

MULTIPARAMETER ANALYSIS OF SENSITIZED EMISSION DATA TO DETECT INTRA- AND INTERMOLECULAR FRET, MATURATION AND ENVIRONMENTAL EFFECTS IN VIVO.

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Methods to detect Fluorescence Resonance Energy Transfer (FRET) comprise photobleaching techniques, Fluorescence Lifetime Imaging Microscopy (FLIM) and measurements of the sensitized emission of the acceptor. Photobleaching and sensitized emission techniques provide only an apparent FRET efficiency. This apparent efficiency is affected by the amount of interacting vs. non-interacting fluorophores and additionally by the measurement conditions in the case of sensitized emission measurements. Methods to obtain information beyond the apparent FRET efficiency using conventional imaging means such as sensitized emission detection would be desirable.

Using descriptions of FRET interactions as linear equations, the contributing factors to sensitized emission measurements can be completely defined. This allows to correct for the contributions by the specific measurement conditions (e.g. illumination, filter properties, exposure times) to the measured FRET value. Apparent FRET efficiencies can thus be obtained for donor as well as acceptor based normalization that consist only of the true FRET efficiency of the molecule pair and the amount of interacting donor or acceptor molecules. Sensitized emission measurements can thus be made instrument independent. They can then be directly compared to FRET experiments made on other instruments and with other methods (e.g. acceptor photobleaching).

Instrument-corrected donor and acceptor normalized sensitized emission data can be further combined into a multiparameter FRET analysis. This approach provides information about changes in the stoichiometries of interacting donor and acceptor molecules in the form of a ratiometric value. As the data for these calculations are taken from sensitized emission measurements, changes in donor/acceptor stoichiometry can thus be easily observed over time in living samples. Knowledge of the true (molecular) FRET efficiency of the donor/acceptor pair is not needed for this analysis.

Multiparameter FRET analysis also allows the detection of non-fluorescent dark states of one of the fluorophores in fluorescent protein tandem constructs. Thus a better calibration of ratiometric measurements as well as correction for the slower maturation of one of the fluorescent proteins during long term observations can be achieved.

Additionally, effects of the environment on one of the fluorophores of a FRET pair can be distinguished from actual changes in FRET efficiency by conformational change. This aspect can be used to correct environmental effects on physiological sensors as well as to study the effects of fixation on the conformation of a FRET pair.

All in all, multiparameter FRET analysis offers many new ways to study dynamic processes in live cell experiments.