Genetically Encoded Fluorescent Redox Biosensors

Subjects: Biochemistry & Molecular Biology

Contributor: Aleksandra Kokova

In the most general sense of this term, a genetically encoded fluorescent redox biosensor is a kind of genetic system that changes a fluorescence in response to a redox event. Most often, such a tool is a chimeric protein consisting of a sensory domain that responds to a redox stimulus, and a domain whose fluorescence changes depending on alterations occurring with the sensory domain. It can also be a single-domain fluorescent protein, the structure of which changes when exposed to a redox stimulus, and the change in the structure leads to a change in fluorescence. In addition, there are transcriptional reporters, the principle of which is based on the alteration of the transcription of the fluorescent protein gene upon exposure to a redox stimulus. This article will focus on protein genetically encoded fluorescent redox biosensors that are currently used in *in vivo* research.

Keywords: fluorescent proteins ; genetically encoded biosensors ; redox biology

1. roGFP type biosensors

Today, the most widely used *in vivo* redox probes belong to the redox-sensitive green FP (roGFP) and HyPer families. The roGFP family includes redox-sensitive proteins, more or less selective to E_{GSH}[1][2][3][4] and probes that are relatively specific to other thiols[5][6][7] or H_2O_2[8][9], which were developed by fusing roGFPs with different adapter proteins. The first roGFP variants were developed in 2004 by introducing redox-active cysteine residues to the GFP β-barrel surface in spatial proximity to the chromophore[1] (a similar design was applied for the first time to yellow FP (YFP) by Winther et al. in 2001, giving the redox-sensitive YFP (rxYFP) probe[10]). Thus, a number of roGFPs were developed, and roGFP1 and roGFP2 gained the most widespread popularity in further research. roGFP1 and roGFP2 demonstrate a ratiometric signal of relatively high amplitude, moreover, the roGFP1 signal is not pH-dependent[1].

Two special improvements in the roGFP1 have subsequently been reported, namely, the roGFP1-Rx[2] and roGFP1-iX[3] groups, with the former group consisting of variants with improved response rate, and the latter group demonstrating less negative midpoint redox potentials, allowing for the use of roGFP1-iN in relatively oxidizing subcellular compartments, such as the ER and Golgi apparatus. In 2008, an important optimization of roGFP2 was published: a fusion protein consisting of human glutaredoxin 1 (Grx1) and roGFP2 showed a significantly higher response rate and selectivity to glutathione[4]. The idea behind the probe was as follows: human Grx1 specifically catalyzes the transfer of oxidative equivalents between the glutathione redox pair and roGFP2, thus, the spatial proximity of the enzyme and the sensor molecule leads to a more rapid and specific equilibration. The obtained probe, Grx1-roGFP2, allowed ratiometric pH-independent (pH 5.5–8.5) measurements and became one of the commonly applied *in vivo* redox biosensors. In a similar way to Grx1-roGFP2, probes to assess the redox potential of *Mycobacterium tuberculosis* mycothiol (Mrx1-roGFP2[5]), *Staphylococcus aureus* bacillithiol (Brx-roGFP2[6]), and trypanosome trypanothione (Tpx-roGFP2[7]), were developed.

The roGFP-based probes specific to H_2O_2, roGFP2-Orp1[8] and roGFP2-Tsa2ΔC_R (and the $\Delta C_R \Delta C_P$ variant)[9], have been described and used *in vivo*. RoGFP2-Orp1[8] was designed as a fusion protein consisting of roGFP2 and Orp1 peroxiredoxin. Orp1 was found to efficiently mediate electron flow between H_2O_2 and roGFP2, creating a special redox relay when placed in close proximity to the target protein. The indicator allows ratiometric measurements and is widely used in different model organisms for H_2O_2 registration. Recently, an ultrasensitive probe with a similar design idea has been reported[9]. The yeast peroxiredoxin Tsa2 was fused to roGFP2 to mediate the oxidation of the latter. The fundamental difference is that Tsa2 is a 2-Cys peroxiredoxin, which are the most efficient thiol peroxidases and react with H_2O_2 100 times faster than Orp1. The indicators roGFP2-Tsa2ΔC_R and $\Delta C_R \Delta C_P$ variant, enable the detection of endogenous basal H_2O_2 levels, which may give new insights into H_2O_2 biology. However, since the probes were developed based on the yeast platform, some optimization may be needed for their use in other model organisms.

