—Photogravure—

Imaging Mitochondrial Redox Environment and Oxidative Stress Using a Redox-Sensitive Fluorescent Protein

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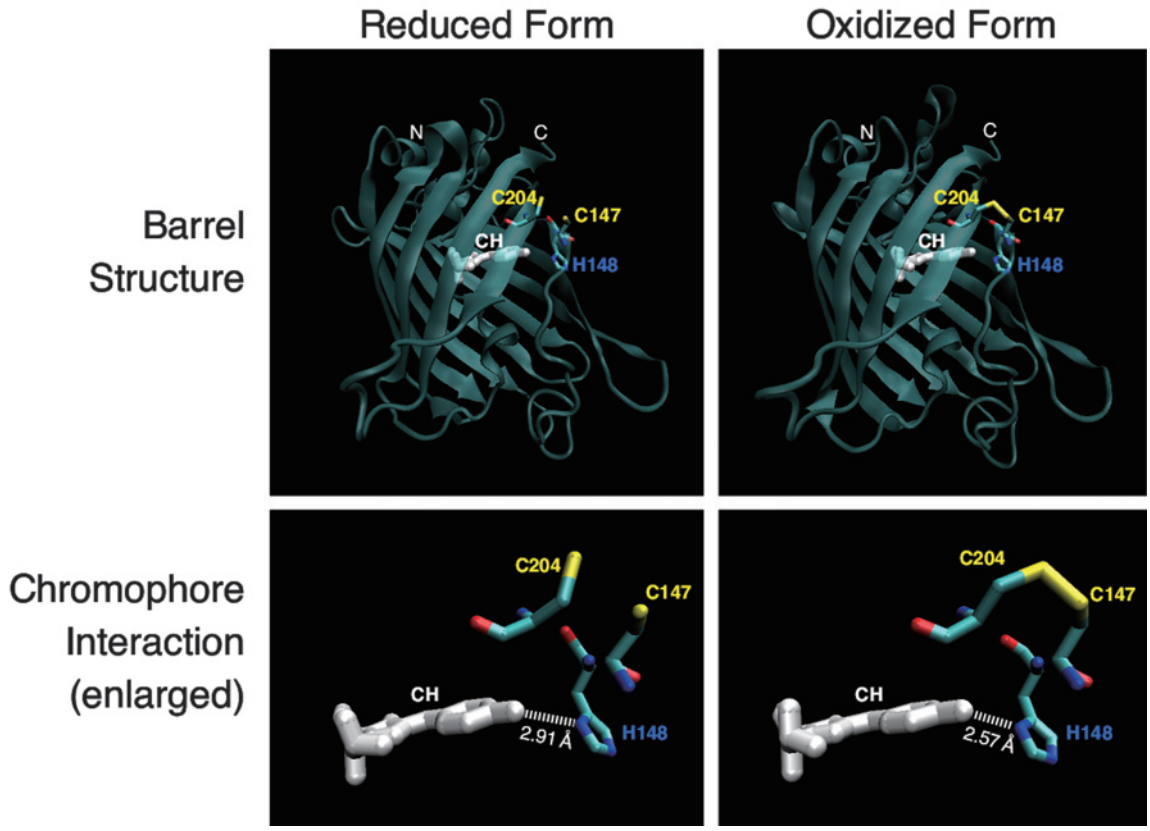

Fig. 1

Abstract

Redox-sensitive green fluorescent protein (roGFP) is a fluorescent protein in which two cysteines are placed adjacently in the barrel structure. Disulfide formation (oxidation) increases the absorption at short wavelengths (410 nm) at the expense of absorption at longer wavelengths (490 nm). The fluorescence ratio indicates reduction/oxidation, i.e., the redox potential at specific cellular locations.

