specificity, to identify protease inhibitors, or to map protease substrate preference *in vitro* or *in vivo*. No chemical synthesis, co-factors or co-substrates are required for the operation of Cut-N-Glow. Additionally, this assay requires only two reagents and both are proteins that can be easily obtained following over-expression in *E. coli*. The two proteins are engineered, fluorogenic fragments of green fluorescent protein. Site-specific proteolysis of one of these fragments releases a configurational restraint, allowing it to be bound by the complementary fragment. The GFP complex then undergoes a chromophore-forming reaction that leads to the emission of green light. Light emission can be followed quantitatively using a fluorimeter, or qualitatively by eye as the assay solution turns green. In addition to the distinct advantage of emitting a fluorescent signal in the presence of proteases, Cut-N-Glow has been functionally validated as a substrate for representative enzymes from the three major protease classes: serine, cysteine, and aspartic acid.

Proteases occur naturally in all organisms, serving as initiators of cell signaling, as regulators of immune responses, and as virulence factors of diverse infectious agents. Accordingly, proteases are valuable biomarkers for medical diagnostics. Additionally, proteases are widely employed as reagents for biomedical research and for industrial applications. The Cut-N-Glow system, with its operational simplicity and *in vitro/in vivo* versatility, could find use in all of the areas outlined below.

Applications:

Human clinical diagnostic for protease-based disease detection:

- In microbial infections such as *M. tuberculosis*, *Clostridium botulinum*, *Vibrio Cholera*, *Cryptosporidium parvum*, *Plasmodium falciparum*, and *Trypanosoma cruzi*.

- Detecting secreted proteases associated with cancer such as human kallikrein-3, commonly known as prostate specific antigen (PSA).
- Sensing the HIV protease, for monitoring AIDS therapy.
- Detecting cell-associated matrix metalloproteinases's (MMPs) involved in tissue remodeling such as morphogenesis, angiogenesis, cirrhosis and arthritis.

Potential utility as a tool for protease research:

- 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 has a persistently high signal-to-noise level 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.

State of Development:

Early research stage with diagnostic assay available

Patents:

Provisional patent 61/514,074

Licensing Potential:

Health Research, Inc. is seeking companies interested in commercializing protease activity detection assay's for diagnostic and research *in vitro* and *in vivo*. This invention is available for license.

The Inventors:

Brian Callahan, Ph.D.

Brian Callahan obtained his B.Sc. from the State University of New York in Cortland (1996). He worked as a laboratory technician (1997-1999), before pursuing a Ph.D. in Biochemistry and Biophysics at the University of North Carolina in Chapel Hill (2000-2005). In 2006, Brian received an NIH-funded fellowship in Biodefense and Emerging Infectious Diseases from the Wadsworth Center in Albany, N.Y., where he is currently a postdoctoral associate in Professor Marlene Belfort's laboratory.



Marlene Belfort, Ph.D.

Marlene Belfort is a distinguished scientist and has won many awards and honors within the scientific community. She directs her own laboratory at the New York State Department of Health, Wadsworth Center, called The Belfort Laboratory. She is an Adjunct Scientist for the Marine Biological Laboratory in Woods Hole, MA and founding member of Mobile DNA Cluster; an Adjunct Professor in the Department of Chemical and Biological Engineering & the Department of Biology with Rensselaer Polytechnic Institute in Troy, NY; an Adjunct Professor for the Biology Department with SUNY at Albany in Albany, NY; a Professor for the School of Public Health at SUNY at Albany and the New York State Department of Health. Marlene Belfort holds two patents for intein technology and recently applied for a third.

Awards & Honors

Distinguished Scientist, Wadsworth Center, Molecular Genetics
Fellow, American Association for Advancement of Science
Doctor Philosophiae Honoris Causa, Hebrew University
Distinguished Professor, School of Public Health, Biomedical Sciences
Alice Evans Award, American Society for Microbiology
MERIT Award NIH
Fellow, American Academy of Microbiology
Member, National Academy of Sciences (NAS)
Fellow, American Academy of Arts and Sciences

Education

Ph.D., University of California at Irvine (1972)
Postdoctoral training, Hebrew University, Jerusalem

Publications:

Callahan, Brian P., Stanger, Matthew, J., Belfort, Marlene: *Protease Activation of Split Green Fluorescent Protein*: ChemBioChem - Chemistry Clinical Biochemistry
10-13-2010 DOI: 10.1002/cbic.201000453

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