In 2017, some limitations for *in vivo* application of the Grx1-roGFP2 and roGFP2-Orp1 probes were observed by Müller at al.[11]. It was found that micromolar amounts of hypochlorous acid and polysulfides contribute to roGFP2-based probe responses in vitro. Peroxynitrite may also influence the probes redox state, especially for roGFP2-Orp1. The required

concentrations of HOCl, for instance, can be produced during the phagocyte respiratory burst. These facts imply that these indicators should be used carefully in physiological conditions, pathological conditions, or in specific cell types, where high amounts of the aforementioned oxidants are generated[11].

In addition to roGFP family probes, there are indicators constructed in a similar way. Among these are oxidation-sensitive Oba-Q reporters[12], the fluorescence of which is drastically quenched upon oxidation, as reported by Sugiura et al. The Oba-Q indicators are based on blue-shifted FPs, so multiparameter imaging is possible using Oba-Q in combination with biosensors of different colors. Two red probes, redox sensitive red FP (rxRFP)[13] and Grx1-roCherry[14] have been applied *in vivo*. RxRFP was based on circularly permuted (cp) red FP cp-mApple with cysteine residues introduced on its mutated N- and C-termini[13]. Grx1-roCherry was constructed as a fusion protein consisting of human Grx1 and the redox-sensitive red FP roCherry[14]. Both probes are redox-sensitive, and Grx1-roCherry demonstrates a sufficient response rate and relative specificity to glutathione redox potential due to the Grx1 domain. Red probes are preferable in some conditions because, firstly, their excitation and emission light is less toxic for living objects, gives less autofluorescence, and penetrates tissues better, and secondly, red indicators allow for multiparameter imaging when used in combination with blue and green indicators.

A novel redox-sensitive probe, which was based on the Japanese eel FP UnaG[15] has been reported by Hu et al.[16]. Like other redox indicators, redox-sensitive UnaG (roUnaG) contains a pair of cysteines on the β-barrel surface, which form a disulfide bond and change the chromophore spectral properties. Interestingly, UnaG emits light only upon binding of bilirubin, which acts as an external chromophore. This makes it possible to use roUnaG to monitor the redox state in hypoxic conditions, which may be a good alternative to GFP-based proteins because the GFP chromophore requires the molecular oxygen for maturation. However, there may be some limitations due to the necessity of bilirubin introduction.

2. HyPer type biosensors

The HyPer family is a group of H_2O_2 sensing probes[17][18][19][20][21]. The first HyPer was designed as a chimera consisting of a regulatory domain of the *Escherichia coli* H_2O_2 sensing transcriptional factor OxyR (OxyR-RD) and cpYFP, integrated into a flexible region of the former[18]. In the primary structure of OxyR-RD there are two key cysteine residues, which form a disulfide bond under H_2O_2-mediated oxidation, with consequent dramatic conformational changes in the protein structure. The conformational rearrangement triggers a change in the cpYFP chromophore microenvironment and, accordingly, fluorescence intensity. Two upgrades of HyPer, namely HyPer-2[19] and HyPer-3[17], with improved maximum response amplitude and kinetic properties were subsequently reported. In 2020, an ultrasensitive pH-stable probe HyPer7, characterized by significantly enhanced brightness, kinetics, and sensitivity was described by Pak et al.[20]. The fundamental difference of HyPer7 from previous HyPers is that its sensory domain is represented by *Neisseria meningitidis* OxyR-RD, which presumably has a higher sensitivity to H_2O_2 than its *E. coli* homolog, although this fact has not been known a priori. The red probe HyPerRed[21], which is based on cp-mApple, also has also been applied *in vivo*. Except for HyPer7, all HyPers respond to pH alterations, so experiments with the H_2O_2-insensitive HyPers-C199S, or SypHers, are needed to control for possible artifacts. In HyPers-C199S one of the two key cysteines is replaced with serine, which makes the probe unresponsive to H_2O_2, retaining the same pH dependency of fluorescence ratio. HyPer-3, HyPer7, and HyPerRed could be used in combinations to study several redox processes simultaneously. While the application of HyPerRed with either green variant allows real-time imaging in different organelles, HyPer7 and HyPer-3 allow H_2O_2 dynamics to be monitored in conditions where different sensitivity of the indicator is needed.

