Examination of Förster resonance energy transfer (FRET) characteristics of fluorescent dyes with DNA-oligomer hybridization

The main purpose of this present work is to identify fluorescent dye pairs that can participate in FRET reactions as donors and acceptors, among the numerous labels, that were synthetized in our laboratory. At the outset, we selected 12 dyes covering a broad spectral range.

For measuring the FRET efficiency, we labelled 17 base pair long single-stranded DNA oligomers that were complementary to each other, with the proper fluorescent dyes. The spectral properties of these preparations were recorded and, we identified theoretically functioning FRET dyes in pairs. From the 121 combinatorically possible pairs, 48 seemed to be a possible functioning pair. After that, we reduced this number with those pairs, where the overlap between the emission spectrum of the donor molecule, and the excitation spectrum of the acceptor fluorophore was less than 20%. Plus, we added an extra criterium that excluded all the cases, where the cross-talk or the bleed-through phenomenon is significant according to the spectral analysis. This process resulted in 27 dye pairs that were tested for their FRET efficacy.

During the investigations, we synthetized 19 DNA-oligomer preparations, with the use of 10 possible donor labels, and 9 possible acceptor labels. We kept the donor concentration on a relatively constant level, compared to the acceptor concentration, that was continuously added to the solution during the FRET titrations, and we monitored the changes of the fluorescence intensity and could calculate the efficacy.

Our initial goal consisted of three standards: to measure less fluorescence intensity on the emission spectrum of the donor in the present of the acceptor dye, to measure more fluorescence intensity on the acceptor molecule at the same time, and the excitation spectrum of the donor is on a larger wavelength range, because it is desired to avoid the phenomenon of autofluorescence, while examining biological samples, for further research purposes.

We could not identify a dye pair, that succeeded all of the three standards above, specifically, we could not measure significant increasements on the intensity levels at the acceptor molecules side. But we could not exclude that with a device, that has a different sensitivity, such as the microscope, could also detect a better signal from the acceptor form this dye examination pool. While, in case of the donors, the decrease of the fluorescence intensity often approximates or outperforms our 72% reference value from the literature.