Time-resolved fluorescence anisotropy and fluorescence lifetime imaging of fluorescent molecular rotors in living cells

Klaus Suhling[1], Pei-Hua Chung[1], James A. Levitt[1,2], Emilie Steinmark[1], Marina K. Kuimova[3], Carolyn Tregidgo[1,4], Gokhan Yahioglu[5]

[1]Department of Physics, King's College London, Strand Campus, Strand, London, WC2R 2LS, UK
[2] Present address: Randall Centre for Cell and Molecular Biophysics, King's College London, Guy's Campus, London SE1 1UL, UK
[3]Department of Chemistry, Imperial College London, South Kensington, London SW7 2AZ, UK
[4] Present address: Genomics England
[5]Anikor Biopharma Ltd, Stevenage Bioscience Catalyst, Gunnels Wood Road, Stevenage, SG1 2FX, UK
Klaus.suhling@kcl.ac.uk, http://www.kcl.ac.uk/nms/depts/physics/people/academicstaff/suhling.aspx

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We present polarization-resolved fluorescence lifetime measurements of the fluorescent molecular rotor bodipy-C_{12} in solutions of varying viscosity and in living cells. We study the anisotropy decay behaviour of the dye in solvents of varying viscosity, and find that the rotational correlation time obeys the Stokes-Einstein-Debye formula. From the integrated fluorescence decays, we calculate the steady-state anisotropy and plot it in a modified Perrin-Weber plot for fluorescent molecular rotors. We find that the initial anisotropy of bodipy-C_{12} is lower than 0.4, in agreement with time-resolved measurements. To understand the fluorescence anisotropy decays in bodipy-C_{12} in a heterogeneous medium such as the cell, we use polarization-resolved FLIM in solutions of varying viscosity in a multiwell plate. We create a model system by combining fluorescence decay curves from two wells to result in a dip-and-rise profile of the time-resolved fluorescence anisotropy decay. This approach allows us to understand the time-resolved fluorescence anisotropy of bodipy-C_{12} in lipid droplets of HeLa cells, which also shows a dip-and-rise profile. On the basis of the rotational correlation time, we find that the viscosity in lipid droplets is lower than in other regions of the cell. This is consistent with fluorescence lifetime measurements.