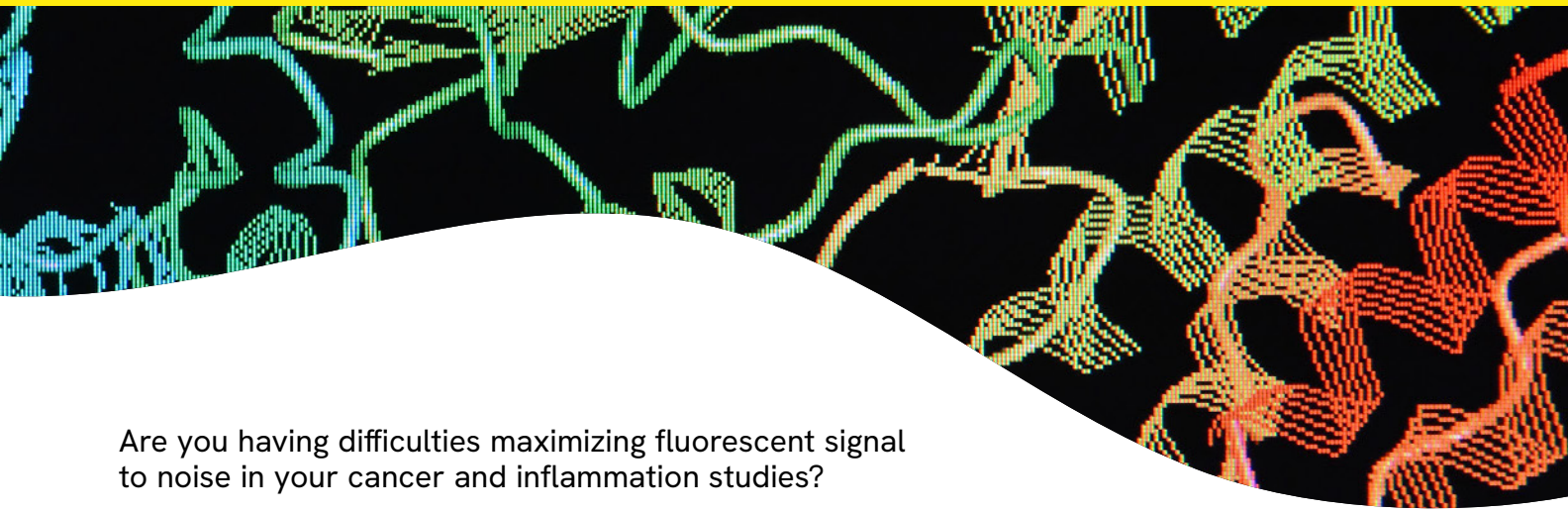
Detect and monitor protease-related biological processes *in vivo* with IVISense Pan Cathepsin fluorescent probes.



Are you having difficulties maximizing fluorescent signal to noise in your cancer and inflammation studies?

Traditional preclinical mouse models rely primarily on *ex vivo* measurements of disease morphology and histologic analysis for the assessment of tumors and other manifestations of disease. These measurements limit the amount of information obtainable and may not show the most relevant biology. In contrast, *in vivo* fluorescence imaging allows for a maximized number of endpoints and readings to obtain the most relevant information from a set of animals in a study, but may still be challenged by a low signal to noise ratio. With IVISense™ Pan Cathepsin NIR fluorescent probes, both challenges can be met, for optimal cancer and inflammation-related cathepsin visualization. Sensitive detection of lysosomal cathepsin activity, which may change with disease progression and therapeutic response, can be visualized non-invasively over time, and with maximized fluorescent signal to noise.

What are proteases, and what is their function?

Proteases have long been known to perform the crucial tasks of specific and nonspecific proteolysis. Found in all forms of life, including bacteria and viruses, proteases have evolved multiple times, enabling the emergence of different classes of protease. One important class, cysteine cathepsin family, is essential in controlling protein lifespan as well as activity. Expression and inhibition is regulated in exquisite balance with the protein cleavage and degradation functions they perform in a healthy biological system.

Within the human lysosomal cysteine cathepsin family, there are 11 members, many of which have overlapping functions. Some of the more common lysosomal cathepsins include cathepsin B, L, S, and Plasmin. The activity of these cysteine cathepsins is limited almost entirely to within the slightly acidic low pH environment of the lysosome. Dysregulation and overexpression of cathepsins into the extracellular space has been demonstrated to be a hallmark of many pathological conditions, including:

- Inflammation
- Cancer, Tumor Metastasis
- Atherosclerosis
- Cardiovascular disease
- Rheumatoid Arthritis, Autoimmunity, Osteoarthritis
- Pulmonary Disease
- Neuroinflammation/Hyperalgesia