Using HyPer, HyPer-2, HyPer-3, and HyPer7 as an example, one can see how the gradual improvement of the biosensor makes it more and more optimal for its use *in vivo*. HyPer-2 differs from HyPer in its doubled response amplitude, which improves the target signal-to-noise ratio in living systems, especially at low H_2O_2 concentrations. HyPer-3 retained the high amplitude and showed faster oxidation/reduction dynamics, which made it possible to track changes in H_2O_2 levels more quickly. HyPer7 is rather brighter compared to its predecessors, which is an important improvement because it significantly facilitates its visualization in tissues. Moreover, the pH stability of the probe allows its use without pH-control, and this is rather important in animal models, because the more complex the organism, the less likely it is to make good control for the experiment since different individuals differ more from each other.

TriPer, a probe that reports H_2O_2 dynamics in the thiol-oxidizing environment of the ER, was developed by Melo et al.[22]. The original HyPers are fully oxidized in such conditions, and the solution found by the authors was as follows: the third cysteine was introduced into the OxyR-RD moiety, which initially contains two redox active cysteines that form a disulfide bond under oxidation. The introduction of the third cysteine may permit rearrangement of the disulfide bonding pattern and preserve a fraction of the indicator in its reduced form even in oxidizing conditions. Another H_2O_2-sensing probe, NeonOxIrr, has been published recently[23]. NeonOxIrr is characterized by a slow reduction rate and allows H_2O_2 registration *ex vivo* in fixed samples.

3. Biosensors that report NAD(H) redox state

Besides H_2O_2 and glutathione redox state sensing probes, sensors for NADH/NAD^+ registration are commonly used, as this ratio serves as a metabolic and redox marker. Most representatives of this group are based on the bacterial repressor protein Rex. In the ligand binding site of Rex, competition between NAD^+ and NADH occurs, and when the NADH/NAD^+ ratio changes, the protein undergoes dramatic conformational rearrangement[24]. Peredox was constructed by inserting the circularly permuted FP cpT-Sapphire between two *Thermus aquaticus* Rex (T-Rex) subunits with subsequent optimization and fusing mCherry to the construct for fluorescence signal normalization[25]. The probe had remarkably high affinity to NADH. This may be beneficial as Peredox senses minimal changes in NADH levels, but in some conditions with initially high NADH levels it could limit the application of the probe due to saturation. This could happen when using the indicator in mitochondrial matrix[25] or in bacteria[26] for instance.

In a similar manner to Peredox, Frex and FrexH were developed by integration of cpYFP between one full-length and one truncated *Bacillus subtilis* Rex subunits, although, these probes are specific to NADH only[27]. These indicators have an intensiometric response and are different in affinity to NADH and as well as in the direction and amplitude of response, so one could use either of them in appropriate experimental conditions. RexYFP is an intensiometric NADH/NAD^+ reporter, which was based on a single T-Rex subunit with cpYFP integrated between DNA- and ligand-binding T-Rex domains[28]. Thus, the RexYFP protein molecule is smaller than that of Peredox and Frex, which may facilitate its use when targeting to different subcellular compartments. The affinity of RexYFP to NADH (K' = 180 nM) allows its implementation both in cytoplasm and mitochondria. The indicator also demonstrates low affinity to NADPH (K' = 6.2 µM), which should not interfere in most experiments. However, since the NADP(H) pool is more reduced than NAD(H) pool in the cytoplasm, sensitivity to NADPH could alter the signal under certain conditions. Another probe, SoNar, was also constructed by inserting cpYFP into a T-Rex monomer but in this case the fluorescent core was placed in the surface loop of the nucleotide-binding domain, while the DNA-binding domain was truncated[29]. The distinct feature of SoNar is the remarkably high amplitude of its ratiometric response (~15-fold) that makes its use *in vivo* rather preferable. Later, SoNar specificity was altered by rational mutagenesis and iNaps, which are NADPH sensors with different affinities, were reported [30]. Like SoNar, iNaps are characterized by ratiometricity of the fluorescent signal and high response amplitudes. Recently, an NAD^+/AXP reporter was developed by Zou et al.[31] where AXP refers to the total pool of ATP and ADP. FiNad was obtained by optimizing one of the versions that preceded SoNar via random mutagenesis of short amino acid linkers between T-Rex and cpYFP. Then, mCherry was fused to the sensor to achieve a ratiometric fluorescence response. The authors suggest that FiNad actually reports NAD^+ level shifts since the total physiological AXP pool is generally maintained in homeostasis.

