THE IMPACT OF THE SUBCELLULAR LOCALISATION OF FLUORESCENCE PROTEINS ON THEIR PHOTOPHYSICAL PROPERTIES

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Since the extraction, identification and genetic encoding of the autofluorescent green fluorescence protein (GFP) from Aequorea victoria about 15 years ago, fluorescence proteins (FPs) became useful tools in cell biology [1]. FPs fused to target proteins serve as useful markers to determine their subcellular localisation. Furthermore, they can be utilised to display intermolecular protein interactions like colocalisation as well as intramolecular conformational changes of proteins to e.g. read out phosphorylation events [2] by taking advantage of photophysical effects like Förster resonance energy transfer (FRET) [3], fluorescence lifetime changes (FLIM) or FRET induced FLIM alterations (FIFA). For this, their detailed photophysical properties such as fluorescence spectra or fluorescence lifetimes are of fundamental importance.

We investigated HEK-293 cells expressing FPs (CFP, GFP, YFP, DsRed) localised to various subcellular compartments, e.g. mitochondria, golgi complex, cytoplasm and plasma membrane. Living cells were optically analysed in cells 24-48 hours after transfection. 3D-confocal microscopy (QLC-100, VisiTech international) was used to verify the subcellular localisation of the FPs. The spectral properties of single cells were measured by attaching a fluorescence spectrometer (USB2000, Ocean Optics) to an inverted microscope (TE2000, Nikon). To determine the fluorescence lifetime a streak-camera-based set-up was used (C9136, Hamamatsu Photonics), in which lifetime is translated into spatial information.

Photophysical properties of the FPs at different subcellular localisation were compared to FPs in solution.

Here we report recorded differences in excitation and/or emission spectra of the FP localised to various intracellular targets, discuss the impact of these findings on spectral unmixing techniques and the effects of localisation on fluorescence lifetimes.

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