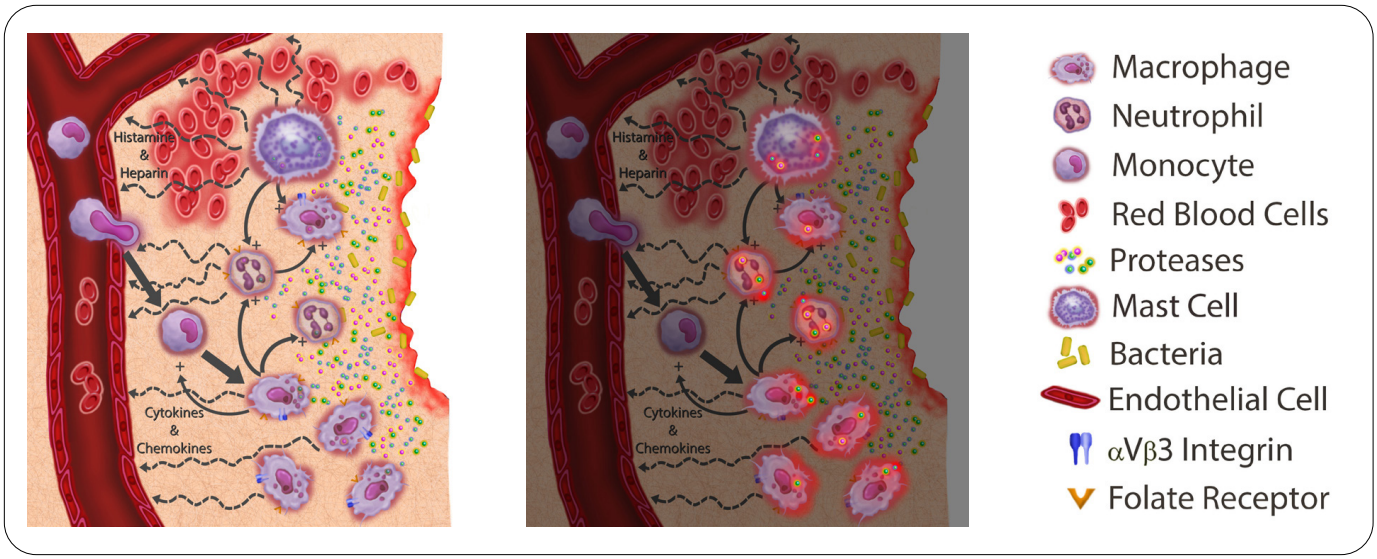


Figure 1. Proteases and cysteine cathepsins have characterized activity in the lysosomal and extracellular space. IVISense Pan Cathepsin fluorescent activation is represented in the figure on the right.

How do IVISense Pan Cathepsin fluorescent agents work?

IVISense Pan Cathepsin NIR fluorescent probes were developed to detect broad cathepsin family activity as they are key disease associated proteases. Optically silent in their intact state, IVISense Pan Cathepsin probes use a novel patented technology* to visualized active protease activity, becoming highly fluorescent following protease-mediated cleavage and activation.

IVISense Pan Cathepsin probes can be used to detect and quantify normal as well as abnormal expression of proteases, including cathepsins, *in vivo* in real time. Taken up via pinocytosis into the lysosomal/endosomal pathway, this probe allows the detection of proteases inside the lysosomes of tumor or inflammatory cells such as macrophages, neutrophils, and mast cells, with no inhibition of protease activity. IVISense Pan Cathepsin probe has minimal up-take and activation by resting macrophages, making it particularly useful for inflammation studies, such as pulmonary inflammation and acute paw inflammation, where it detects a broad range of active inflammatory cells.

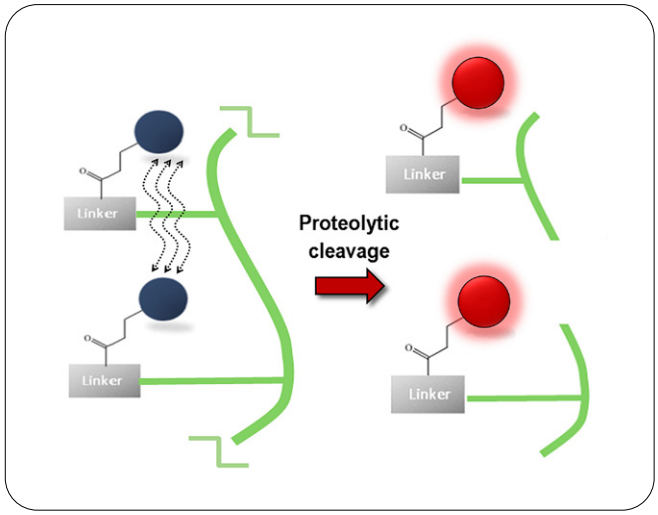


Figure 2. IVISense Pan Cathepsin probe activation. (left) Inactive probe with fluorophores in close approximation, (right) protease cleavage separates the fluorophores allowing activation.

How is IVISense Pan Cathespin used?

Here we show two case studies demonstrating possible applications of IVISense Pan Cathepsin fluorescent probes.