4. Biosensors specific to other redox parameters

Along with redox-sensitive proteins and families of indicators specific to H_2O_2 and NAD(H), there are several probes that are specific to other redox parameters. Indicators for R- and S-diastereomers of methionine sulfoxide were reported by Tarrago et al.[32]. Methionine sulfoxide is formed during oxidation of methionine by biological oxidants both under physiological and pathological conditions and may serve as redox biomarker. Each probe, MetSOx or MetROx, was constructed as a chimera consisting of *Saccharomyces cerevisiae* methionine sulfoxide reductase (MSRA or MSRB, specific to the diastereomer), its specific thioredoxin (Trx1 or Trx3) and cpYFP located between them. The following mechanism of action is expected for MetSOx and MetROx: under the influence of methionine sulfoxide, two redox active cysteines of MSR form a disulfide bond, which is subsequently reduced by a corresponding thioredoxin (Trx). During the process of reduction, a new disulfide is formed between Trx and methionine sulfoxide reductase, and this is accompanied by conformational changes that trigger a shift in the fluorescence signal. A probe that enables a visualization of organic hydroperoxides in living systems was developed by Simen Zhao et al.[33]. In OHSer, cpVenus was placed in the most conformationally mobile region of the bacterial transcriptional factor OhrR, which is specific to organic hydroperoxides. Thus, the conformational change that occurs in the OhrR moiety after its interaction with organic hydroperoxides leads to a change in the spatial structure of cpVenus and the fluorescence signal.

5. FRET-based biosensors

Besides single FP-based biosensors, there are a number of Förster resonance energy transfer (FRET)-based redox-sensitive probes used *in vivo*. Redoxfluor, reported by Yano et al., has been used to monitor the redox states of peroxisomes[34]. The biosensor molecule consists of Cerulean and Citrine FPs and a tandem repeat of part of the yeast transcriptional factor Yap1 between them. The Yap1 part is capable of undergoing conformational rearrangement during oxidation-reduction events represented by internal disulfide bond formation, and this results in a FRET response of the probe. A specific $NADP^+$-sensing FRET-based probe, NADPsor[35], was designed by inserting the $NADP^+$-reporter

element, ketopantoate reductase, between the FRET pair cyan FP and YFP. When binding to $NADP^+$, ketopantoate reductase changes its conformation from relatively closed to open, so FPs move away from each other and the FRET between them weakens. A probe for measurements of the thioredoxin redox state in chloroplasts, CROST[36], has been recently published by Sugiura et al. CROST contains the mTurquoise/cp-mVenus pair and partial sequence from CP12, a redox-sensitive regulatory and thioredoxin-targeted protein in chloroplasts, between FPs. As in other fluorescent biosensors, the reduction of internal cysteines in the CP12-derived domain by thioredoxin leads to conformational alterations in the molecule, and corresponding changes in the FRET ratio.

