SIMULTANEOUS FLIM-PLIM IMAGING USING DUAL pH-O_2 MOLECULAR PROBE

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Luminescent bioimaging is a rapidly developing and highly promising method for the non-invasive visualization of biological systems [1]. Luminescent compounds can be sensitive to corresponding media parameters and possess specific photophysical characteristics, e.g. lifetime of excited state (LT), in definite conditions. New microscopy methods PLIM and FLIM (phosphorescence and fluorescence lifetime imaging microscopy) allow mapping of the microscopic image according to the lifetime of the sensor probe in each pixel. Using two sensors conjugated with some vector can afford us to get unique information on these parameters in each point of the image and therefore monitor and study processes in living cells and tissues in real-time.

Herein, we report the first example of simultaneous PLIM-FLIM cellular imaging using a dual pH-O_2 probe (Figure 1). The probe was built on the basis of HSA (human serum albumin), the most common protein in the human body, which is known and widely used as the carrier for hydrophobic compounds in cells [2]. Fluorescein (FITC) and iridium complex (Ir) were substantially conjugated to HSA. Fluorescein, fluorophore, possesses strong pH dependency of photophysical properties upon acidity in the physiological pH region (4,5 - 8,5), whereas the triplet emission intensity of Ir-complex is highly sensitive to the presence of molecular oxygen. Thus, the dual conjugate Ir-HSA-FITC is simultaneously sensitive to pH and O_2.

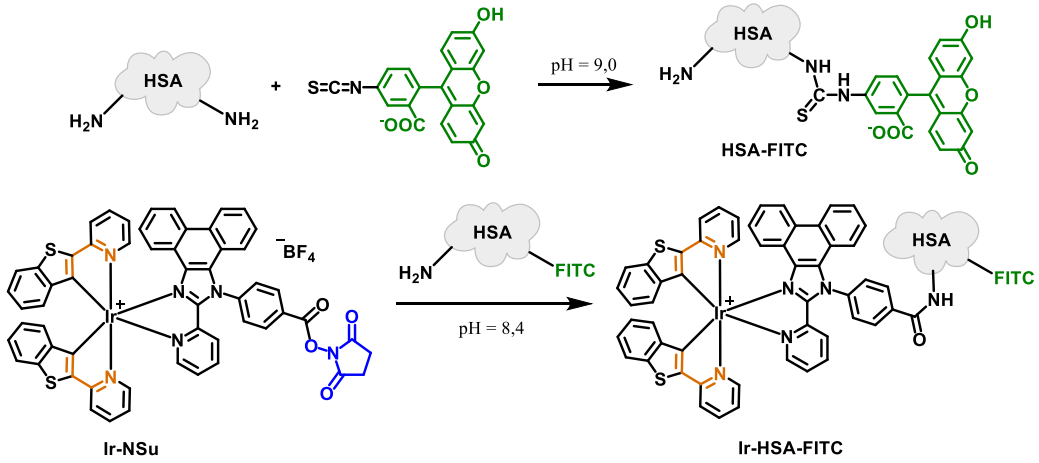

Figure 1. Synthesis of the dual probe Ir-HSA-FITC.

Calibration curves of singlet emission lifetime upon pH and of triplet emission lifetime upon [O_2] were measured in buffer solution and growing media. The conjugate does not demonstrate cytotoxicity on CHO cells up to 150 μM. The compound localizes in lysosomes and possesses very low FITC emission intensity, which can be explained by quenching of the fluorescence in highly acetic conditions of lysosomes. Ir phosphorescence is sensitive to molecular oxygen concentration, which allows using the sensor as a pH-O_2 probe for simultaneous PLIM-FLIM imaging.

References

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