

A novel biosensor to detect DNA damage in real time

March 13 2023

A Nobel Biosensor for the Real-Time Detection of DNA Damage in Living Specimens

Double-strand breaks (DSBs) are a severe type of DNA damage that negatively impact cellular function and survival

Cell nucleus

H2AX protein accumulates at sites of DSBs

Currently, DSBs are detected in isolated cell samples, making it difficult to analyze the DNA damage in real-time in live specimens

How can we create a real-time biosensor to detect DSBs based on H2AX accumulation at the site?

A fluorescence resonance energy transfer (FRET) biosensor for real-time detection of DSBs

Plasmid with H2AX protein

H2AX substrate

Yellow fluorescent protein

Enhanced cyan fluorescent protein

BRCT1

BRCT1 domain

Conformational change between fluorescent proteins

Phosphorylation of H2AX protein to γ H2AX at substrate

Change in FRET signal

DSB

Dephosphorylation of H2AX

Substrate drawn to BRCT1 domain

- Non-invasive method
- Real-time detection of DSBs in live specimens
- High specificity and sensitivity

The FRET-based biosensor can detect DSBs in living specimens, in real-time, making it valuable for cancer treatment, drug discovery, and identification of DNA-damaging agents

A novel DNA double-strand breaks biosensor based on fluorescence resonance energy transfer
Suh et al. (2023) | Biomaterials Research | DOI: 10.1186/s40824-023-00354-1

Lab website: <https://tkim77.wixsite.com/ncid>
ORCID ID: 0000-0001-7678-0478

PUSAN NATIONAL UNIVERSITY

The biosensor consists of fluorescent proteins that change intensity when DSBs occur, allowing scientists to detect DNA damage in living cells and evaluate cellular response to radiation therapy and other DNA damaging factors. Credit: Tae-Jin Kim from Pusan National University

Double-strand breaks (DSBs) are a type of DNA damage where both strands of DNA break at the same location. They can adversely affect

cell growth and functioning. Currently, DSBs are detected by immunostaining techniques, which identify markers that accompany DNA damage, such as the protein γ H2AX. However, these methods are tedious, and cannot be used to detect DSBs in real time in living specimens.

In a 2023 study, published in *Biomaterials Research*, researchers describe a [fluorescence resonance energy transfer](#) (FRET) biosensor that can detect DSBs in real time, and provide time- and location-based information on γ H2AX. "The biosensor we have designed could be useful in areas such as [cancer treatment](#) and [drug discovery](#)," says Associate Professor Tae-Jin Kim, from Pusan National University, Korea, who led the study.

FRET sensors consist of two fluorescent proteins or dyes—a donor and an acceptor—which investigate interactions between [biological molecules](#). The energy transfer, and consequently, the amount of emitted light (the FRET signal) depends on the distance and orientation between the two dyes.

The researchers attached the fluorescent dyes with proteins that are involved in the cellular response to DNA damage, namely the H2AX substrate and BRCT1 domain. The H2AX substrate is a target for the H2AX protein to bind and become phosphorylated (forming γ H2AX).

On the other hand, the BRCT1 domain acts as a site for the accumulation of repair proteins, including γ H2AX. Thus, when a DSB occurs, γ H2AX is attracted to the BRCT1 domain, leading to a conformational change in the fluorescent proteins, thereby causing a change in the FRET signal.

The researchers then confirmed the validity of the sensor by introducing plasmids (DNA that, here, contain instructions to make the FRET sensor

inside the cells) encoding the FRET sensor into human embryonic kidney cells (HEK293T) cells. Compared to conventional immunostaining techniques, this biosensor was more sensitive at reacting to the presence of γ H2AX, making it more effective at detecting drug- and radiation-induced DSBs.

"Moreover, as changes in the FRET signal give useful indications of the extent of the DNA damage, the sensor can also be used to examine DNA damage and repair mechanisms, optimize cancer treatments, discover and assess DNA repair drugs, and identify DNA damaging factors in the environment," concludes Associate Prof. Kim.

More information: Jung-Soo Suh et al, A novel DNA double-strand breaks biosensor based on fluorescence resonance energy transfer, *Biomaterials Research* (2023). [DOI: 10.1186/s40824-023-00354-1](https://doi.org/10.1186/s40824-023-00354-1)

Provided by Pusan National University

Citation: A novel biosensor to detect DNA damage in real time (2023, March 13) retrieved 2 May 2024 from <https://phys.org/news/2023-03-biosensor-dna-real.html>

<p>This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.</p>
--