References

1. George T. Hanson; Robert Aggeler; Devin Oglesbee; Mark Cannon; Roderick A. Capaldi; Roger Y. Tsien; S. James Remington; Investigating Mitochondrial Redox Potential with Redox-sensitive Green Fluorescent Protein Indicators. *Journal of Biological Chemistry* **2004**, *279*, 13044-13053, 10.1074/jbc.m312846200.
2. Mark B. Cannon; S James Remington; Re-engineering redox-sensitive green fluorescent protein for improved response rate. *Protein Science* **2006**, *15*, 45-57, 10.1110/ps.051734306.
3. Jeremy R. Lohman; S. James Remington; Development of a Family of Redox-Sensitive Green Fluorescent Protein Indicators for Use in Relatively Oxidizing Subcellular Environments†‡. *Biochemistry* **2008**, *47*, 8678-8688, 10.1021/bi800498g.
4. Marcus Gutscher; Anne-Laure Pauleau; Laurent Marty; Thorsten Brach; Guido H Wabnitz; Yvonne Samstag; Andreas J Meyer; Tobias P Dick; Real-time imaging of the intracellular glutathione redox potential. *Nature Methods* **2008**, *5*, 553-559, 10.1038/nmeth.1212.
5. Ashima Bhaskar; Manbeena Chawla; Mansi Mehta; Pankti Parikh; Pallavi Chandra; Devayani Bhave; Dhiraj Kumar; Kate S. Carroll; Amit Singh; Reengineering Redox Sensitive GFP to Measure Mycothiol Redox Potential of Mycobacterium tuberculosis during Infection. *PLOS Pathogens* **2014**, *10*, e1003902, 10.1371/journal.ppat.1003902.
6. Vu Van Loi; Manuela Harms; Marret Müller; Nguyen Thi Thu Huyen; Christopher J. Hamilton; Falko Hochgräfe; Jan Pané-Farré; Haike Antelmann; Real-Time Imaging of the Bacillithiol Redox Potential in the Human PathogenStaphylococcus aureusUsing a Genetically Encoded Bacilliredoxin-Fused Redox Biosensor. *Antioxidants & Redox Signaling* **2017**, *26*, 835-848, 10.1089/ars.2016.6733.
7. Samantha Ebersoll; Marta Bogacz; Lina M Günter; Tobias P Dick; R. Luise Krauth-Siegel; A tryparedoxin-coupled biosensor reveals a mitochondrial trypanothione metabolism in trypanosomes. *eLife* **2020**, *9*, e53227, 10.7554/eLife.53227.
8. Marcus Gutscher; Mirko C. Sobotta; Guido H. Wabnitz; Seda Ballikaya; Andreas J. Meyer; Yvonne Samstag; Tobias P Dick; Proximity-based Protein Thiol Oxidation by H2O2-scavenging Peroxidases. *Journal of Biological Chemistry* **2009**, *284*, 31532-31540, 10.1074/jbc.m109.059246.
9. Bruce Morgan; Koen Van Laer; Theresa N E Owusu; Daria Ezeriņa; Daniel Pastor-Flores; Prince Saforo Amponsah; Anja Tursch; Tobias P Dick; Real-time monitoring of basal H2O2 levels with peroxiredoxin-based probes. *Nature Chemical Biology* **2016**, *12*, 437-443, 10.1038/nchembio.2067.
10. Henrik Østergaard; Anette Henriksen; Flemming G. Hansen; Jakob R. Winther; Shedding light on disulfide bond formation: engineering a redox switch in green fluorescent protein. *The EMBO Journal* **2001**, *20*, 5853-5862, 10.1093/emboj/20.21.5853.
11. Alexandra Müller; Jannis F. Schneider; Adriana Degrossoli; Nataliya Lupilova; Tobias P. Dick; Lars I. Leichert; Systematic in vitro assessment of responses of roGFP2-based probes to physiologically relevant oxidant species. *Free Radical Biology and Medicine* **2017**, *106*, 329-338, 10.1016/j.freeradbiomed.2017.02.044.
12. Kazunori Sugiura; Takeharu Nagai; Masahiro Nakano; Hiroshi Ichinose; Takakazu Nakabayashi; Nobuhiro Ohta; Toru Hisabori; Redox sensor proteins for highly sensitive direct imaging of intracellular redox state. *Biochemical and Biophysical Research Communications* **2015**, *457*, 242-248, 10.1016/j.bbrc.2014.12.095.
13. Yichong Fan; Zhijie Chen; Hui-Wang Ai; Monitoring Redox Dynamics in Living Cells with a Redox-Sensitive Red Fluorescent Protein. *Analytical Chemistry* **2015**, *87*, 2802-2810, 10.1021/ac5041988.
14. Arina G. Shokhina; Alexander I. Kostyuk; Yulia G. Ermakova; Anastasiya S. Panova; Dmitry B. Staroverov; Evgeny S. Egorov; Mikhail S. Baranov; Gijsbert J. Van Belle; Dörthe M. Katschinski; Vsevolod V. Belousov; et al. Red fluorescent redox-sensitive biosensor Grx1-roCherry. *Redox Biology* **2019**, *21*, 101071, 10.1016/j.redox.2018.101071.
15. Akiko Kumagai; Ryoko Ando; Hideyuki Miyatake; Peter Greimel; Toshihide Kobayashi; Yoshio Hirabayashi; Tomomi Shimogori; Atsushi Miyawaki; A Bilirubin-Inducible Fluorescent Protein from Eel Muscle. *Cell* **2013**, *153*, 1602-1611, 10.1016/j.cell.2013.05.038.

16. Hanyang Hu; Aoxue Wang; Li Huang; Yejun Zou; Yanfang Gu; Xianjun Chen; Yuzheng Zhao; Yi Yang; Monitoring cellul ar redox state under hypoxia using a fluorescent sensor based on eel fluorescent protein. *Free Radical Biology and Me dicine* **2018**, *120*, 255-265, 10.1016/j.freeradbiomed.2018.03.041.

