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A Ratiometric Fluorescent Sensor for Intracellular Nitric Oxide Detection

Lucia Bellanova [1], Arne Franzen [2], Stefano Bruno [1], Antonio Scarano [1], Stefania Abbruzzetti [1], Pietro Delcanale [1], Cristiano Viappiani [1], Thomas Gensch [2]

[1] Università di Parma, Parma, Italy

[2] Forschungszentrum Jülich, Jülich, Germany

Monitoring intracellular nitric oxide (NO) dynamics is essential to understand its role in several pathological and physiological processes. These studies have been hampered by the scarce availability of suitable NO probes. Recently, Genetically Encoded Fluorescent Sensors (GES) have been developed to this aim; nonetheless, this field remains poorly explored and only a limited number of GES have been proposed for intracellular NO sensing. In this work, we present a chimeric construct consisting of two fluorescence protein variants that can be used as a ratiometric sensor for NO. In this construct, we have fused the blue fluorescent protein mTagBFP2, with the red emitting protein mCherry. The fluorescence emission intensity and decay lifetime of mTagBFP2 are strongly dependent on NO concentration in the micromolar range, whereas mCherry fluorescence is unaffected. We find that upon exposure to NO, fluorescence intensity and lifetime of the mTagBFP2 domain decreases, while mCherry emission is unchanged. The above properties provide the basis for the development of a ratiometric NO sensor. Further experiments on bacterial and mammalian cells expressing the chimeric construc are underway.

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