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Analysis of the Spectroscopic Aspects of the Cationic Dye Basic Orange 21 in bulk, and particularly in leukocytes

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The ability to differentiate between leukocyte types by fluorescence properties of the cationic dye Basic Orange 21 (BO21) were tested. The fluorescence properties of BO21 in solutions, in solids and within leukocytes were examined. The quantum yield of BO21 was found to be less than 0.2% with extremely high fluorescence polarization (p=0.465). A thorough analytical and experimental investigation of these phenomena indicated that the very short lifetime of BO21 in water (τ=10ps) is the most probable reason for the high fluorescence polarization of BO21 in water. However, upon addition of heparin, a large anionic molecule within basophil cells, to aqua BO21, the fluorescence polarization dramatically dropped (p=0.465→p=0.195) and fluorescence lifetime lengthened (τ=10ps→τ=56ps). The low quantum yield increased up to 70 times by altering viscosity of the host glycerol in a controlled manner, hence supporting the perception that BO21 is a molecular rotor – a finding which is suggested for the first time and was assessed by a time-dependent DFT. The fluorescence properties of BO21 in the RBL, the Molt-4 and the U937 cell lines, which are established from leukocyte populations of basophils, T-lymphoblast and monocytes, respectively, were investigated at single-cell resolution. In RBL, BO21 exhibits a red shift of the absorption spectra from 485nm to 550nm, while in the Molt-4 and U937 absorption is not observed. However, a green fluorescence (excitation at 480nm) is observed in all three cell types, while a red fluorescence (excitation at 550nm) is obsereved mainly in basophils. From the differences in the absorption and fluorescence properties of BO21 in the three leukocyte types, a SVM (support vector machine) classifier was created, which produced a high success rate.

Biography

Zehavit Eizig received her B.Sc in Biomedical Engineering in 2007 from Lev Academic Center, Jerusalem College of Technology (JCT), Israel. She received her M.Sc at the Biophysical Schottenstein Center, Bar-Ilan University, Physics Department and is currently in the final stages of completing studies for her Ph.D. degree.

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