17. Dmitry S. Bilan; Luke Pase; Linda Joosen; Andrey Yu. Gorokhovatsky; Yulia G. Ermakova; Theodorus W. J. Gadella; Cl emens Grabher; Carsten Schultz; Sergey Lukyanov; Vsevolod V. Belousov; et al. HyPer-3: A Genetically Encoded H2O 2 Probe with Improved Performance for Ratiometric and Fluorescence Lifetime Imaging. *ACS Chemical Biology* **2013**, *8*, 535-542, 10.1021/cb300625g.

18. Vsevolod V Belousov; Arkady F Fradkov; Konstantin A Lukyanov; Dmitry B Staroverov; Konstantin S Shakhbazov; Alex ey V Terskikh; Sergey Lukyanov; Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nature Methods* **2006**, *3*, 281-286, 10.1038/nmeth866.

19. Kseniya N. Markvicheva; Dmitry S. Bilan; Natalia M. Mishina; Andrey Yu. Gorokhovatsky; Leonid M. Vinokurov; Sergey Lukyanov; Vsevolod V. Belousov; A genetically encoded sensor for H2O2 with expanded dynamic range. *Bioorganic & Medicinal Chemistry* **2011**, *19*, 1079-1084, 10.1016/j.bmc.2010.07.014.

20. Valeriy V. Pak; Daria Ezeriņa; Olga G. Lyublinskaya; Brandán Pedre; Pyotr A. Tyurin-Kuzmin; Natalie M. Mishina; Mario n Thauvin; David Young; Khadija Wahni; Santiago Agustín Martínez Gache; et al. Ultrasensitive Genetically Encoded In dicator for Hydrogen Peroxide Identifies Roles for the Oxidant in Cell Migration and Mitochondrial Function. *Cell Metab olism* **2020**, *31*, 642-653.e6, 10.1016/j.cmet.2020.02.003.

21. Yulia G. Ermakova; Dmitry S. Bilan; Mikhail E. Matlashov; Natalia M. Mishina; Ksenia N. Markvicheva; Oksana M. Suba ch; Fedor V. Subach; Ivan Bogeski; Markus Hoth; Grigori Enikolopov; et al. Red fluorescent genetically encoded indicat or for intracellular hydrogen peroxide. *Nature Communications* **2014**, *5*, 1-9, 10.1038/ncomms6222.

22. Eduardo Pinho Melo; Carlos Lopes; Peter Gollwitzer; Stephan Lortz; Sigurd Lenzen; Ilir Mehmeti; Clemens F. Kaminsk i; David Ron; Edward Avezov; TriPer, an optical probe tuned to the endoplasmic reticulum tracks changes in luminal H2 O2. *BMC Biology* **2017**, *15*, 1-15, 10.1186/s12915-017-0367-5.

23. O. M. Subach; Tatiana A. Kunitsyna; Olga A. Mineyeva; Alexander A. Lazutkin; Dmitri V. Bezryadnov; Natalia V. Barykin a; K. D. Piatkevich; Yulia G. Ermakova; Dmitry S. Bilan; Vsevolod V. Belousov; et al. Slowly Reducible Genetically Enco ded Green Fluorescent Indicator for In Vivo and Ex Vivo Visualization of Hydrogen Peroxide. *International Journal of M olecular Sciences* **2019**, *20*, 3138, 10.3390/ijms20133138.

24. Krystle J. McLaughlin; Claire M. Strain-Damerell; Kefang Xie; Dimitris Brekasis; Alexei S. Soares; Mark S.B. Paget; Cla ra L. Kielkopf; Structural Basis for NADH/NAD+ Redox Sensing by a Rex Family Repressor. *Molecular Cell* **2010**, *38*, 5 63-575, 10.1016/j.molcel.2010.05.006.

25. Yin Pun Hung; John G. Albeck; Mathew Tantama; Gary Yellen; Imaging Cytosolic NADH-NAD+ Redox State with a Gen etically Encoded Fluorescent Biosensor. *Cell Metabolism* **2011**, *14*, 545-554, 10.1016/j.cmet.2011.08.012.