Case study 1 Asthma

Monitoring arthritis progression and treatment in vivo using IVISense Pan Cathepsin 680

Asthma is an inflammatory disease process characterized by reversible airway obstruction and airway hyperresponsiveness. This disease process is driven by activated T lymphocytes and eosinophils that are recruited to the lung upon inhalation of triggering allergens. These cells release inflammatory mediators, activate mast and epithelial cells, and stimulate mucus secretion, which ultimately leads to airway obstruction. The allergic reaction can be induced in mice by provoking an immune response to ovalbumin in the lungs by first immunizing mice with ovalbumin. Three weeks later, mice are given an intranasal challenge of ovalbumin. As seen in Figure 3, an allergic response characterized by changes in airway hyperreactivity then develops in the lungs, resulting from a large influx of eosinophils and the induction of cytokines and immune factors, also typical of those in human asthma (for example, interleukin (IL)-4, histamine and IgE).

Measurement of these parameters generally requires surgical procedures (for airway hyperreactivity), assessment of sacrificed mice (for bronchoalveolar lavage [BAL] eosinophil count), and extensive sample handling and preparation (for serum and BAL fluid microplate assays). In contrast, non-invasive imaging with IVISense Pan Cathepsin allows one to track the disease and monitor the progression at multiple time points in the same animal.

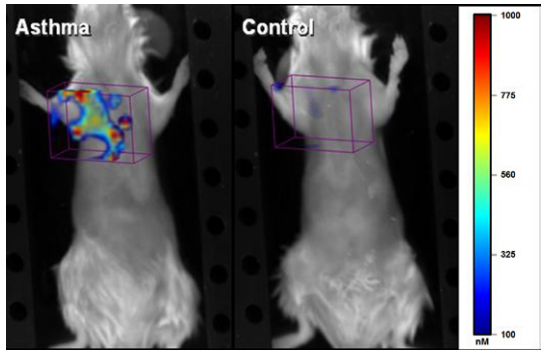


Figure 3. Asthmatic mouse (left) injected with IVISense Pan Cathepsin 680, shows broad distribution of fluorescence and inflammation in the lung. Control mouse (right) has almost no fluorescence signal. Imaged using the FMT® fluorescence tomography system.

Case study 2 Oncology

Monitoring tumor development in a metastatic lung tumor model using implanted 4T1 tumor cells

Metastatic lung tumors were established by intravenous injection of 4T1 mouse mammary carcinoma cells. Tumors were allowed to grow for two weeks prior to administration of IVISense Pan Cathepsin 750 probe. The fluorescent non-invasive imaging results as seen in Figure 4 were robust and correlated well with terminal assessments, such as changes in gross lung weight, *ex vivo* tissue imaging, and histologic assessment. Such an imaging approach may be shown to help visualize and quantitate steps in cancer progression or metastatic cascade *in vivo*, and may be beneficial in developing novel therapeutic treatments.

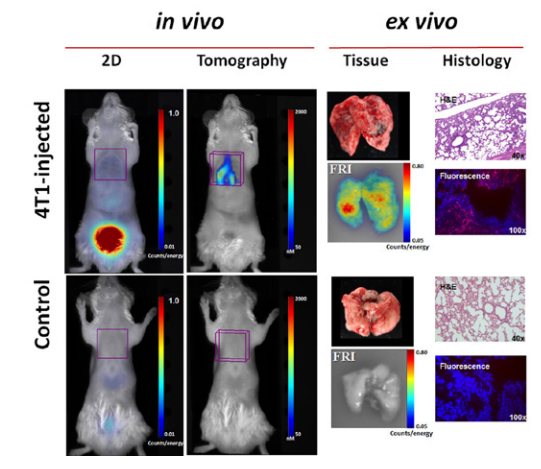


Figure 4. Two weeks following injection of $5X10^5$ 4T1 cells IVISense Pan Cathepsin 750 fluorescent probe was injected, animals were imaged, and organs were removed for *ex vivo* confirmation. Imaged using the FMT® fluorescence molecular tomography imaging system.

Use IVISense Pan Cathepsin as part of a complete experimental solution package

Revvity provides complete *in vivo* imaging solutions including reagents, instrumentation and support expertise that can help you monitor and design experiments to understand the progression of diseases and their related processes, or to evaluate the potential therapeutic efficacy of drugs targeting the underlying mechanisms involved in disease.

IVISense Pan Cathepsin is available in three wavelengths; 680, 750, and 750 FAST. IVISense Pan Cathepsin FAST (Fluorescent Activatable Sensor Technology), has an improved pharmacokinetic profile with earlier time points offering higher target specific signal with reduced background while also reducing the optimal imaging time after injection.

Cat #	Product	
NEV10003	IVISense Pan Cathepsin 680	The original "Smart" cathepsin probe; rather than targeting all protease, smart probes have activatable fluorescence that keeps background down and allows detection of activated protease
NEV10001EX	IVISense Pan Cathepsin 750	Offers cathepsin detection at an alternate wavelength
NEV11171	IVISense Pan Cathepsin 750 FAST	Faster activation after injection, and can also be imaged at earlier times for acute inflammation models

*Patent 9574085 - Biocompatible N, N-disubstituted sulfonamide-containing fluorescent dye labels.

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