STRATEGIES TO IMPROVE PHOTOSTABILITIES IN ULTRASENSITIVE FLUORESCENCE SPECTROSCOPY AND IMAGING

**Dept. NanoBiophotonics, MPI Biophys. Chem., Göttingen, Germany;
*** Institut für Physikalische Chemie, Heinrich-Heine Universität, Düsseldorf, Germany
E-mail: andriy@biomolphysics.kth.se

KEY WORDS: Fluorescence, spectroscopy, imaging, photobleaching, photophysics, triplet states, antioxidants, triplet state quenchers, Rhodamine 6G, ascorbic acid, MEA, COT, nPG

Given the particular importance of dye photostability for single-molecule and fluorescence fluctuation spectroscopy investigations, refined strategies were explored for how to chemically retard dye photobleaching. These strategies will be useful for fluorescence correlation spectroscopy (FCS), fluorescence-based confocal single-molecule detection (SMD) and related techniques. In particular, the effects on the addition of two main categories of antifading compounds, antioxidants (n-propyl gallate, nPG, ascorbic acid, AA) and triplet state quenchers (mercaptoethylamine, MEA, cyclo-octatetraene, COT), were investigated, and the relevant rate parameters involved were determined for the dye Rhodamine 6G. Addition of each of the compound categories resulted in significant improvements in the fluorescence brightness of the monitored fluorescent molecules in FCS measurements. For antioxidants, we identify the balance between reduction of photoionized fluorophores on the one hand and that of intact fluorophores on the other as an important guideline for the amount of concentration needed to be added for optimal fluorescence generation in FCS and SMD experiments. For nPG/AA, this optimal concentration was found to be in the lower micromolar range, which is considerably less than what has previously been suggested. Also, for MEA, which is a compound known as a triplet state quencher, it is eventually its antioxidative properties and the balance between reduction of fluorophore cation radicals and that of intact fluorophores that defines the optimal added concentration. Interestingly, in this optimal concentration range the triplet state quenching is still far from sufficient to fully minimize the triplet populations. We identify photoionization as the main mechanism of photobleaching within typical transit times of fluorescent molecules through the detection volume in a confocal FCS or SMD instrument (<1-20 ms), and it manifests its generation via both one- and multistep excitation processes. Apart from reflecting a major pathway for photobleaching, our results also suggest the exploitation of the photoinduced ionization and the subsequent reduction by antioxidants for biomolecular monitoring purposes and as a possible switching mechanism with applications in high-resolution microscopy.