Silica Nanobeads Create Fast Enzyme Sensor

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Proteases are an important family of enzymes involved in many key biochemical processes, including the metastatic spread of cancer cells from a primary tumor.

Because of the importance of these enzymes in a variety of disease processes, researchers have developed a wide range of protease sensors, but few are sensitive enough to monitor real-time activity of protease activity either in the body or in clinically useful diagnostic assays. But a new assay, using silica nanobeads, appears to overcome this limitation.

The investigators published their work in the journal *Sensors and Actuators B*.

Shiela Grant, Ph.D., of the University of Missouri, Columbia, and colleagues took advantage of a physical effect known as fluorescence resonance energy transfer, or FRET, to create the new sensor. FRET is a process in which one molecule absorbs light energy and passes it to a nearby molecule that then releases the energy in the form of fluorescent light. If the pair of molecules become physically separated, FRET does not occur and the fluorescent signal disappears.

In this work, the investigators anchored the twin components of a FRET system to the surface of a silica nanobead using a small peptide linker molecule that can be severed by a pre-specified protease. In this case, the investigators synthesized peptide linkers that can be severed by the protein known as trypsin. As a control, the researchers also attached the FRET components to silica nanobeads using a peptide that trypsin

cannot cleave. The large surface area of the silica nanobeads allowed the researchers to attach hundreds of FRET pairs to each nanobeads sensor, enabling the bead to fluoresce brightly when not in the presence of trypsin.

However, when the investigators added trypsin to a solution of the nanobeads, they saw a sharp decline in fluorescence within two minutes. As expected, fluorescence remained stable with the control nanobeads. The sensor nanobeads were able to detect trypsin concentrations as low as 12 micrograms of enzyme per milliliter of solution, well within a range that would be useful for clinical measurements. Nevertheless, the researchers note that they are developing new linkers that should further increase the sensitivity of this assay.

This work is detailed in a paper titled, “Development of a protease biosensor utilizing silica nanobeads.” This paper was published online in advance of print publication. An abstract is available at the journal’s website.

Source: National Cancer Institute