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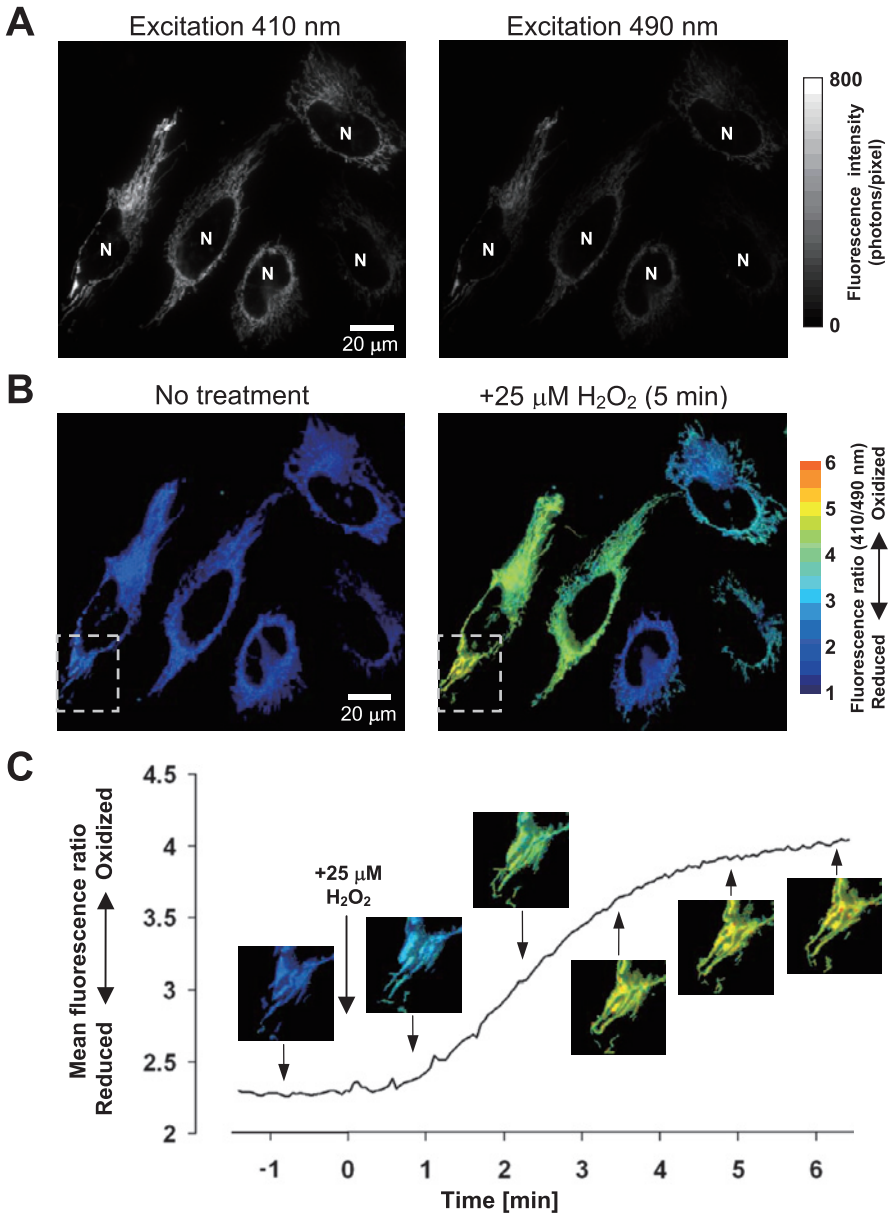

Fig. 2

Fig. 1 Rendered visualization of the reduced and oxidized roGFP barrel structure (green, semitransparent) protecting the chromophore (CH, white), the location of the two cysteines (C147 and C204), and the histidine (H148) involved in the chromophore interaction. The enlargement shows how the formation of a disulfide bridge (yellow) brings histidine 148 closer to the chromophore, changing the absorption spectrum of the protein.

Fig. 2 (A) Fluorescence images of HeLa cells expressing roGFP targeted to the mitochondrial matrix (N: nucleus). roGFP excitation is higher at 410 nm. (B) Visualization of redox state by dividing fluorescence intensities and assigning color according to a scale. Hydrogen peroxide induced oxidation of the mitochondrial matrix. (C) Time course of the mean fluorescence ratio of the cells in B, showing gradual matrix oxidation after addition of H_2O_2. Inset magnifications (boxed area in B) show the dynamic shift of the mitochondrial redox potential.

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