

Development of a Förster resonance energy transfer sensor for realtime imaging of intracellular redox dynamics

In work published on page 681 of this issue of *Experimental Biology and Medicine*, Kolossov, Spring and their co-investigators – a multidisciplinary team within the Institute for Genomic Biology at the University of Illinois – have transferred the concept of redox-sensitive green fluorescent proteins (GFPs) to a quantitative Förster resonance energy transfer (FRET) imaging platform. For the FRET-based sensors, a change in redox induces a conformational change in a redox-sensitive switch that links two fluorescent proteins (the donor and acceptor), changing their distance, which in turn causes a detectable change in FRET efficiency. In its oxidized state, the wavelength spectrum of the sensor's fluorescence emission is red-shifted (due to increased acceptor fluorescence), independent of variations in the local sensor concentration or in the intensity of the excitation light. As explained by Robert Clegg, a pioneer in the development of novel applications of optical microscopy in the biological sciences and key collaborator on the study, 'FRET-based sensors circumvent the complications associated with imaging methods based on fluorescence intensity, since the increase in the FRET acceptor molecule's fluorescence can only take place if there is a change in the efficiency of energy transfer. This specific and discriminatory feature of FRET is one of the driving motives behind our development of a FRET-based assay rather than relying only on changes in the fluorescent intensity of a single component.'

The current publication builds on the authors' previous work, where they reported a series of first-generation redox-sensitive linkers flanked by FRET donor and acceptor GFP variants. As summarized by co-author Vladimir Kolossov, 'The major advance in the current study is an improved dynamic range of the spectroscopic signal; in other words, a greater difference between fully reduced and oxidized states. Increasing the dynamic range leads to better discrimination between the redox states of the probe in complex biological specimens. Furthermore, the highly oxidative midpoint potential of the novel probe is ideal for measuring glutathione redox potentials in oxidative compartments of mammalian cells.'

Recently, a different innovative ratiometric probe – a redox-sensitive GFP (roGFP) – has been developed in

another lab. The measurement with the roGFP sensor involves the ratio of intensities of two sequential images, acquired at two different excitation wavelengths. Two thiol groups form/break a disulfide bond that modulates the peak excitation wavelength of the roGFP chromophore in response to the redox environment. Bryan Spring, a co-author, notes, 'The roGFP and the FRET-based sensors have contrasting characteristics. The FRET-based sensor may prove advantageous for intravital microscopy studies, because only a single laser line is required. In contrast, roGFP requires sequential scanning of two laser lines, which slows the frame rate of image acquisition; also, the images must be compensated for the different laser intensities in order to correct for wavelength-dependent tissue scattering, and the measurement relies on the optical alignment of two excitation light beams. However, the roGFP probe is sensitive to a different range of oxidation–reduction potentials than our FRET probe, possibly leading to complementary applications.' Spring adds, 'We look forward to further exciting innovations for optimizing the performance of oxidation-reduction-based sensors.'

Dr Rex Gaskins, who led the project remarked, 'Distinct advantages of the FRET-based approach include: (1) the ability to quantify the change in redox state; (2) independence of sensor concentration; and (3) modularity, the ability to precisely tune the redox sensitivity and range by exchange of the switch or the fluorophore modules in the probe. We expect that newly developed redox-sensitive probes could potentially be critical to a better understanding of the pharmacologic and toxicological actions of chemotherapeutic drugs and oxidants.'

Dr Steven R Goodman, Editor-in-Chief of *Experimental Biology and Medicine*, said 'This multidisciplinary group has developed a novel FRET-based biosensor which is a major advance in the measurement of oxidative stress in living cells in realtime. This will allow the measurement of intraorganellar glutathione potentials in living cells.'

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