26. Vijay Tejwani; Franz-Josef Schmitt; Svea Wilkening; Ingo Zebger; Marius Horch; Oliver Lenz; Thomas Friedrich; Investi gation of the NADH/NAD + ratio in Ralstonia eutropha using the fluorescence reporter protein Peredox. *Biochimica et B iophysica Acta (BBA) - Molecular Cell Research* **2017**, *1858*, 86-94, 10.1016/j.bbabio.2016.11.001.

27. Yuzheng Zhao; Jing Jin; Qingxun Hu; Hai-Meng Zhou; Jing Yi; Zhenhang Yu; Lei Xu; Xue Wang; Yi Yang; Joseph Losc alzo; et al. Genetically Encoded Fluorescent Sensors for Intracellular NADH Detection. *Cell Metabolism* **2011**, *14*, 555-566, 10.1016/j.cmet.2011.09.004.

28. Dmitry S. Bilan; Mikhail E. Matlashov; Andrey Yu. Gorokhovatsky; Carsten Schultz; Grigori Enikolopov; Vsevolod V. Bel ousov; Genetically encoded fluorescent indicator for imaging NAD+/NADH ratio changes in different cellular compartm ents. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2014**, *1840*, 951-957, 10.1016/j.bbagen.2013.11.018.

29. Yuzheng Zhao; Qingxun Hu; Feixiong Cheng; Ni Su; Aoxue Wang; Yejun Zou; Hanyang Hu; Xianjun Chen; Hai-Meng Z hou; Xinzhi Huang; et al. SoNar, a Highly Responsive NAD+/NADH Sensor, Allows High-Throughput Metabolic Screeni ng of Anti-tumor Agents. *Cell Metabolism* **2015**, *21*, 777-789, 10.1016/j.cmet.2015.04.009.

30. Rongkun Tao; Yuzheng Zhao; Huanyu Chu; Aoxue Wang; Jiahuan Zhu; Yuzheng Zhao Xianjun Chen; Yejun Zou; Mei S hi; Renmei Liu; Ni Su; et al. Genetically encoded fluorescent sensors reveal dynamic regulation of NADPH metabolism. *Nature Methods* **2017**, *14*, 720-728, 10.1038/nmeth.4306.

31. Yejun Zou; Aoxue Wang; Li Huang; Xudong Zhu; Qingxun Hu; Yinan Zhang; Xianjun Chen; Fengwen Li; Qiaohui Wang; Hu Wang; et al. Illuminating NAD+ Metabolism in Live Cells and In Vivo Using a Genetically Encoded Fluorescent Sens or. *Developmental Cell* **2020**, *53*, 240-252.e7, 10.1016/j.devcel.2020.02.017.

32. Lionel Tarrago; Zalán Péterfi; Byung Cheon Lee; Thomas Michel; Vadim N. Gladyshev; Monitoring methionine sulfoxide with stereospecific mechanism-based fluorescent sensors. *Nature Chemical Biology* **2015**, *11*, 332-338, 10.1038/nche mbio.1787.

33. Boxuan Simen Zhao; Yujie Liang; Yanqun Song; Chunhong Zheng; Ziyang Hao; Peng R. Chen; A Highly Selective Fluorescent Probe for Visualization of Organic Hydroperoxides in Living Cells. *Journal of the American Chemical Society* **2010**, *132*, 17065-17067, 10.1021/ja1071114.
34. Taisuke Yano; Masahide Oku; Natsuko Akeyama; Akinori Itoyama; Hiroya Yurimoto; Shusuke Kuge; Yukio Fujiki; Yasuyoshi Sakai; A Novel Fluorescent Sensor Protein for Visualization of Redox States in the Cytoplasm and in Peroxisomes. *Molecular and Cellular Biology* **2010**, *30*, 3758-3766, 10.1128/mcb.00121-10.
35. Feng-Lan Zhao; Chang Zhang; Chen Zhang; Yun Tang; Bang-Ce Ye; A genetically encoded biosensor for in vitro and in vivo detection of NADP +. *Biosensors and Bioelectronics* **2016**, *77*, 901-906, 10.1016/j.bios.2015.10.063.
36. Kazunori Sugiura; Yuichi Yokochi; Nae Fu; Yuki Fukaya; Keisuke Yoshida; Shoko Mihara; Toru Hisabori; The thioredoxin (Trx) redox state sensor protein can visualize Trx activities in the light/dark response in chloroplasts. *Journal of Biological Chemistry* **2019**, *294*, 12091-12098, 10.1074/jbc.ra119.007616.
