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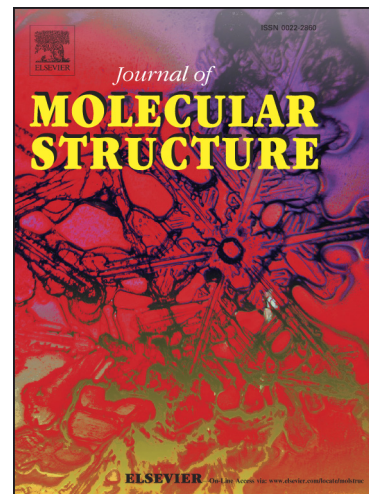
Alexander P. Demchenko

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Practical aspects of wavelength ratiometry in the studies of intermolecular interactions

Alexander P. Demchenko

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovicha st. 9, Kyiv 01601, Ukraine, alexdem@ukr.net

Highlights:

- The wavelength ratiometry is the simplest but the most efficient internally calibrated method to study intermolecular interactions based on fluorescence
- Different versions of this method can be realized, with single, double and multiple reporters
- Different photophysical mechanisms can be used to generate ratiometric response
- The method allows obtaining rich multiparametric information on molecular scale

Abstract

Wavelength ratiometry (often abbreviated as λ -ratiometry) is one of the most popular methods to study intermolecular interactions that allows obtaining both thermodynamic and kinetic parameters and can be applied in a variety of sensing and imaging technologies. In comparison with simple recording of intensity it allows avoiding many technical problems and realizing many new possibilities. Here we briefly analyze different approaches in λ -ratiometric sensing that use single, double and multiple fluorescence emitters that are based on different mechanisms producing spectroscopic change and concentrate on their practical aspects. Finally we present several examples of successful application of this method and discuss the prospects for its further development.

Keywords: Fluorescence analysis; intermolecular interactions; Wavelength ratiometry; Excited-state reactions; Sensing technologies; FRET

Abbreviations: BMFC - 6-bromomethyl-2-(2-furanyl)-3-hydroxychromone; ESIPT – excited-state intramolecular proton transfer; FA - 2-(6-diethylaminobenzo[b]furan-2-yl)-3-hydroxychromone; FRET – Förster resonance energy transfer; GFP – green fluorescent protein; 3HC – 3-hydroxychromone; ICT – intramolecular charge transfer; PET – photoinduced electron transfer.

Corresponding author:

Prof. Alexander P. Demchenko

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovicha st. 9, Kyiv 01601, Ukraine,

Tel. +380 44 234 11 06,

alexdem@ukr.net

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