Engineering chimeric polypeptides to illuminate cellular redox states

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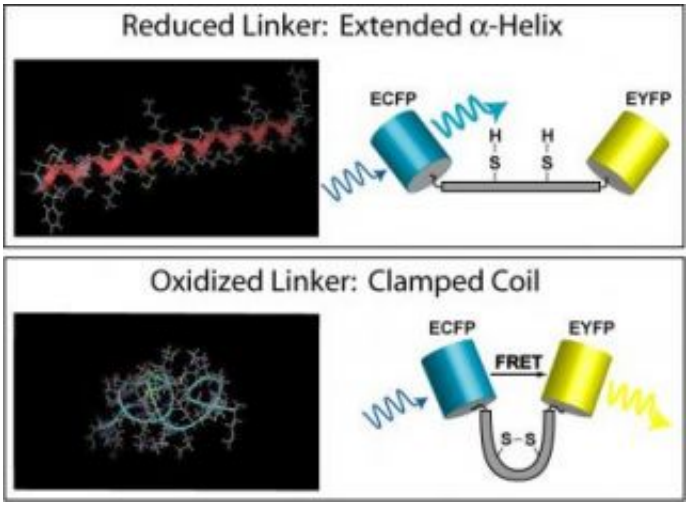

The principle by which the proposed redox biosensors work is illustrated in the figure above. Credit: Society for Experimental Biology and Medicine

UIUC interdisciplinary team reports the design of chimeric polypeptides leading to development of noninvasive biosensors for potential application in biomedical research.

Reduction/oxidation (redox) systems research is reaching a stage where domains that traditionally belonged to the physical sciences, chemistry, and molecular biology are coming together to offer new synergistic opportunities for understanding and manipulating basic cellular processes that underlie complex biomedical problems (e.g.,

tumorigenesis).

Parallel with this advance is the emerging recognition that the intracellular redox environment exerts a profound influence on the normal cellular processes of DNA synthesis, enzyme activation, selective gene expression, cell cycle progression, proliferation, differentiation, and apoptosis. However, this is a difficult area of study and molecular mechanisms mediating redox sensitivity are poorly defined.

An interdisciplinary research team from the University of Illinois' Institute for Genomic Biology (IGB) report in the February issue of the journal Experimental Biology and Medicine the engineering of novel peptide sequences that are sensitive to redox conditions inside cells.

"Attachment of linkers between a special pair of green fluorescent proteins (GFP) shows great promise for developing genetically encoded redox sensitive biosensors," said Vladimir L. Kolossov, corresponding author. To detect oxidation and reduction, the biosensor uses a powerful optical technique called Förster resonance energy transfer (FRET).

The absence of polypeptide linkers able to sense the redox state by undergoing a conformational change was the major obstacle to a FRET-based redox sensor. The researchers designed the linker sequence such that in its reduced state the linker is an α-helix. Thiol groups, strategically placed throughout the linker, sense the redox potential of the environment and form disulfide bonds upon oxidation.

Under oxidative conditions intramolecular disulfide bonds can form, shifting the free energy minimum from the α-helix, to a "clamped-coil" state (similar to a helix-coil transition). The coiled state allows the two fluorescent proteins to approach closer than in the extended helix state, where they can more efficiently exchange excitation energy (i.e., a high FRET state). The extent of energy transfer is easily quantified from the

increased emission of the acceptor.

This is the first step towards development of a FRET-based biosensor for visualizing redox potentials and oxidative stress in live cells and tissues via optical microscopy.

“We employed a sensitive technique for measuring FRET to screen our linkers. This methodology greatly expedited the quantitative analysis and development of the linkers and will be very useful for the development of other FRET-based sensors,” said Bryan Q. Spring, a doctoral student and co-author of the publication. Given the importance of the intracellular redox state in determining a cell’s fate, and the increasing evidence that perturbations in the redox state are associated with cancer and various inflammatory disorders as well as aging, FRET-based redox sensors offer significant promise for understanding molecular mechanisms underlying human health and disease.

Dr. Steven R. Goodman, Editor-in-Chief of Experimental Biology and Medicine, said “Altered redox status is a hallmark of many diseases ranging from neurological disorders, such as Alzheimer’s Disease, to hematologic disorders such as Sickle Cell Disease. The development of a FRET-based biosensor to measure oxidative stress in living cells would be of enormous benefit to biomedical researchers working in many diverse fields. This is precisely the type of interdisciplinary effort that the new Experimental Biology and Medicine hopes to provide to the international scientific community.”

Source: Society for Experimental Biology and Medicine

https://phys.org/news/2008-01-chimeric-polypeptides-illuminate-cellular-redox.html